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Introduction and Establishment of Cyrtobagous salviniae Calder and Sands (Coleoptera: Curculionidae) for the Control of Salvinia minima Baker (Salviniaceae), and Interspecies Interactions Possibly Limiting Successful Control in Louisiana

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INTRODUCTION AND ESTABLISHMENT OF *CYRTOBAGOUS SALVINIAE* CALDER AND SANDS (COLEOPTERA: CURCULIONIDAE) FOR THE CONTROL OF *SALVINIA MINIMA* BAKER (SALVINIACEAE)*,* AND INTERSPECIES INTERACTIONS POSSIBLY LIMITING SUCCESSFUL CONTROL IN LOUISIANA.

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ABSTRACT

Common salvinia, *Salvinia minima* Baker, is a floating aquatic invasive macrophyte that

obstructs waterways and causes problems in wetlands across Louisiana and Texas. The salvinia weevil,

Cyrtobagous salviniae Calder and Sands, has been released in over 14 countries around the world for the

biological control of *Salvinia* spp. We successfully monitored the introduction and establishment of *C.*

salviniae on *S. minima* in southern Louisiana between 2006 and 2010. *Cyrtobagous salviniae* significantly

lowered the biomass of *S. minima* and increased the number of terminal buds damaged but had no

significant impact on pH, dissolved oxygen, conductivity, surface temperature, percent of surface

coverage, or percent of the mat that was green. Restricting access to the *S. minima* mat from the red

imported fire ant, *Solenopsis invicta* Buren, significantly increased the number of *C. salviniae,* suggesting

that *S. invicta* should be controlled where possible to maximize the success of the biological control

program. While collecting arthropods associated with *S. minima*, we identified 5,773 individuals that

represent 176 species within 62 families and seven orders including four currently undescribed species,

and seven species of semi-aquatic Curculionidae (five of which have been used in biological control

programs). We collected higher numbers of taxa than previous studies, but most of the species are

previously known as hydro- or hygrophilous, indicating the differences may have been due to collection

methods. Collecting locations were clustered into five groups based on secondary aquatic vegetation

and evaluated by cluster for community composition and similarity. We found no support for the spatial

heterogeneity hypothesis, as our most diverse community is one of the least spatially complex,

suggesting other factors are affecting community composition. Findings included successful

establishment of *C. salviniae*, recommendation to control *S. invicta* around *C. salviniae* release sites,

improved methods for collecting insects associated with floating aquatic vegetation, and contributions

to the knowledge of the biodiversity of Louisiana's backwater swamps. In addition to providing new

information on interactions between *C. salviniae* and *S. minima*, these studies will be useful in designing,

evaluating, and monitoring releases of other biological control agents on floating aquatic macrophytes.

CHAPTER 1
INTRODUCTION AND REVIEW OF THE LITERATURE

THE GENUS *SALVINIA*

The floating fern genus *Salvinia* (Salviniaceae) was named for the botanist A.M. Salvini and erected for the type species *Salvinia natans* in 1754 (Seguir 1754). More than 31 species have been described within the genus, but of those only 10 are currently accepted and valid (de la Sota 1977, Schneller 1990). Of the 10 currently recognized species, 7 are endemic to tropical America (de la Sota 1976).

Members of the genus *Salvinia* are characterized as rootless with whorled leaves around a central rhizome, having two green dorsal entire simple leaves and a third ventral highly dissected leaf (Croxdale 1978, 1979, 1981). The adaxial sides of the dorsal leaves are covered by epicuticular wax and dense hydrophobic trichomes that exhibit four different species group specific architectures (Barthlott et al. 1994, 2009). Plants can survive fully submerged underwater for up to 60 days in some species (Barthlott et al. 2009). Most members of the genus are polyploid with a chromosomal base number n=9 (Schneller 1981). *S. molesta* is a pentaploid hybrid and sterile due to irregular spore formation, but reproduces vegetatively like other members of the genus (Loyal and Grewal 1966).

The first reports of *Salvinia* in the United States come from Fredrick Pursh's *Flora americae septentrionalis; or A Systematic Arrangement and Description of The Plants of North America* (1814) listing *S. natans* (L.) Alloni as "Floating, like *Lemna,* on the surface of stagnant waters: in several of the small lakes in the western parts of New York and Canada". A second record of *S. natans* from Missouri at Dixon's Lake in 1886 was confirmed by Dr. Asa Gray at a location where "land is low, somewhat swampy and subjected to over flow by the water of the Mississippi River" (Gray 1890, Weatherby 1921). A third population of *S. natans* was found at Sweeney's Twin Lakes, four miles west of the city of Minneapolis, Minnesota (MacMillan 1891). Additional records for *S. natans* in Staten Island (New York) were reported in 1893 and 1898 (Craig 1893, Hollick 1898). Rosendhal and Butters (1916) disputed the

MacMillian (1891) Minnesota record and noted that while the plant colony flourished in the greenhouse, *S. natans* was never recovered again from Sweeney's Twin Lakes and could have been the result of contamination from other projects in the greenhouse. The Minnesota herbarium material was reexamined by Butters (1921) and the identification changed on the specimens from the name *S. natans* to the *S. auriculata* var. *Olfersiana*. The Saint Johns River and associated tributaries in Florida were listed as having a population of *S. auriculata* from an undetermined introduction (Small 1931).

Herzog (1935) restructured the genus *Salvinia* to contain 8 species, keeping several of the original names listed in Baker (1887) and resurrecting *S. rotundifolia* Willdenow. He separated the species commonly found in the United States by the structure of the trichomes with a complex of species including *S. auriculata* having trichomes that are split and rejoined at the tips. After Herzog's review of the genus, Weatherby (1937) reevaluated the North American collections from the Gray Herbarium and the United States National Herbarium upon which previous records of *S. natans* were based. He concluded that only the record from Missouri (which was never found to have established a permanent population) was truly *S. natans*, with all of the other specimens and records (including *S. olfersiana* pictured in <u>Flora of Bermuda</u>) were truly *S. rotundifolia* based on trichome architecture (Britton 1918, Weatherby 1937). In 1967, Morton examined the holotype for *S. rotundifolia* and discovered it had trichomes with joined hairs and synonymized the name, recommending that the name *S. minima* be resurrected for specimens with branched trichomes (Morton 1967).

The Florida population of *S. minima* referenced in Small (1931) is the only introduction known to survive and successfully establish (Weatherby 1921, 1937). By the end of 1967 *S. minima* was reported from 14 drainage basins across the states of Florida and Georgia (Jacono et al. 2001). Nauman (1978) reported four counties in Florida with records. Its range had expanded to west of the Mississippi River in Louisiana by 1981 (Landry 1981). *Salvinia minima* was discovered outside Mobile, Alabama in 1982

(Haynes and Jacono 2000) and by 1993 it was reported in drainages across Florida, Alabama, Georgia, and Louisiana (Nauman 1993). Continued spread and infestation of waterways were reported in Texas in 1995 (Hatch 1995), Arkansas in 1998 (Peck 1999, 2001), followed by Mississippi and South Carolina in 1999 (Jacono et al. 2001).

Salvinia molesta was first reported in North America in 1995, occurring in a small pond in South Carolina and controlled through eradication (Johnson 1995). It was reported again outside of Houston Texas in 1997 and positively identified as Kariba weed in 1998 (Jacono 1999a). Additional established sites in Texas and Louisiana were discovered at the Toledo Bend Reservoir, Swinney Lake, Sabine River, and Trinity River (Jacono 1999b). It has now spread to waterways in Florida, Alabama, Mississippi, Georgia, and a reach of the lower Colorado River between Arizona and California (Jacono et al. 2001).

THE SALVINIA WEEVIL: *CYRTOBAGOUS SALVINIAE* CALDER AND SANDS (COLEOPTERA: CURCULIONIDAE)

Cyrtobagous salviniae Calder and Sands (Coleoptera: Curculionidae) is a semi-aquatic weevil native to southern Brazil, Bolivia, Paraguay, Uruguay, and Argentina (Wibmer and O'Brien 1986). It has been introduced into 16 countries worldwide to control infestations of *S. molesta* (Julien and Griffiths 1998, Julien et al. 2002). While previously used to control *S. molesta*, *C. salviniae* can also complete its life cycle on *S. minima* (Tipping and Center 2005a). A population of the weevil was identified in Florida in 1962 occurring on *S. minima* and originally identified as a related species, *Cyrtobagous singularis* Hulst (Kissinger 1966). Calder and Sands (1985) later identified the Florida weevil population as *C. salviniae* during attempts to find a biological control agent and noted a size difference between North and South American populations, with the South American populations being considerably larger. *Cyrtobagous salviniae* from South America were identified as a control agent for *S. molesta* and were released in Australia with great success (Room et al. 1981).

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Goolsby et al. (2000) provided evidence that the Florida population was genetically different from larger individuals released in Australia, and suggested the two populations were cryptic species but did not include an outgroup. More recent studies by Madeira et al. (2006) suggested that the size difference between populations is more likely a case of two different ecotypes of the same species than of two differing cryptic species after analyzing differing sizes of *C. salviniae* as well as *C. singularis*. In addition, both *C. singularis* and *C. salviniae* were collected from a variety of species of *Salvinia* within the native overlapping ranges of the species, supporting Sands et al. (1986) theory of niche differentiation (Madeira et al. 2006). Tipping and Center (2005b) found that when given a choice, adults of different ecotypes both preferred larger sized plants, but did not discriminate between species of *Salvinia.* Recent research showed that the smaller Florida ecotype damages both *S. minima* and *S. molesta* more than the larger Brazilian ecotype, suggesting the need for a reanalysis of management strategies for *S. molesta* infestations in the United States (Tipping et al. 2010).

The Florida populations of *C. salviniae* are credited with keeping *S. minima* under control across southern drainages. The explosive growth of *Salvinia spp.* in Texas and Louisiana is attributed to the absence of *C. salviniae* (Jacono et al. 2001). Individuals from the Florida population of *C. salviniae* were introduced into both Texas and Louisiana starting in 2000 to help control infestations of *S. molesta* (Goolsby et al. 2000). After several years of monitoring sites in both locations, the introduction failed and *C. salviniae* had not established in the area (Tipping and Center 2003) but were finally successful (Tipping et al. 2008). Tewari and Johnson (2011) noted that damage from Florida ecotype *C. salviniae* worked in conjunction with a native herbivore in Southern Louisiana, *Samea multiplicalis* (Gunee)(Lepidoptera: Pyralidae) and lowered the biomass of *S. minima* more than either herbivore alone. A single pathogen, *Helicosporidium sp.*, has been isolated and identified from *C. salviniae* individuals in Florida and occurs there at very low densities (White et al. 2007).

THE RED IMPORTED FIRE ANT: *SOLENOPSIS INVICTA* BUREN (HYMENOPTERA: FORMICIDAE)

Ants are an important component of many terrestrial ecosystems, though their role in aquatic systems has not yet been quantified. In terrestrial systems ants often play a major role in nutrient exchange, seed dispersal, and energy movement (Handel et al. 1981). In many areas they are a significant portion of the animal biomass (Holldobler and Wilson 1990).

The Red Imported Fire Ant (RIFA), *Solenopsis invicta* Buren, was introduced into Mobile, Alabama in the 1930s from South America (Buren 1972, Buren et al. 1974) and have continued to spread across the southeastern United States (Gotelli and Arnett 2000, Tschinkel 2006). Large areas of disturbance have contributed to the continued range expansion of RIFA populations which thrive in these areas (Tschinkel 1988, King and Tschinkel 2008). In the United States approximately 132 million ha are infested in 13 states (AL, AR, CA, FL, GA, LA, MS, NC, NM, OK, SC, TN, TX) and Puerto Rico are included in the USDA APHIS Quarantine area (Allen et al. 2004).

In their native range, *S. invicta* populations are kept in check by competition, predators, and natural enemies (Buren et al. 1974, Jouvenaz 1983, Porter et al. 1997). Outside their native range they are often the dominant ant species due to aggressive foraging, lack of natural enemies, and high reproductive rates. They easily colonize disturbed habitats (Taber 2000) and form colonies or "mounds" which are highly territorial. Colony size is directly related to the space the colony controls (Tschinkel et al. 1995). The diet of *S. invicta* is broad and often indiscriminate (Tschinkel 2006), and can have negative effects on beneficial insects and other arthropods (Eubanks 2001). Porter and Savignano (1990) reported that invading *S. invicta* polygyne ants decreased species richness and all but eliminated some native ant species. In addition these colonies reduce diversity across the community landscape long past their initial invasion (Morrison 2002).

Solenopsis invicta has been observed foraging on the mat S. minima in Southern Louisiana regardless of multiple applications of Amdro ™ (AMBrands 2004) on surrounding terrestrial areas. The foraging behavior of S. invicta is not well documented on S. minima, as in terrestrial systems they forage in multiple dimensions including extensive underground tunneling. Porter and Tschinkel (1987) found that S. invicta foraging behavior is directly related to weather and seasonality. Temperature also has been documented to play a role in foraging in many other species of ants (Bernstein 1979).

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CHAPTER 2
INTRODUCTION AND ESTABLISHMENT OF *CYRTOBAGOUS SALVINIAE* CALDER AND SANDS FOR THE CONTROL OF COMMON SALVINIA (*SALVINIA MINIMA* BAKER), IN LOUISIANA.

INTRODUCTION

Salvinia minima Baker is a free floating aquatic fern in the family Salviniaceae, one of over ten species which occur worldwide in the genus *Salvinia* (Mitchell 1972). *Salvinia minima* is one of two non-native species currently found in North America (Jacono et al. 2001). It has been commonly misidentified in older literature as a variety of other *Salvinia* species including: *S. rotundifolia, S. natans*, or *S. auriculata* Aublet (Jacono et al. 2001). It spread progressively from an introduction in the Saint Johns River in Florida throughout the state of Florida, and across the southeastern U.S. (Small 1931).

S. minima can be identified from its larger relative not only by size, but by the presence of divided hairs on the abaxial leaf surface that are free and not joined at the tips (Julien et al. 2002). *Salvinia molesta* Mitchell, the only other member of the genus currently established in North America, has "egg beater" shaped hairs which are joined at the tips (Mitchell 1972). Plants in the genus *Salvinia* are believed to be sterile, do not produce viable spores, and are thought to be of hybrid origin (Mitchell 1972, Schneller 1980). Plants in the genus *Salvinia* reproduce vegetatively from plant fragments (Loyal and Grewal 1966). Madeira et al. (2003) determined through genetic analysis that all of the populations of *S. minima* in the southern United States were closely related with the exception of a more recent secondary introduction in Mississippi. *Salvinia minima* has been recorded from 14 states (USDA and NRCS 2011) and is considered a problematic weed in both Texas and Louisiana (Jacono et al. 2001). Salvinia *minima* has a history of being freely sold and traded as part of the nursery trade (Forno et al. 1983) and is still widely available on the internet in spite of its invasive nature (Kay and Hoyle 2001).

S. minima forms dense mats of plant material that decrease aesthetic value and impede access to and use of many aquatic areas (Montz 1989). Both commercial and recreational uses of lakes and ponds are impaired by its presence and habitat for game birds is altered because *S. minima* outcompetes native plants for resources and shades out submersed vegetation (Hatch 1995). These

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freshwater areas contributed a total positive economic effect of $1,293,172,571 to the state of Louisiana in 2006 through freshwater activities like fishing, migratory bird hunting, and alligator harvests (Southwick Associates 2008). Uncontrolled growth of *S. minima* not only causes mats that obstruct waterways but can decrease light availability, reduce available dissolved oxygen, and alter pH levels (Richards 2003, Flores and Carlson 2006). These thick mats of vegetation can also contribute to human health issues as they provide breeding areas for *Mansonia* sp. disease transmitting mosquitoes (Chow et al. 1955, Ramachandran 1960). Species of *Mansonia* that occur in the US have been implicated in the spread of St. Louis encephalitis and Venezuelan equine encephalitis (Lounibos et al. 1990).

Salvinia spp. prefers lentic freshwater locations, especially marshes and low-lying forested woodlands. Louisiana is home to over 664,898 ha of freshwater marshes and 2,783,023 ha of forested wetlands all of which could potentially be susceptible to infestation by *Salvinia* sp. (Coreil 1993). Like other aquatic weeds, *S.minima* reproduces asexually from small plant fragments, and boats can easily infect clean waterways if not cleaned prior to being moved from infested waterways (Johnstone et al. 1985, Miller and Wilson 1989). Weather can also contribute to the spread of *Salvinia* as mats can fragment and move with flooding and water movement (Harley and Mitchell 1981, Room 1983, Room 1990).

Chemical control is impractical and cannot be recommended against *Salvinia spp*. Currently available chemical control options include Rodeo® (glyphosate) and Reward® (diquat) which are both non-selective, broad spectrum herbicides for aquatic vegetation (Dow Agriscience 2004, Syngenta Crop Protection 2005). In addition to being non-selective, the price for herbicides for chemical control of *S. minima* can range from $198 to $297 /ha (Tewari and Johnson 2011). Chemical applications can also be limited by local laws regarding groundwater and drinking water contamination. Mechanical control is not a feasible control solution as chopping the plants results in larger growth from vegetative fragments, and complete removal by hand is impossible in most areas. Biological control has been successful on a

related species, *Salvinia molesta* Mitchell, and was significantly less expensive than other methods of control (Chikwenhere and Keswani 1997).

Cyrtobagous salviniae, a semi-aquatic weevil native to Brazil, Paraguay, and Bolivia (Wibmer and O'Brien 1986) has been introduced into 16 countries worldwide for control of *S. molesta* (Julien and Griffiths 1998, Julien et al. 2002). While widely used to control *S. molesta*, *C. salviniae* can also complete its life cycle on *S. minima* (Tipping and Center 2005). A population of weevils was identified in Florida occurring on *S. minima*, and was identified as *Cyrtobagous singularis* Hulst, a related species (Kissinger 1966). Calder and Sands (1985) identified the Florida weevil population as a new species, *Cyrtobagous salviniae,* and noted a size difference between North and South American populations, with the South American populations being considerably larger. Recent studies by Madeira (2006) implied that the size difference between populations is more likely a case of two different ecotypes.

Florida populations of *C. salviniae* are credited with keeping *S. minima* under control while the explosive growth of *Salvinia spp*. in Texas and Louisiana is attributed to the absence of the weevil (Jacono et al. 2001). Individuals from a Florida population of *C. salviniae* were first introduced into both Texas and Louisiana in 2000 to help control infestations of *S. molesta* (Goolsby et al. 2000). The goal of this project was to successfully introduce and establish a population of *C. salviniae* that could overwinter in Louisiana and control local infestations of *S. minima*.

MATERIALS AND METHODS

ORIGINS OF *C. SALVINIAE* POPULATIONS

Initial populations of the Florida ecotype of *C. salviniae* were collected in 2002 by Dr. Phil Tipping (USDA-ARS) from the Ft. Lauderdale area in southern Florida. The colony at Louisiana State University was established from 300 weevils sent in two shipments, and reared in a greenhouse on the main campus in Baton Rouge. Dr. Tipping mailed additional weevils during 2004, 2005, and 2006 to

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increase the LSU colony. During September 2005, a trip was made from Louisiana to northern Florida to collect *C. salviniae*. Approximately 200 weevils were collected by hand and an additional 600 were recovered by berlese funnels off *S. minima* from Lake Talquin in Quincy, FL and used to establish a second greenhouse colony from northern Florida at LSU. Field collections were made in northern Florida from Lake Talquin (2005 and 2006) and Lake Miccosukee (2007 and 2008) to collect *C. salviniae* to replenish the greenhouse colonies for releases. Approximately 1400 weevils were collected from Lake Talquin in 2006 while over 2000 weevils were collected by a combination of hand picking and berlese funnels from Lake Miccosukee.

RELEASE LOCATIONS

Cyrtobagous salviniae was first released in Louisiana on *S. minima* in June 2002 at Jean Lafitte National Historical Park and Preserve in Barataria, LA by the USDA-ARS Invasive Plant Research Laboratory in cooperation with the U.S. National Park Service. Additional releases were made at the same locations until 2005, and while populations established during the year, no overwintering was observed. During August 2003, Louisiana State University released south Florida weevils at four sites across southern Louisiana: Henderson Swamp, St. James, Joyce Wildlife Management Area, and Maurepas Wildlife Management Area (Table 2.1, Figure 2.1). During spring 2004, three of the four sites showed no signs of establishment of *C. salviniae* and Henderson Swamp was not sampled due to inaccessibility. Between May 29th and July 29th, 2004, a total of 1,319 weevils were released at five sites across southern Louisiana. The use of Henderson Swamp was discontinued, and two new locations were established: McElroy Swamp, which is near Maurepas WMA, and Cypress Lake at Moss Bluff in St. Charles. Again in the spring of 2005, none of the sites established the prior year showed signs of establishment*. Cyrtobagous salviniae* was released at eight locations during the late spring of 2005 near Gramercy, Louisiana in August 2005 as part of a study on *Samea multiplicalis*.

Table 2.1. Release sites for *C. salviniae* in Southern Louisiana. Numbers listed are per quadrat, number of release locations at a site is listed in parentheses next to the site name.

Release Location	2003	2004	2005	2006	2007	2008	2009	2010
Henderson Swamp (1)	100-200	--	--	--	--	--	--	--
St. James (1)	100-200	100	--	125+150	--	--	--	--
Joyce WMA (1)	100-200	275	--	125+150	--	--	--	--
Maurepas WMA (1)	100-200	309	--	125+150	--	--	--	--
McElroy Swamp (1)	--	315	--	--	--	--	--	--
Cypress Lake (1)	--	307	--	50	--	--	--	--
Gramercy (multiple sites)	--	--	90	150	500	300	--	--
Tunica (x8)	--	--	--	--	--	--	150	150
Vacherie (x8)	--	--	--	--	--	150	--	--
Alligator Bayou (1)	--	--	--	125+150	--	--	--	--
Hammond (1)	--	--	--	--	--	--	--	150
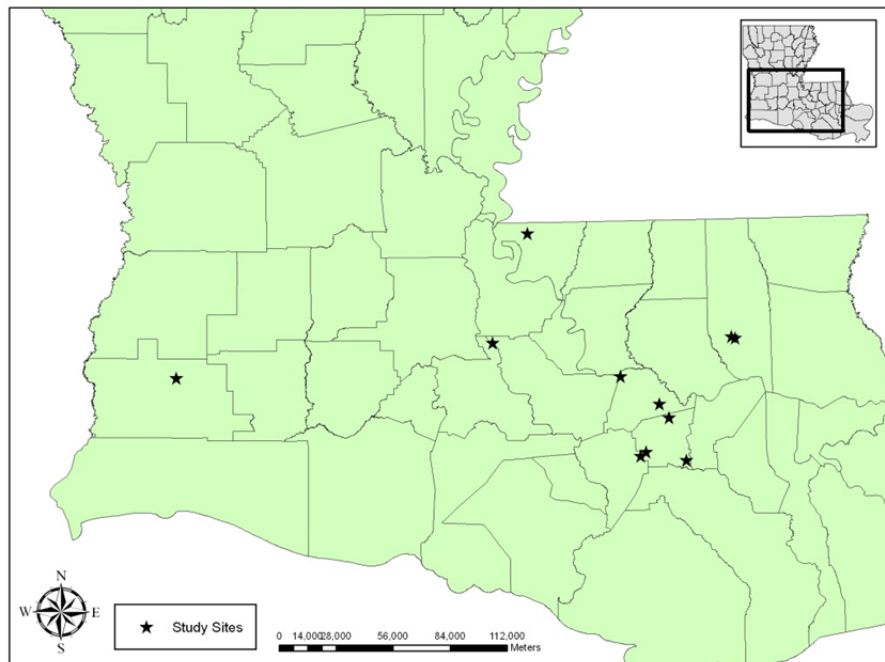
Figure 2.1. Map showing study site locations across southern Louisiana.

Weevils were released in May of 2006 at a total of 11 locations across 6 sites in southern

Louisiana: four locations specifically for this study, eight locations in Gramercy as part of a different

study (Tewari and Johnson 2011), with an additional release at Cypress Lake. An additional release was

made in mid-September at the four release sites for this study. Of those four sites, two were lost for

research when Alligator Bayou became infested with water hyacinth (*Eichornia crassipes* (Mart.) Solm.)

and the site at Joyce was destroyed by vandalism. The number of release sites for 2007 was reduced to

two locations in Gramercy in order to concentrate release numbers of *C. salviniae* and restrict access to

research sites. A third release site was added at Gramercy in 2008 and those three sites were kept

through the end of the experiment. Additional releases were made as part of other studies in Vacherie,

Tunica, and Hammond between 2008 and 2010.

SAMPLING

We constructed 1 m^2 experimental frames from 5.08 cm dia SCH 40 PVC that were sealed to

float and serve as experimental plots (quadrats). Two quadrats were placed at each field location, at

least 500 m apart in similar habitat areas, and anchored with a nylon rope tied to two bricks. Of the

paired quadrats, one was designated a release site and received *C. salviniae* while the other was

maintained as a control site. Any vegetation other than *S. minima* was removed by hand weeding where

and when possible.

Sampling for damage from *C. salviniae* and populations was performed monthly by placing three

0.1 m^2 quadrats made from 2.5 cm SCH 40 PVC into the larger 1 m^2 quadrat. Plant material from the

smaller quadrats was removed from the quadrats and placed into 1 gal plastic bags. Since wet weights

and dry weights of *S. minima* are correlated (Tewari and Johnson 2011), each bag was lightly squeezed

to remove water and weighed. In addition, we randomly chose 100 rhizomes of *S. minima* from within

the larger quadrat and counted adult *C. salviniae* and damage to terminal buds. All sampled material

was returned to the quadrat. The area within each 1 m^2 quadrat was visually assessed for the percentage of the water inside the quadrat that was covered in *S. minima* and an estimation of the percent of the mat that was green in color. Since water quality is impacted by the presence of aquatic vegetation, we also took additional measurements for surface water temperature, dissolved oxygen, pH, and conductivity. Quadrats were sprayed with a microbial insecticide (Thuricide containing *Bacillus thuringiensis kurstaki,* equivalent to 4000 *Spodoptera* units or six million viable spores per milligram) weekly to eliminate confounding effects of feeding damage by *Samea multiplicalis* (Guneé)(Lepidoptera: Crambidae), a native herbivore. This solution was chosen to elminate *S. multiplicalis* because it has no effect on Coleoptera (MacIntosh et al. 1990).

STATISTICAL ANALYSIS

We used a mixed linear model to evaluate and compare the biomass from the release and control plots, focusing on the plots that were part of this research (2006-2010). The data was analyzed as a one-factor completely randomized design. The loss of study sites due to environmental changes and human interference reduced our statistical power within the study, so sites were pooled by year for analysis. Differences in the number of terminal buds damaged between treatment and control, surface water temperature, dissolved oxygen, pH, and conductivity were analyzed by t-test.

RESULTS AND DISCUSSION

While releases of *C. salviniae* were made in southern Louisiana on *S. minima* as early as 2002, data and analysis presented here is from 2006 through 2010. Prior to this study, none of the releases of *C. salviniae* had established in Louisiana and survived through the winter to the following year. Additionally, neither of the two surviving field sites from 2006 had any establishment. Field sites established in Gramercy during 2007 were inoculated with *C. salviniae* individuals from northern Florida instead of southern Florida.

Figure 2.2. Overwintered *C. salviniae* adult, June 2008 in Gramercy, LA.

The first evidence of *C. salviniae* successfully overwintering and establishing on *S. minima* in Louisiana was a single individual found on April 14, 2008 in Gramercy. Additional individuals were observed at both sites in Gramercy on June 17th, 2008 (Figure 2.2) along with visible damage to the *S. minima* mat (Figure 2.3). Material was brought back to the lab from the field on that date and processed through the Berlese funnel for a more thorough population evaluation. Site 1 had 12 *C. salviniae* / kg of *S. minima* while site 2 had 136 *C. salviniae* / kg of plant material. While they had been reported as unestablished, adult *C. salviniae* were observed at mutiple sites in Jean Lafitte National Park during the summer of 2008. We assume these *C. salviniae* were a residual population from the USDA's releases between 2002 and 2005. In the fall of 2008, Hurricane Gustav and Hurricane Ike backed up several feet of storm surge onto the property at Gramercy, limiting access through the fall. The field release sites survived both hurricanes.

Again in 2009, *C. salviniae* successfully overwintered at all three sites in Gramercy that were established for the 08-09 year as well as Jean Lafitte National Park. All sites where we had made releases the previous year were sampled for weevil populations. *Cyrtobagous salviniae* had successfully established in low numbers in Gramercy (18 *C. salvinae*/kg), the site in Vacherie from another study (15 *C. salviniae*/kg), and were located again during sampling at Jean Lafitte National Park (4 *C. salviniae*/kg). Sampling at Gramercy was continued through 2009 until freezing temperatures sank the majority of the mat of *S. minima*. Throughout November and December 2009 low air temperatures with frosts occured, culminating in a low of -5 °C January 11, 2010. Our water surface temperature data recorder for Gramercy was lost during the winter for 2009/2010. The closest USGS data available lists water surface temperatures of 2.8°C January 13, 14, 15, 18, 2010 (54km from reasearch site)(USGS 2010). The mat did not rebound from the freezing temperatures and cover the water's surface again until June 2010.
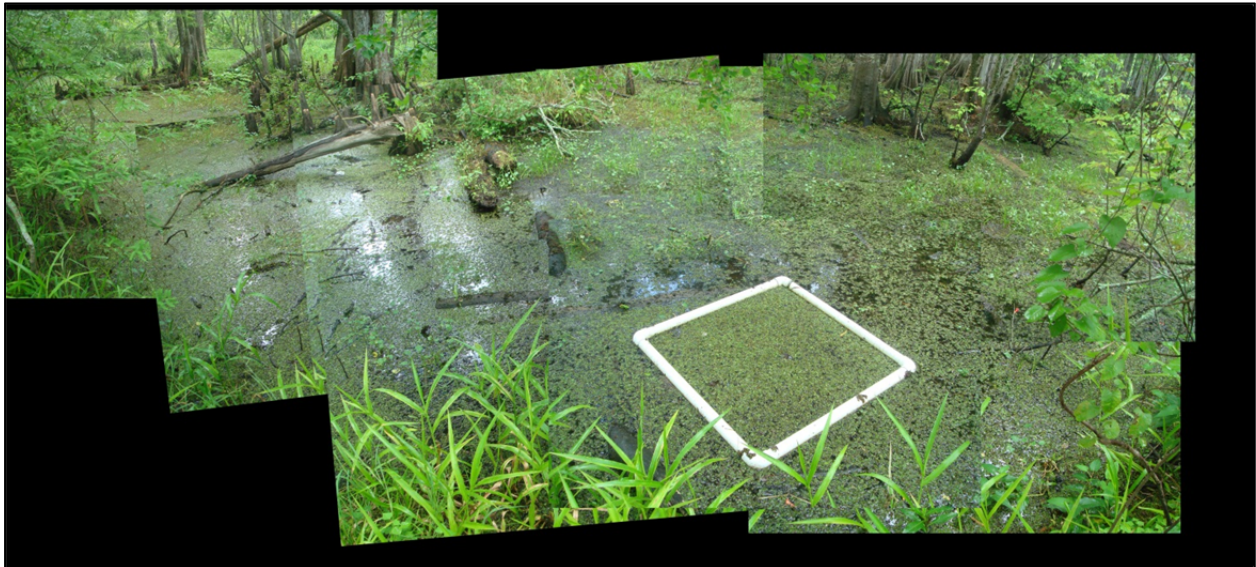
Figure 2.3. June 2008- Montage photograph showing open water within the mat of *S. minima* in Gramercy, LA.
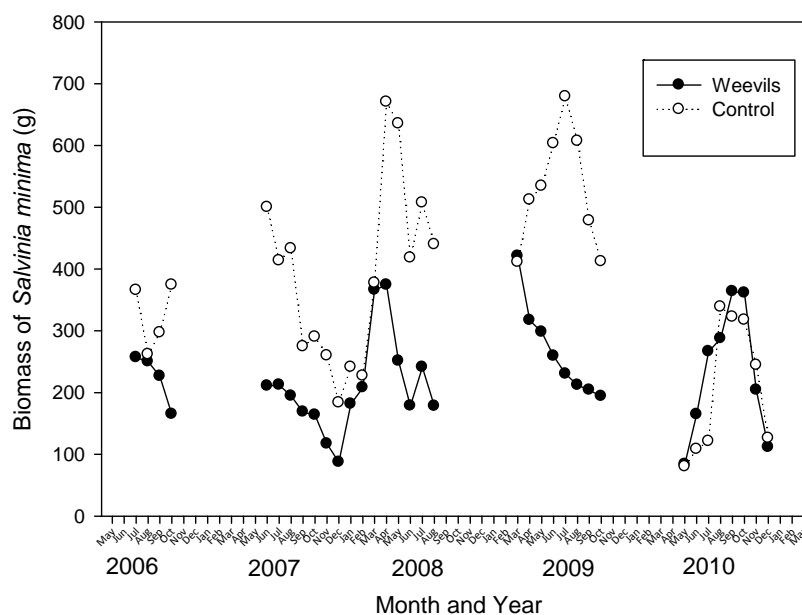
Figure 2.4. Average biomass per 0.1 m^2 area removed from both treatment and control quadrats July 2006-December 2010.

The mean fresh weight biomass of *S. minima* varied over the years, ranging from a minimum average weight of 80.22 g /0.1 m^2 (release plots, May 2010) to a maximum average of 679 g /0.1 m^2 (control plots, July 2009). The mixed linear model indicated that introducing *C. salviniae* into our field sites significantly impacted the fresh weight biomass of *S. minima* (F=461.75, df=1,138, p<0.0001) (Figure 2.4). A significant between month effect was documented (F=28.44, df=34,138, p<0.0001). Our treatment * month term was also highly significant, indicating changes in treatment over the course of time (F=13.67, df=34,138, p<0.0001).

The mean number of terminal buds damaged ranged from a high of 66/100 (release plots, September 2007) to a low of 1.5/100 (control plots, January to March 2008). The difference in the number of terminal buds sampled between quadrats with *C. salviniae* and those without was also significant (t=5.572, df=70, p<0.0001) (Figure 2.5). None of the other variables measured were significantly different between treatment and control: percentage of the mat covering the quadrat (t=-0.92, df=70,

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p=0.358), percentage of the mat that was green (t=-1.164, df=70, p=0.112), pH (t=1.161, df=70, p=0.25), dissolved oxygen (t=0.564, df=70, p=0.588), conductivity (t=1.172, df=70, p=0.254), or temperature at the water's surface (t=-0.487, df=70, p=0.28).
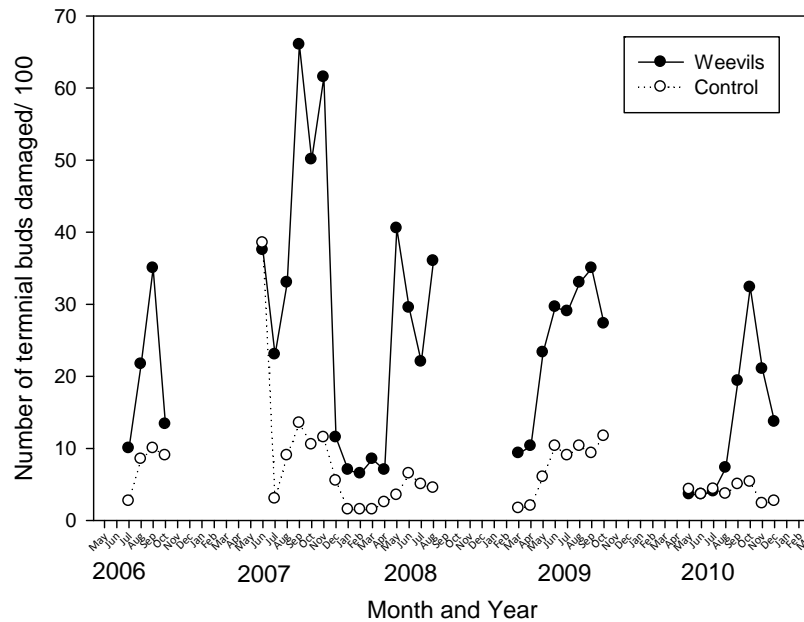
Figure 2.5. Average number of terminal buds of *S. minima* damaged per 100 randomly checked from both treatment and control sites from July 2006- December 2010.

The original population of *C. salviniae* brought to Louisiana from Ft. Lauderdale in southern Florida may not have been temperately adapted to the cooler climate in Louisiana. The population of *C. salviniae* collected from Lakes Talquin and Miccosukee in northern Florida was introduced in 2006 under the assumption that it would be better adapted to the local climate. *Cyrtobagous salviniae* from northern Florida successfully established on *S. minima* here in Louisiana from 2007-2010. We later found *C. salviniae* in low numbers at Jean Lafitte during 2008, suggesting that original releases by the USDA had become established but had not been successful in biological control of *S. minima*. While we did not collect any *C. salviniae* adults during 2010, the mat of *S. minima* in Gramercy was sparse and never

recolonized to the pre-frost extent, having lost most of the former mat's area to water hyacinth invasion (*Eichornia crassipes* (Mart.) Solm.). It is a strong possibility that a population was successful in establishing, as *Salvinia* maintains a warmer temperature than the surrounding air (Room and Kerr 1983). *Cyrtobagous salviniae* has also successfully established on *S. molesta* here in Louisiana and in an areas of temperate Australia that experience similar winter temperatures and conditions to those recorded here in Louisiana (Tipping and Center 2003, Sullivan and Postle 2010, Sullivan et al. 2011).

While *C. salviniae* was sucessfully introduced and established in southern Louisiana, and it significantly lowered the biomass of *S. minima*, it failed to completely control the infestation at any of our field sites. We suspect that other factors may be influencing the effectiveness of *C. salviniae* as a herbivore, possibly including nutrient limiting or interspecies interactions with other arthropods. The literature reports predation on *C. salviniae* by both *Hydrochus* sp. (Coleoptera: Hydrophilidae) as well by an unidentified spider during mass rearing in Parc National du Diawling, Mauritania (Sands et al. 1986, Triplet et al. 2000), as well as competition with an additional herbivore of *S. minima*, *S. multiplicalis* (Tewari and Johnson 2011). Additional possibilites include impacts from predation by the red imported fire ant (*Solenopsis invicta* Buren) which has been known to prey on other biological control agents for aquatic plants (Dray et al. 2001, Cuda et al. 2004).

This study illustrates that while *C. salviniae* did not completely control *S. minima* at our field sites, it had a significant impact on both the biomass and terminal buds damaged in southern Louisiana. We saw no significant differences between release quadrats with *C. salviniae* and those without when we measured pH, dissolved oxygen, percentage of the mat covered, percentage of the plant material green, and conductivity. Working in tandem, *C. salviniae* and the native herbivore *S. multiplicalis* can provide an ecologically sound and economically practical alternative to chemical or mechanical control of *S. minima* in southern Louisiana.

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CHAPTER 3
IMPACTS OF THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA* BUREN, ON THE SUCCESS OF *CYRTOBAGOUS SALVINIAE* CALDER AND SANDS AS A BIOLOGICAL CONTROL AGENT IN THE FIELD

INTRODUCTION

The Red Imported Fire Ant (RIFA), *Solenopsis invicta* Buren, is an invasive exotic pest introduced into the port of Mobile, Alabama in the 1930s (Buren 1972). RIFA are native to South America where populations are limited by competition and parasitoid pressure (Buren et al. 1974, Jouvenaz 1983). The RIFA have a broad omnivorous diet, eating both plants and animals with other invertebrates making up a large part of their diet (Vinson 1997). While their voracious appetite and broad indiscriminate diet has earned RIFA a reputation as beneficial for eating other pests in some agricultural systems, their diet is hard to predict and can include other beneficial insects (Sterling 1978, Eubanks 2001).

RIFA exploit any resource available including aquatic areas. They are known to use water to disperse (Morrill 1974) and display unique rafting behavior when flooded (Mlot et al. 2011). Freed and Neitman (1988) first noted RIFA using aquatic vegetation to forage over water. They were recorded crossing long leaf pondweed (*Potamotegon nodosus),* and foraging up to 15 m from the shore in a Texas pond (Patrock 2007). Tewari (2007) found RIFA foraging extensively on *Salvinia minima* Baker mats in both forested wetlands and canals in southern Louisiana up to 80 m from the levee. They have been known to venture into wet habitats to prey on intertidal polychaetes (Palomo et al. 2003), young sea turtles (Allen et al. 2001), neonate alligators (Allen et al. 1997), and a variety of other wildlife (Allen et al. 2004).

Cuda et al. (2004) documented the impact of RIFA on *Stenopelmus rufinasus* Gyllenhal, a native control agent of the floating fern *Azolla.* Plant biomass decreased when the ants were excluded from the pond. Fire ants were also observed preying on *Spodoptera pectinicornis* Hampson*,* an introduced biological control agent released for control of waterlettuce, *Pistia stratiotes* L. (Dray et al. 2001). The goal of this study was to determine whether RIFA are negatively affecting the success of the biological control of *S. minima* in southern Louisiana.

MATERIALS AND METHODS:

The experimental site was an artificial pond heavily infested with *S. minima* on privately owned

property located near the town of Tunica, Louisiana (30.951656, -91.480719). The levee adjacent to the

pond had known populations of RIFA and the property owner agreed not to treat or control for them

during the course of our study. Eight exclusion quadrats were constructed and transported to Tunica for

placement in the water. Each consisted of two nested square quadrats made from 5.08 cm diameter

SCH40 PVC pipe. The inner quadrat measured 1 m^2 while the outer was 1.5 m^2, leaving a 0.25 m moat

between the inner and outer quadrats. The two quadrats were rigidly connected to each other in four

locations 0.125 m underwater by 12.7 mm SCH40 PVC to prevent the moat from being compromised.

Assembled quadrats were placed in the water, anchored to the levee on the bank of the pond, and

cleared of all plant material.

In both 2009 and 2010 each of the inner quadrats on all 8 quadrats was filled with 3 kg of *S.*

minima plant material and seeded with 150 *Cyrtobagous salviniae* Calder and Sands individuals (In 2010

S. minima was transported from another location to prevent established populations of *C. salviniae* from

confounding results). Every other quadrat's moat (4 of 8) was filled with an additional 3 kg of plant

material to allow RIFA access to the inner quadrat (Figures 3.1, 3.2). Moated release quadrats were

cleared of any introduced plant material and plants growing in the quadrat other than *S. minima* were

removed every other week. Inner quadrats also received a floating pitfall trap to monitor for RIFA.

During 2009, *C. salviniae* were introduced in early July and allowed to establish for two months

until sampling commenced in September. Quadrats were sampled once a month for two months before

heavy rain caused the pond to overtop the levee, ruining the mat and exclusions. The 2009/2010 winter

was unusually cold, with the pond's water surface reaching a low of 1.18 °C. In 2010 the experiment was

not reestablished until the month of July when the mat of *S. minima* had reformed across the water's
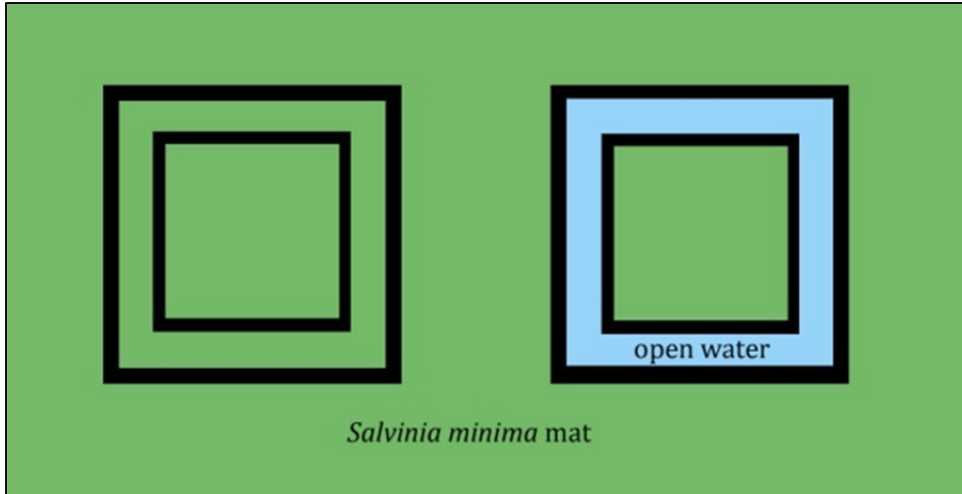
Figure 3.1 Diagram showing a plan for exclusion quadrats, with one displaying a 0.25 m open moat.

Figure 3.2. Exclusion quadrats in the field at the site in Tunica, Louisiana.

surface. *Cyrtobagous salviniae* were again allowed to establish for two months before beginning

sampling in September. Sampling the mat of *S. minima* was done by randomly placing three 0.1 m^2 mini-

quadrats constructed from 2.5 cm dia SCH40 PVC pipe. We removed the plant material from within each

of the mini-quadrats and hand squeezed the samples to remove excess water and weighed to determine

biomass. Tewari and Johnson (2011) established that wet weights of *S. minima* samples were

significantly correlated with dry weights, suggesting that wet weights were an efficient and reliable way

of comparing treatments. Of the three samples taken for biomass, two were returned to the inner

quadrat and one sample was returned to the lab and submerged for 24 hrs. to count *C. salviniae*

present. Both the plant material and weevils were returned to the original quadrat following counting

individuals of *C. salviniae*. In addition to the samples of plant material, each floating pitfall trap was

serviced at the same time and catches returned to the lab.

The presence of *Samea multiplicalis* (Guneé), a native herbivore documented to impact the

biomass of *S. minima* in Louisiana was also noted at the field site (Tewari and Johnson 2011). We

sprayed quadrats with a microbial insecticide when larvae and adults were noticed at the field site

(Thuricide concentrate, active ingredient: *Bacillus thuringiensis kurstaki,* approximately 4000 *Spodoptera*

units/six million viable spores per milligram). Data was analyzed as a one-factor completely randomized

design, and all data analysis was calculated using SAS 9.2 software using PROC MIXED.

RESULTS AND DISCUSSION

The number of RIFA recovered from the floating pitfall traps in 2009 and 2010 (Figures 3.3 and

3.4) were not normally distributed, and data were log transformed before analysis. The number of

individuals caught were significantly different between treatments for both years as a group, indicating

that the moat was successful in keeping most individuals from accessing the inner area (F=6.69; df= 1, 6;

P=0.0414). The treatment * date statement was also significant, suggesting that the treatment effect on

the number of ants differed between trapping periods (F=3.56; df= 7, 28; P=0.0073). The significant

treatment * date effect is likely a response to changes in foraging behavior as a response to abiotic

factors such as rainfall or temperature change. Other studies have found that optimum foraging occurs

between 22 and 36°C (Porter and Tschinkel 1987). While moats were an effective method to

significantly decrease the number of RIFA within the quadrat, several individuals managed to cross the

moat. Possibly, vegetation from the bank provided temporary access, we were diligent to clear any

possible bridge substrates from the area. RIFA are well documented to survive water and flooding so it is

not surprising that some individuals might make it across 0.25 m of open water (Morrill 1974, Mlot et al.

2011). No other species of ants were collected from the traps in either year.

Sampled populations of *C. salviniae* during the study were non-normal and heavily skewed. We

broke the data into two sets by year, and the data for 2009 was still non-normal and log transformed

during analysis while the 2010 data were normal. During 2009, numbers of *C. salviniae* per sample were

not significantly different between treatments (F=0.16; df= 1, 6; P= 0.7) (Figure 3.5). Looking closer at

the data for 2010, one data point (one quadrat from October 2010) stood out from the others as over 6

standard deviations from the mean, possibly indicating a severe outlier. Data for 2010 was analyzed

using Dixon's Q, which suggested rejecting the outlier from analysis with a 95% confidence level

(Q=0.86) (Dean and Dixon 1951, Rorabacher 1991). After removing the outlier from the 2010 data, the

number of individuals of *C. salviniae* per sample of *S. minima* was significantly different between

treatments (F=11.46; df= 1, 6; P=0.0148) (Figure 3.6), indicating that the presence of RIFA is related to

the population level of *C. salviniae*. When pooled for the whole study, data from 2009 and 2010

(without the outlier) were non-normal and log transformed during analysis. No significant treatment

effect (F=0.75; df= 1, 6; P=0.4190) was detected.
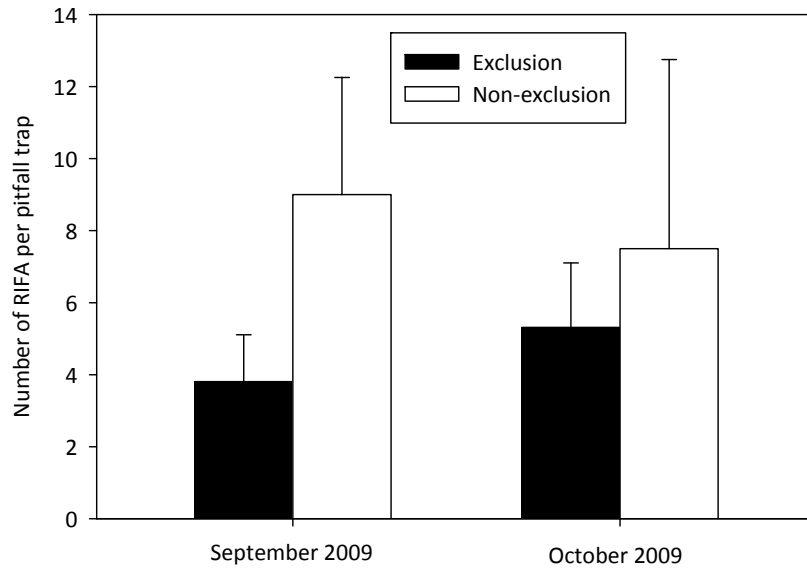
Figure 3.3. Number of individual RIFA recovered from a floating pitfall trap placed within the inner-quadrat for two sampling dates from September-October of 2009.
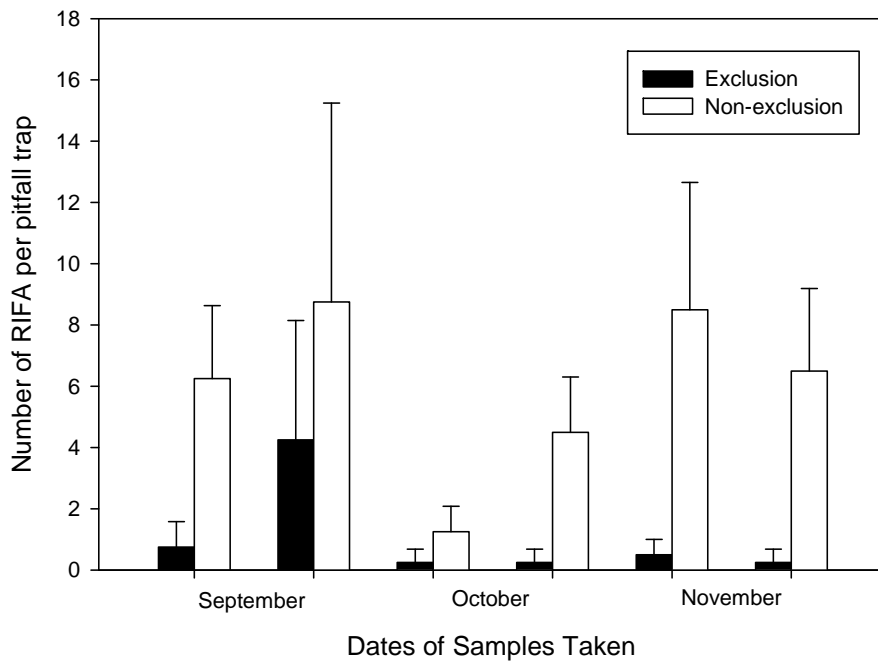
Figure 3.4. Number of individual RIFA recovered from a floating pitfall trap placed within the inner-quadrat over six sampling dates (two per month) from September-November of 2010.
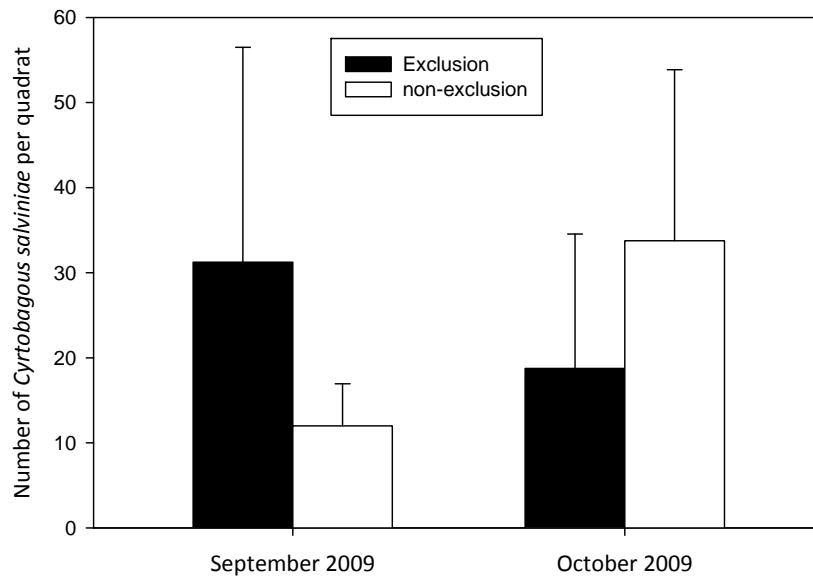
Figure 3.5. Number of individuals of *Cyrtobagous salviniae* removed from 0.1 m^2 area of *Salvinia minima* from two sampling dates, September-October of 2009.

Weights of the of the *S. minima* samples (Figure 3.7) were only analyzed for 2010 and were normally distributed. No significant difference between treatments (F=3.34; df= 1, 6; P=0.1175) was documented. Data do show a trend that quadrats moated for exclusion had slightly lower biomass than those where they RIFA had access. Differences in weight variation may have been suppressed by the presence of *S. multiplicalis* that we observed even after spraying quadrats with Thuricide.

Many ant species interfere with biological control programs, both by defending food sources and through intra-guild predation (Cudjoe et al. 1993, Stechmann et al. 1996, González-Hernández et al. 1999, Eubanks et al. 2002, Kaplan and Eubanks 2002, Wyckhuys et al. 2007). Discussion of predation on weevils from other arthropods is scattered in the literature across a wide variety of ecological systems (Barney et al. 1979, Alfaro and Borden 1980, Barney and Armbrust 1980, Richman et al. 1983, Barker et al. 1989). The best documented impact of arthropod predation on a weevil is RIFA as a control agent for the boll weevil (Sterling 1978, Jones and Sterling 1979, Fillman and Sterling 1983). RIFA has also been
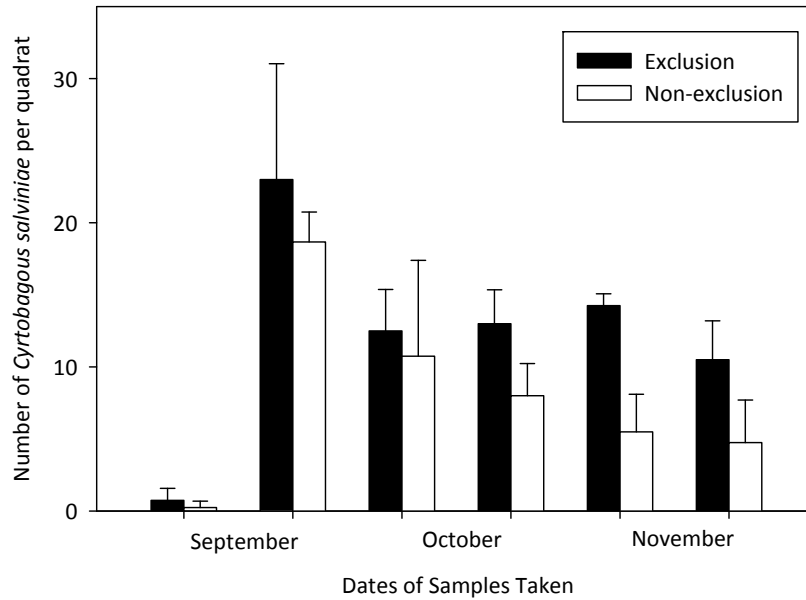
Figure 3.6. Number of individuals of *Cyrtobagous salviniae* removed from 0.1M^2 area of *Salvinia minima* over six sampling dates (two per month) from September-November of 2010 (Adjusted using Dixon's Q).
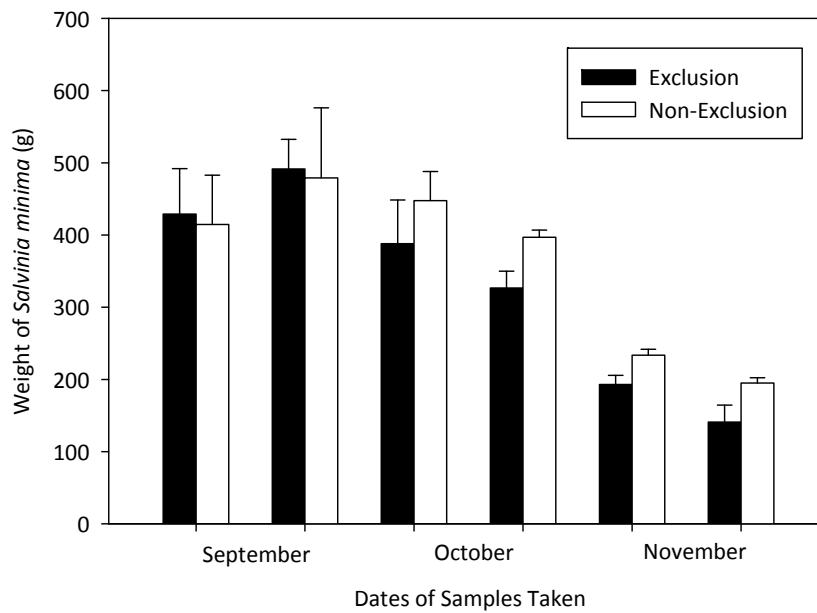
Figure 3.7. Changes in weight of 0.1 m^2 of *Salvinia minima* over six sampling dates (two per month) from September-November of 2010.

documented impacting the *Azolla* weevil, *S. rufinasus* (Cuda et al. 2004) which is of a similar adult size (2 mm) to *C. salviniae* (Hill 1998, Tipping et al. 2010).

While not addressed in this study, RIFA that were actively foraging on the mat of *S. minima* were most likely also preying on both larvae and eggs of *S. multiplicalis* as well. RIFA preys on lepidopteran eggs and larvae in a variety of terrestrial systems (Reagan et al. 1972, McDaniel and Sterling 1979, Eger et al. 1983, Elvin et al. 1983, Eubanks 2001, Seagraves and McPherson 2006). Also, RIFA interfered with the introduction and establishment of *S. pecticornis* for the control of waterlettuce (Dray et al. 2001). While we sprayed Thuricide to control *S. multiplicalis* within our study area, the majority of the mat was untreated and supported large populations of larvae as a food source. While *S. multiplicalis* had a negligible effect on *S. minima* in Florida (Tipping and Center 2005), Tewari and Johnson (2011) documented that *S. multiplicalis* had a significant negative effect on the biomass of *S. minima* in Louisiana.

This study suggests RIFA is actively impacting the success of the biological control of *S. minima* by decreasing the population of *C. salviniae*. In combination with probable predation on the only other herbivore, *S. multiplicalis*, RIFA should be controlled where possible to increase the success of biological control. While not efficient or plausible for backswamp wetland releases or large water bodies, controlling RIFA around smaller infestations of *S. minima* when infested with *C. salviniae* should increase chances of successful biological control.

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CHAPTER 4
DEVELOPMENT OF FLOATING PITFALL TRAP FOR COLLECTING ARTHROPODS ASSOCIATED WITH AQUATIC VEGETATION

INTRODUCTION

Collecting insects in wetlands with dense vegetation is difficult at best due to standing water,

dense vegetation, and high structural complexity. Unfortunately no sampling method is currently

available for a comprehensive analysis of wetland invertebrates (Henke 2005). Almost as many sampling

methods are available for sampling aquatic habitats as there are types of water bodies (Cummings

1962). Merritt et al. (2008) provided the most recent comprehensive guide to aquatic sampling methods

and listed over 30 different methods to collect arthropods in aquatic and emergent vegetation. The

number and type of taxa collected in an aquatic sampling program depends on the type of collection

strategy used and the local vegetation (Turner and Trexler 1997).

Floating pitfall traps have been commonly used to sample both amphibians (Jones 1986) and

spiders (Graham et al. 2003) but rarely for insects. Pitfall trapping has a well-established history in

entomology and is commonly used in terrestrial systems for biodiversity studies (Triplehorn and Johnson

2005, Aguilar Julio 2010). Pitfall traps operate continuously, are inexpensive and easy to use, and result

in large species-rich samples (Clark and Blom 1992). Forests and agricultural areas are the most

frequently sampled habitats with pitfall traps, and they are rarely used in areas that could potentially be

flooded (Mertens et al. 2007).

There have been a variety of floating trap designs published in the literature. Grigarick (1959)

provided the first floating trap designed to sample *Hydrellia* in rice fields using a round 20.3 cm diameter

by 3.18 cm deep aluminum pan inserted into a piece of wood. The trap was filled with water and a

wetting agent and checked every 1-3 days. It was non-selective and caught a wide variety of insects as

well as the occasional animal and due to the shallow design, was swamped easily by water movement.

Additional designs consisted of a 0.35 L small pot (unspecified type) weighted with wax and lead, and

inserted into a 20 by 12 cm piece of cork (Ruzicka 1982) and a 3.5 x 8 cm vial inserted into a 12 cm

square board (Renner 1986). Graham et al. (2003) constructed a floating pitfall trap with a double cup, the smaller cup for collecting fluid nested in a larger outer cup (10 cm dia) weighted with mud and rocks, these were then inserted into a 15 cm piece of square styrofoam.

We constructed a variety of published traps before designing our own. Traps based on Grigarick (1959) were quickly sunk by turtles at our field site, and those constructed from Renner (1986) would not stay level with the water's surface. The design from Ruzicka (1982) fared slightly better, but the cork used for the float degraded quickly in the water, and Louisiana's high summer temperatures melted the wax in the cup fouling samples. The last design, Graham et al. (2003), had problems with the Styrofoam float falling apart as well as many of the traps getting water between the two trap cups resulting in the inner collecting cup's rim being several cm above the level of the water.

We aimed to design an inexpensive, robust trapping system to effectively sample insects associated with emergent and floating aquatic pleuston vegetation that could be deployed in the field for long periods of time without maintenance. We successfully collected several insects previously released for biological control of aquatic invasive vegetation as well as a wide variety of hydrophilous and hygrophilious insects.

MATERIAL AND METHODS

TRAP DESIGN

Pitfall traps in terrestrial systems consist of two major parts: a base that includes the trapping container and a cover. Our trap design includes an anchoring stake with tether as an additional component to compensate for both horizontal and vertical water movement (Figure 4.1). The floating base consists of a Ball® standard mouth 236.58 ml glass canning jar with 85.05 g of lead fishing weights placed in the bottom. The weights were encased in FloraCraft® liquid acrylic resin poured over them into the bottom
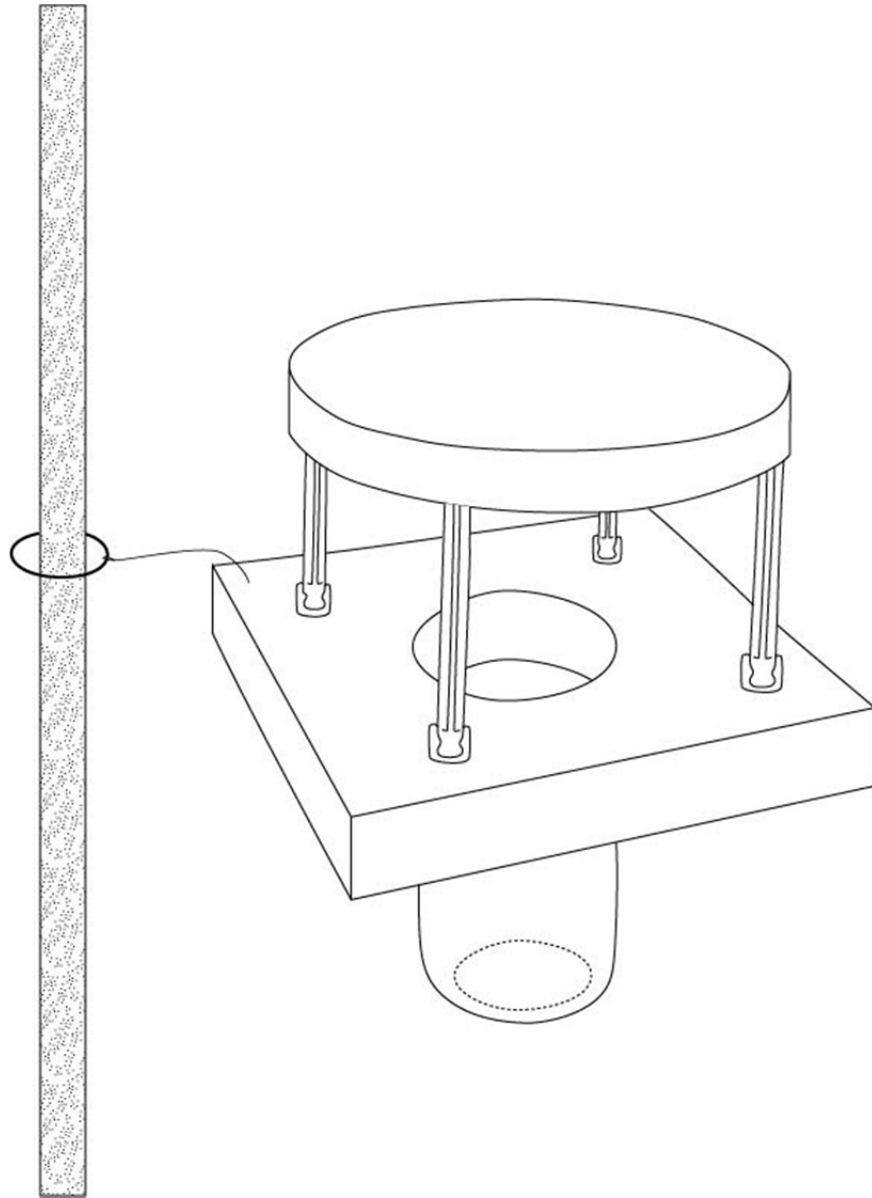
Figure 4.1. Line drawing depicting our design for floating pitfall traps (does not show lead weights).

of the jar to prevent lead contamination in the environment. These jars were hot glued into a 15 cm x 15

cm piece of 2.5 cm- thick black polyethylene packaging foam with a 6.75 cm diameter hole cut in the

center. Trial traps were built with a several thicknesses of foam, and a 2.5 cm thick piece was ideal to

have the trap stay at the surface without the edge being above the surface vegetation, though this could

vary in other habitats. The rod ends of four K'nex® "Standard Black Rod/Connectors" (Part #90914) were

hot glued into the foam, rod down, 3.75 cm from the hole of the jar. These serve to connect the cover to the base. The cover was made from a Fisher® clear polystyrene petri dish (15 mm x 150 mm) with four K'nex® "Standard Yellow Rods 3 $^7/_{16}$" (Part #90953) spaced evenly on the inside of the edge of the petri dish and hot glued in place. The fully assembled trap was anchored by placing a ring at the end of a 30 cm piece of 49-strand nylon-covered steel jewelry wire over a Gardener's Blue Ribbon® 2 m Plastic-Coated Steel Landscape Stake. This attachment prevented horizontal movement from the designated site while allowing vertical movement with changes in water level.

Figure 4.2. Trap *in situ,* approximately .5 m deep water with surface covered by aquatic vegetation.

PRESERVATIVE

Jars were filled half full with approximately 75 ml of Prestone® Extended Life Antifreeze/Coolant (ethylene glycol) for each trap date. This quantity of fluid lowered the edges of the polyethylene foam level with the top of the pleuston vegetation (Figure 4.2). While propylene glycol has gained popularity

in terrestrial pitfall trapping methods due to low mammalian toxicity, it has a much higher biological

oxygen demand in freshwater systems than Ethylene Glycol and could increase oxygen depletion if

spilled (EPA 2009). A few drops of Palmolive® Safe + Clear dish detergent were added to each trap to

increase wetting and minimize arthropod escape from traps (Topping and Luff 1995).

RESULTS

PRODUCTION/INTEGRITY

These traps cost approximately $9 each to build (including the landscaping anchor stakes) and

were constructed in stages over several weeks in the lab. Assembling an individual trap from beginning

to end took less than an hour. After being placed in the field, the traps were serviced every two weeks.

During the time that the traps were in the field, those that failed were found to have a variety of

problems including: animals eating the foam, falling branches, and turtles using them as a platform.

Traps that became submerged stayed buoyant at the water's surface and still contained insects in the

sample, even after the jar became flooded. Less than 50 individual trap catches out of 1300 were lost

due to being overturned or submerged. After a year of environmental exposure in the water, the foam

on some traps began to lose buoyancy and was replaced in preparation for another field season.

PERFORMANCE

A total of 5,773 adult insect specimens representing 176 species within 62 families and 7 orders

were collected during 2009. The Coleoptera were the most species rich (137 sp.) and the most abundant

(3409 individuals). Full biodiversity analysis and a complete taxa list are presented in Chapter 5.

DISCUSSION

Evaluating collection methods and designing a robust collecting program in wetlands with heavy

vegetation requires knowledge of the target taxa, sampling characteristics of the method(s) selected,

and the amount of time required to process the catch (Turner and Trexler 1997). Both dipnets and core

samplers have been commonly used to take whole plant samples of *Salvinia* spp. in other studies looking

at arthropod communities (Bennett 1966, Forno and Bourne 1984, Herrera et al. 2000, Albertoni and

Palma-Silva 2006, Poi de Neiff and Neiff 2006). Unfortunately, heavily vegetated dipnet and corer

samples can take from 2-5 hours a piece to process (Meyer et al. 2011). It took our field team of four

people an average time of 3-4 hours to service 100 traps in the field, and an additional average

processing time of approximately 15 minutes in the lab. This results in around 20 min of total processing

time invested per trap, which is less time than processing whole plant samples.

This trap design provides an easy and efficient way for making sequential collections of hygro-

and hydrophilous insects associated with emergent vegetation for a wide variety of taxonomic and

ecological studies. Our collection of 176 species of adult insects is much richer than other similar studies

published on arthropods associated with *Salvinia* spp. (Pelli and Barbosa 1998). Use of these traps does

not require removal of vegetation samples or disturbance to the local community and allows for

repeated sampling in the same physical location.

Using floating pitfall traps also allows collection of specimens that are uncommon and could be

missed by other types of aquatic sampling since there is a much longer collecting period. Utilizing

different collection methods in wetlands result in very different taxa (Meyer et al. 2011). Highly mobile

taxa such as Carabidae and Hymenoptera that are using the vegetation incidentally or foraging would

easily be missed using net based sampling methods. As in terrestrial ecosystems, biases in the design of

the floating pitfall traps surely exist (Work et al. 2002) and should be evaluated in future investigations.

Pitfall traps in terrestrial systems depend on movement of individuals and collect based on activity and

density (Topping and Sunderland 1992), so potential biases in a floating pitfall trap include a positive

bias to mobile species that frequent the surface and are associated with emergent vegetation while

under sampling taxa that are restricted to the water column. While we were specifically interested in collecting insects associated with pleuston vegetation, these traps could easily be implemented as a part of a larger sampling regime to complement other collection methods.

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CHAPTER 5
INSECTS ASSOCIATED WITH MAT OF *SALVINIA MINIMA* BAKER IN SOUTHERN LOUISIANA

INTRODUCTION

Aquatic macrophytes contribute to the structure and function of wetlands in a variety of ways including positively affecting diversity among associated groups and providing shelter from predation (Brown et al. 1988, Olson et al. 1994, Batzer 1998). Many species of invertebrates show distinct preferences for aquatic plants based on their structure (Dvorak and Best 1982, Cyr and Downing 1988, Dvorak 1996). However, invasive plants threaten wetland community structure and integrity by forming monotypic stands, changing available habitat, altering diversity, and modifying food webs (Zedler and Kercher 2004). As more non-native species like *Salvinia* spp. invade water ways, the ecosystem functions that macrophytes provide could change (Luken and Thieret 1997).

Older studies examining insects associated with *Salvinia* spp. focused on identifying potential biological control agents for *S. molesta* by examining the *S. auriculata* complex (*S. auriculata* Aubl., *S. molesta* Mitchell, *S. herzogii* de la Sota, and *S. biloba* Raddi.) (Bennett 1966, Forno and Bourne 1984). Several of the more recent studies have examined macroinvertebrates associated with *Salvinia* from a conservation standpoint within its native range (Herrera et al. 2000, Albertoni and Palma-Silva 2006, Poi de Neiff and Neiff 2006). Studies sampling insects and arthropods associated with *Salvinia* spp. have returned results ranging from 10 spp. (*S. biloba*, Forno and Bourne 1984) to 113 spp. (*S. molesta*, Pelli and Barbosa 1998).

No previous investigations into associated invertebrates of either *S. minima* or *S. molesta* in the United States have been undertaken. Pelli and Barbosa (1998) suggested that a rich fauna associated with *Salvinia* in Brazil is possibly a side effect of a rich endemic aquatic fauna that uses *Salvinia* incidentally. Unfortunately, while a common habitat in the southern United States, few studies have been done on invertebrates from swamps and flooded woodlands in relation to other wetland types (Batzer and Wissinger 1996). Fewer studies are available from Louisiana to provide baseline surveys for

non-impacted communities (Ziser 1978, Sklar 1983, 1985). Our goal was to quantify and describe the biodiversity and community of adult insects associated with *S. minima* in southern Louisiana and compare with the results of previously published studies.

MATERIALS AND METHODS

This survey was conducted during the spring of 2009 on a privately owned tract of land just north of Gramercy, Louisiana (30˚09.804'N, 90˚48.643'W) and bordered by Interstate 10 and US-61. This site is classified as a Cypress-Tupelo-Blackgum freshwater swamp, and the landscape is dominated by Baldcypress (*Taxodium distichum* L.). Common salvinia (*Salvinia minima* Baker) has colonized the open water and formed solid dense mats of plant material. Other invasive aquatic plants encountered in smaller patches at the study site include water hyacinth (*Eichornia crassipes* (Martius) Solms), and pennywort (*Hydrocotyle* spp.).

One hundred of the aquatic pitfall traps described in chapter 4 were deployed at the study site to collect insects. Each trap location was marked by neon plastic flagging placed atop the 2 m landscape stakes used to anchor the traps. Traps were placed, loaded, and launched on May 18, 2009 and samples were retrieved every two weeks until November 2, 2009 for a total of 13 sampling periods. Samples were labeled with the trap coordinate location and trap pick up date, and preserved in the lab in ethylene glycol until processed. Specimens were sorted in the lab and all adult insects except Diptera and Lepidoptera were pinned or pointed and properly labeled with full locality information. Neither Diptera or Lepidoptera were well preserved when samples were processed, and specimens were placed in residuals. Plant material, amphibian, and crustacean bycatch were discarded. Residual adult taxa and all immature insects were preserved in 95% ethanol. All preserved specimens were identified to species where possible using relevant literature and help from taxonomic specialists (Arnett and Thomas 2000, Arnett et al. 2002, Epler 2006, Merritt et al. 2008, Epler 2010). Voucher specimens are deposited in

Louisiana State Arthropod Museum, Louisiana State University (Baton Rouge, Louisiana), The

Hymenoptera Institute Collection of the Department of Entomology at the University of Kentucky

(Louisville, Kentucky), the Florida State Collection of Arthropods (Gainesville, Florida), and the United

States National Museum (Washington, D.C.).

RESULTS AND DISCUSSION

The insect community associated with a mat of *Salvinia minima* was represented by 176 species

within 62 families and 7 orders. A total of 5,773 individuals were collected (Table 5.1). Coleoptera was

the most species rich order (137 species: 77.8%), followed by Hymenoptera (26 species: 14.8%),

Hemiptera (16 species: 9.1%), Orthoptera (4 species:2.3%), Odonata (2 species: 1.1%), and

Psocoptera/Blattaria (1 species: 0.5%). Carabidae was the most species rich family (30) followed closely

by the Staphylinidae (29) and the Curculionidae (12). In addition to being the most species rich order

Coleoptera was also the most abundant order (3409: 59.1%), followed again by Hymenoptera (1611:

27.9%), Hemiptera (578: 10.0%), Orthoptera (154: 2.7%), Odonata (7: 0.1%), and Psocoptera/Blattaria (1

individual each). The most abundant family was Carabidae (1161), followed by Ichneumonidae (947),

Scirtidae (794), and Hydrophilidae (744). The most abundant species included an unidentified

Ichneumonid wasp located in the subfamily Cryptinae: tribe Hemigastrini (741), *Scirtes tibialis* Guerin-

Meneville (657) (Scirtidae), *Enochrus* sp. (508) (Hydrophilidae), and *Stenocrepis duodecimstriata*

(Chevrolat) (465) (Carabidae).

Rare species (<5 individuals) made up over half of our identified species (108/176). The majority

of those were singletons (77/176). While singletons made up 43.8% of the richness observed, they only

accounted for 1.5% of our total abundance. Many hypothesis have been presented in the literature to

account for rare species including insufficient sampling efforts, genuinely low populations, and tourist

species (Novotny and Basset 2000, Coddington et al. 2009).

As part of this research we collected three currently undescribed species of Coleoptera and one undescribed Braconid wasp. The unidentified genus (near *Nephanes* sp.) in the family Ptiliidae has been observed from dung and fermenting organic material across the eastern coast of the United States and does not currently match any established name (M. Sörensson, pers. com.). The undescribed staphylinidae is placed within *Hoplandria (Genosema).* Another species *H. pulchra* Kraatz has been collected from feces and organic material. The specimen from our sample is the only one known (J.-S. Park pers. com.) .The undescribed species of *Cyphon* (Scirtidae) is conspecific with Epler's "C. sp.2." Its range encompasses much of the Atlantic and Gulf coasts (Tetrault 1967, Epler 2010). The braconid wasp is being described by M. Sharkey and colleagues (M. Sharkey, pers. com.). Other interesting species of note include: two probable new state records of Curculionidae (*Bagous hydrillae* O'Brien and *Onychylis texanus* Burke*),* a new state record for *Limnichites punctatus* (LeConte)(Limnichidae), as well as several species not previously included in the collection of the Louisiana State Arthropod Museum.

Since *S. minima* is currently the subject of a biological control project in southern Louisiana, the Curculionidae collected by this study are of particular interest. These species were a primary focus of this research, though we were also interested in the total community composition of the habitat. Seven species of semi-aquatic Curculionidae (five of which have been used as biological control agents for aquatic plants) were represented in the samples: *Cyrtobagous salviniae* Calder and Sands, *Bagous hydrillae* O'Brien, *Neochetina bruchi* Hustache*, Neochetina eichorniae* Warner*, Stenopelmus rufinasus* Gyllenhall, *Onychylis texanus* Burke, and *Tanysphyrus lemnae* (Fab.) (Calder and Sands 1985, Haag and Habeck 1988, McConnachie et al. 2004, Wheeler and Center 2007). We also collected *Dyscinetus morator* (Fab.)(Scarabaeidae) which has been documented feeding on water hyacinth (Buckingham and Bennett 1989).

While evaluating α-diversity, we observed a single peak in both abundance and species richness from the collections taken during the month of July 2009 (Figures 5.1, 5.2). Margalef's richness is normalized for sample size, and exhibits the same pattern as raw richness values (Figure 5.3)(Margalef 1958). Many indices for diversity and evenness are available. All of which mathematically weigh variables differently (Hill 1973, Heip and Engels 1974, Peet 1975, Mouillot and Leprêtre 1999). We ran two each of both diversity (Simpson's Index of Diversity and Shannon's Diversity Index) and evenness indices (Pielou's Index of Evenness and McIntosh's Evenness Index) to elucidate any differences between collection dates. Simpson's Index of Diversity ranges in value from 0 as a homogenous community to 1 for the most diverse and is based on probability (Simpson 1949). Values for our collection dates range from 0.79 to 0.95 indicating a very heterogeneous and diverse community due to the high number of species recorded (Figure 5.4). Shannon's Diversity Index calculates values that range from 0 for the most homogenous communities to approximately 4.6 for the most diverse and is more sensitive to the presence of rare species (Shannon 1948). Our values range from 2.1-3.5 (Figure 5.5) and exhibit the same single peak observed in Figures 5.1-5.3, which is not surprising given that the Shannon Index takes into account abundances and evenness. Pielou and McIntosh's evenness indices both show similar tends and values for the same collection dates, and are two different mathematical ways to analyze abundances (Figures 5.6 and 5.7) (Pielou 1966, McIntosh 1967).

It is difficult to evaluate β-diversity and compare our results with other studies associating invertebrates with *Salvinia* spp. due to differences in native fauna between study locations, taxonomic resolution, and sampling strategies. Several of the studies only identified invertebrates to family level (Pelli and Barbosa 1998, Albertoni and Palma-Silva 2006, Mfundisi et al. 2008). Junk (1977) identified specimens to order, and Gopalan and Nair (1975) only identified invertebrates to class. Of the papers that provide taxonomic resolution, Bennett (1966) and Forno and Bourne (1984) both focus solely on herbivorous insects, eliminating some of the larger taxonomic groups we collected during sampling.
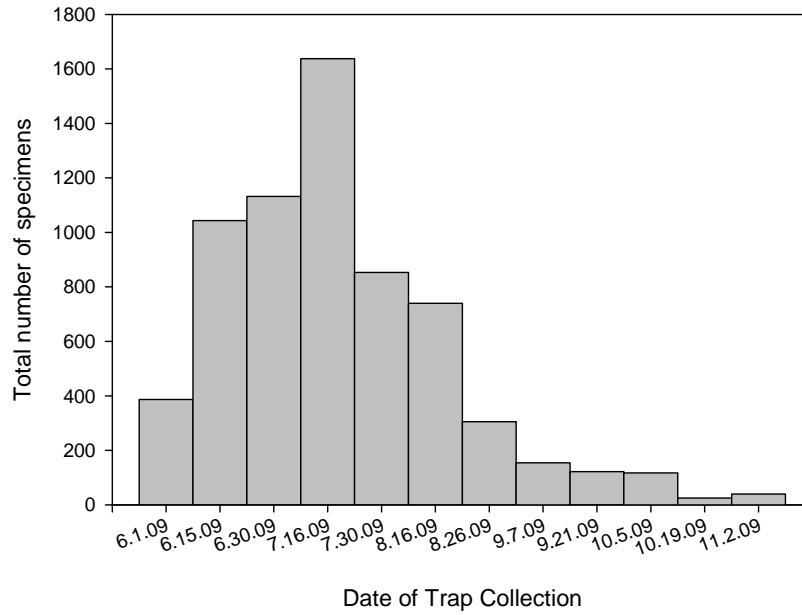
Figure 5.1. Total number of specimens collected from each trap collection period.
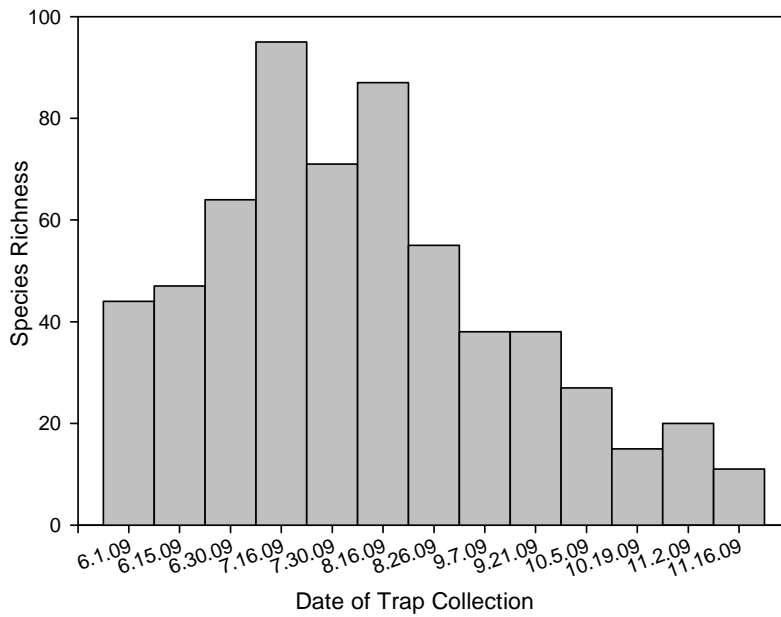
Figure 5.2 Species Richness for each trap collection period.
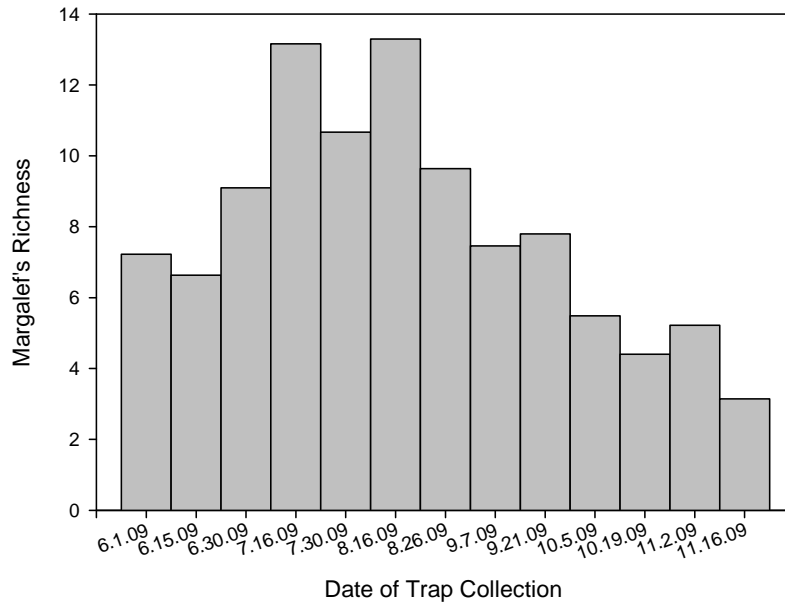
Figure 5.3. Margalef's Richness values for each trap collection period
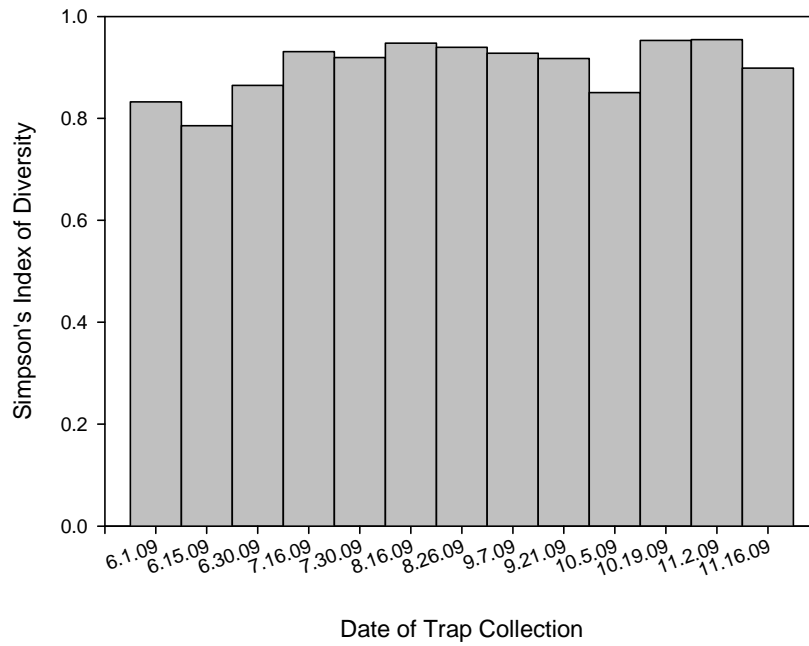
Figure 5.4. Simpson's Index of Diversity for each trap collection period.
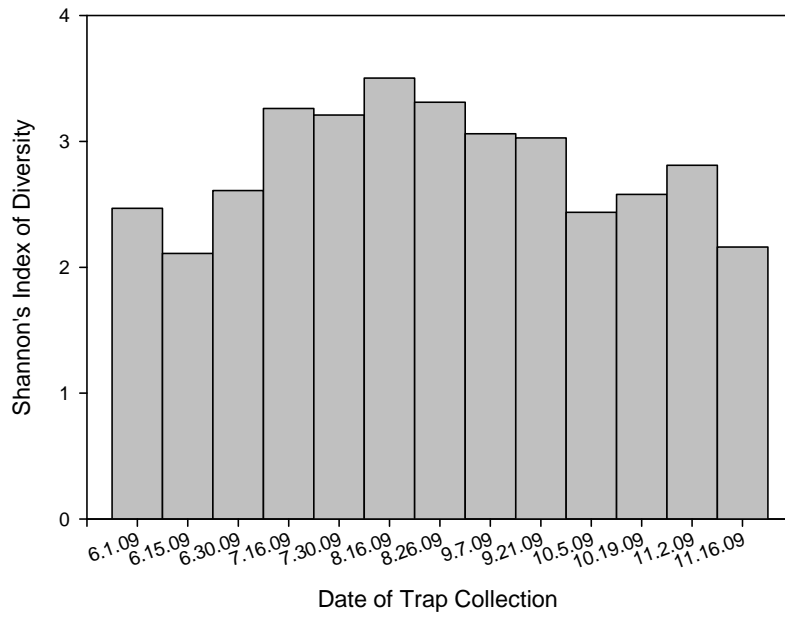
Figure 5.5. Shannon's Diversity Index for each trap collection period.
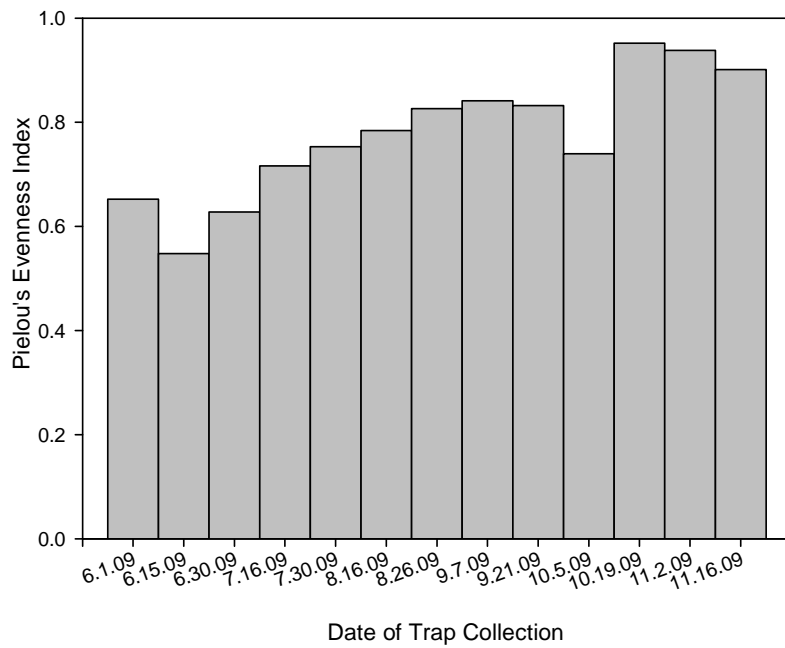
Figure 5.6. Pielou's Evenness Index for each trap collection period.
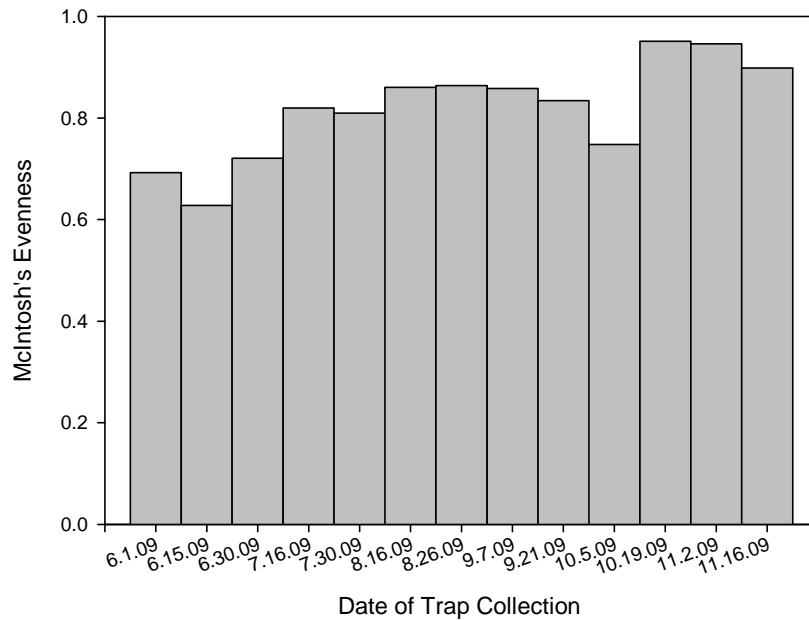
Figure 5.7. McIntosh's Evenness Index for each trap collection period.

Several of the other *Salvinia* invertebrate association studies also report Coleoptera as their most species rich and/or abundant group (Pelli and Barbosa 1998, Herrera et al. 2000, Poi de Neiff and Neiff 2006). Coleoptera represent one of the largest "aquatic" groups in the world (Jåch and Balke 2008). Both Mfundisi et al. (2008) and Albertoni and Palma-Silva (2006) reported larval chironomidae as the most abundant macroinvertebrates. They were absent from our samples due to their fully aquatic nature. Differences in taxa collected are likely from differences in sampling method (Meyer et al. 2011). Our sampling effort for associated insects was much more intensive (1300 samples) than other published studies and focused solely on adult insects.

Sklar's (1983) dissertation provides one of two lists available for macroinvertebrates associated with floating vegetation in Louisiana (*Lemna* spp., pre *Salvinia* infestation) (48 taxa of insects). Ziser (1978) evaluated wetlands adjacent to our field site and collected 55 taxa of insects (mostly larvae and nymphs). Supporting Pelli and Barbosa's (1998) hypothesis that invertebrates already present in an

ecosystem use invasive vegetation incidentally, Sklar (1983) provided the only taxon list that records the presence of Carabidae, our most species rich and abundant family. Sklar (1985) published graphs showing bimodal patterns for Margalef's Richness, Shannon's Diversity Index, and Pielou's Evenness for swamp invertebrates associated with floating vegetation (Figure 5.8). Our graphs for these same indices over a similar time frame (Figures 5.3, 5.5, and 5.6 respectively) show very different patterns and do not exhibit bimodal trends. Ziser (1978) also showed a bimodal graph for abundance of macroinvertebrates/100g plant material, but the Shannon's Diversity index is similar to our results. Overall, our study shows much higher levels of richness and less monthly variation in both diversity and evenness than Sklar's (1983, 1985) or Ziser's (1978) work.

While Pelli and Barbosa's (1998) hypothesis that invertebrates which already exist in a habitat will use invasive vegetation incidentally would account for some increase in richness and abundance, we would expect values to be similar to other studies in similar habitats. During 2009 we observed over triple the number of taxa documented in Sklar (1983) or Ziser (1978), including many predaceous terrestrial Coleoptera and parasitic Hymenoptera. These groups could be a result of using a floating pitfall trap which is a passive trap that relies on arthropod activity instead of disturbing the mat by removing whole plant samples, or the mat of *S. minima* may be supporting a larger community of arthropods foraging out onto a formerly unavailable habitat.

To examine these ideas more closely, we consulted relevant literature for taxa collected during our study to assess lifestyles and habitat associations (Arnett and Thomas 2000, Arnett et al. 2002, Epler 2006, Jåch and Balke 2008, Epler 2010)(Table 5.1). For the Coleoptera, Jåch (1998) defined six ecological groups based on familial associations with water: (1)"True Water Beetles," (2)"False Water Beetles," (3)"Phytophilous Water Beetles," (4)"Parasitic Water Beetles," (5)"Facultative Water Beetles'" and (6)"Shore Beetles." These classifications are roughly associated with the amount of time spent in
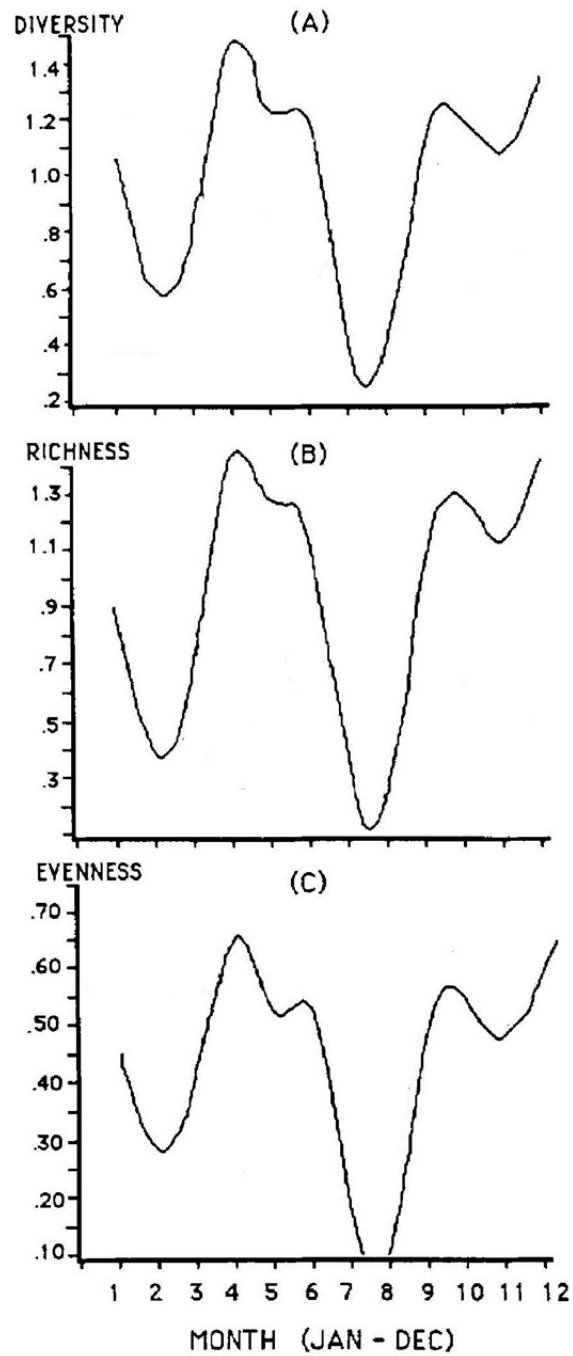
Figure 5.8. Adapted from Sklar (1985). Average monthly variation in diversity (A), richness (B), and evenness (C) in the floating vegetation. Compare (A) to Figure 5.5, (B) to Figure 5.3, and (C) to Figure 5.6.

contact with the water (Table 5.1, "A1-6"). Out of the 137 species of Coleoptera we collected, 87 were

listed as hygrophilic or riparian in one of the references (Jåch 1998, Arnett and Thomas 2000, Arnett et

al. 2002). Intriguingly many of them were also noted as being crepuscular or nocturnal, which could also

explain their absence from lists created from other collection methods. Most of the Hemiptera

collected were aquatic in nature or known to feed on aquatic plants (Epler 2006). Many parasitic

Hymenoptera are semi-aquatic in nature, but we refrained from labeling our taxa list in either direction

without firm identifications. The exception is *Anoplius depressipes* which is known to hunt semi-aquatic

Dolomedes spp. spiders (Roble 1985).

We conclude that most of the taxa collected in our study are previously known to be either

hydro- or hygrophilious through referencing relevant literature, suggesting they were already present in

the general area previous to invasion by *S. minima* and are most likely using the mat incidentally to

exploit new habitat. Utilizing different collection methods results in different taxa collected (Meyer et al.

2011) and the use of a long term non-destructive collection method allowed us to collected crepuscular

and nocturnal insects that are not active during the day when other sampling methods would be used.

Table 5.1. Insects collected from a mat of *Salvinia minima* between May 15 and November 2, 2009. (*)=
previously undescribed species. Lifestyle/food associations are as follows: D= detritus, F= fungus, C=
carnivorous/predaceous, H= herbivorous, and P= Parasitic.

Order/Family	Genus/Species	Number	Food	Habitat
BLATTODEA				
Blattidae	sp.	1	D	T
COLEOPTERA				
Anobiidae	*Byrrhodes* sp.	2	F	T
Anthicidae	*Sapintus pubescens* Leferte	27	F/C	A6
Carabidae	*Acupalpus (Philodes) rectangulus* Chadoir	1	C	A6
	Agonum (s. str.) moerens Dejean	105	C	A5
	Ardistomis obliquatus Putzeys	1	C	A6
	Ardistomis schaumii LeConte	9	C	A6

Table 5.1 continued

	Badister reflexus LeConte	1	C	A6
	Bembidion (Furcacampa) affine Say	3	C	A5
	Bembidion (Notaphus) sp.	1	C	A5
	Bradycellus (Stenocellus) sp.	1	C	A6
	Calleida viridipennis (Say)	1	C	T
	Chlaenius (Agostenus) impunctifrons Say	3	C	A5
	Chlaenius (Agostenus) niger Randall	96	C	A5
	Chlaenius (Agostenus) oxygonus Chaudoir	1	C	A5
	Chlaenius (Agostenus) perplexus Say	1	C	A5
	Chlaenius (s. str.) erythropus Say	1	C	A5
	Chlaenius (s. str.) laticollis Say	28	C	A5
	Clivina (Leucocara) americana Dejean	1	C	A5
	Clivina (Semiclivina) dentipes Dejean	1	C	A5
	Diplocheila major LeConte	6	C	A6
	Elaphropus sp.	13	C	A5
	Loxandrus sp.1	1	C	A6
	Loxandrus sp.2	1	C	A6
	Loxandrus sp.3	1	C	A6
	Oodes amaroides Dejean	93	C	A6
	Oodes americanus Dejean	267	C	A6
	Paratachys sp.	16	C	A6
	Pterostichus (Melanius) ebeninus (Dejean)	58	C	A6
	Scarites quadriceps Chadoir	1	C	A6
	Scarites subterraneus Fabricius	1	C	A6
	Stenocrepis duodecimstriata (Chevrolat)	465	C	A6
	Stenolophus ochropezus (Say)	3	C	A6
Cerambycidae	*Elaphidion mucronatum* (Say)	1	H	T
	Parandra polita Say	1	H	T
	Styloleptus biustus (LeConte)	1	H	T
Chrysomelidae	*Chaetocnema* sp.	1	H	T
	Colaspis sp.	2	H	A3
	Epitrix sp.	1	H	T
	Myochrous sp.	1	H	T
	Nesaecrepida infuscata (Schaeffer)	1	H	T
	Pseudolampis guttata (LeConte)	7	H	A3

Table 5.1 continued

Coccinellidae	*Diomus terminatus* (Say)	2	C	T
Corylophidae	*Clypastraea* sp.	2	F	T
	Orthoperus sp.	2	F	T
Curculionidae	*Bagous hydrillae* O'Brien	3	H	A3
	Chramesus sp.	1	H	T
	Cyrtobagous salviniae Calder and Sands	3	H	A3
	Neochetina bruchi Hustache	12	H	A3
	Neochetina eichorniae Warner	6	H	A3
	Onychylis texanus Burke	2	H	A3
	Platypus flavicornis (Fabricius)	1	H	T
	Scolytinae sp.	16	H	T
	Stenopelmus rufinasus Gyllenhall	76	H	A3
	Tanysphyrus lemnae Fabricius	45	H	A3
	Xyleborus sp.	16	H	T
	Xylosandrus sp.	2	H	T
Dytiscidae	*Prodaticus bimarginatus* (Say)	2	C	A1
	Thermonectus sp.	12	C	A1
Endomychidae	*Rhymbomicrus* sp.	1	F	T
Erotylidae	*Triplax flavicollis* Lacordaire	1	F	T
Eucinetidae	*Eucinetus morio* LeConte	4	F	T
Eucnemidae	*Dirrhagofarsus lewisii* (Fleutiaux)	1	F	T
Haliplidae	*Peltodytes* sp.	1	C	A1
Heteroceridae	*Lanternarius mollinus* (Kiesenwetter)	1	D	A6
	Peditatus texanus Pacheco	1	D	A6
Histeridae	*Euspilotus assimilis* (Paykull)	1	D	A6
Hydraenidae	*Hydraena* sp.	4	--	A2
Hydrophilidae	*Cercyon* sp.	15	D	A6
	Enochrus consortus Green	13	D	A1
	Enochrus interruptus Gunderson	7	D	A1
	Enochrus ochraceus (Melshimer)	524	D	A1
	Hydrobiomorpha casta (Say)	32	D	A1
	Hydrochus callosus LeConte	1	D	A1
	Paracymus sp.	11	D	A1
	Phaenonotum exstriatum (Say)	136	D	A1
	Tropisternus blatchleyi Orchymont	5	D	A1

Table 5.1 continued

Laemophloeidae	*Placonotus* sp.	1	F	T
Latridiidae	*Corticarina* sp.	1	F	T
	Cortinicara sp.	1	F	T
	Enicmus sp.	1	F	T
	Melanophthalma sp.	6	F	T
Limnichidae	*Eulimnichus* sp.	20	H	A6
	Limnichites punctatus (LeConte)	1	H	A6
	Limnichoderus sp.	11	H	A6
Melandryidae	*Dircaea liturata* (LeConte)	1	F	T
	Microscapha clavicornis (LeConte)	3	H	T
Mordellidae	*Mordellistena* sp.	3	H	T
Nitidulidae	*Carpophilus dimidiatus* (Fabricius)	1	H	T
	Stelidota sp.	1	H	T
Noteridae	*Hydrocanthus* sp.	10	C	A1
	Suphisellus bicolor (Say)	3	C	A1
Phalacridae	*Stilbus* sp.	1	F	T
Ptiliidae	*Smicrus americanus* Casey	2	--	A6
	Unidentified genus (near *Nephanes*) sp.*	1	--	A6
Ptilodactylidae	*Ptilodactyla* sp.	1	D	A2
Scarabaeidae	*Dyscinetus morator* Fabricius	61	H	A5
	Euphoria sepulcralis (Fabricius)	1	H	T
Scirtidae	*Cyphon* sp.*	137	D	A2
	Scirtes tibialis Guerin-Meneville	657	D	A2
Sphindidae	*Sphindus* sp.	1	F	T
Staphylinidae	*Acylophorus* sp.	12	C	A6
	Adinopsis sp.	6	C	A6
	Aleocharinae sp.	5	C	--
	Anaquedius sp.	3	C	A6
	Anotylus sp.	7	C	A6
	Atanygnathus sp.	1	C	A6
	Athetini sp.	1	C	--
	Baeocera sp.	3	C	T
	Bibloplectus sp.	1	C	T
	Carpelimus sp.	155	C	A6
	Coproporus sp.	2	C	T

Table 5.1 continued

Staphylinidae	*Euaesthetus* sp.	10	C	A6
	Euconnus (Psomophora) sp.	10	D	T
	Euconnus (s. str.) sp.	1	D	T
	Homaeotarsus sp.	1	C	A6
	Hoplandria (*Genosema*) *pulchra* Kraatz	10	C	T
	Hoplandria (Genosema) sp.*	1	C	T
	Myllaena sp.	25	C	A6
	Neobisnius sp.	3	C	A6
	Philonthus sp.	3	C	A6
	Pinophilus sp.	1	C	T
	Scopaeus sp.	6	C	A6
	Scydmaeninae sp.	4	C	T
	Staphylininae sp.	1	C	--
	Stenus sp.	14	C	A6
	Tachinus sp.	1	C	T
	Tachyporus sp.	1	C	A6
	Thinobius sp.	1	C	A6
	Thoracophorus sp.	1	C	T
Tenebrionidae	*Lobopoda* sp.	1	F/H	T
	Platydema sp.	1	F/H	T
Tetratomidae	*Eustrophopsis bicolor* (Fabricius)	1	F	T
Thanerocleridae	*Ababa tantilla* LeConte	1	F	T
Throscidae	*Trixagus horni* Blanchard	1	F	T
HEMIPTERA				
Anthocoridae	sp.	1	C	T
Belostomatidae	*Belostoma lutarium* (Stäl)	23	C	A
	Belostoma testaceum (Leidy)	5	C	A
	Lethocerus uhleri (Montandon)	1	C	A
Cicadellidae	*Draeculacephela* sp.	60	H	T
Delphacidae	sp.	2	H	T
Gelastocoridae	*Gelastocoris oculatus* (Fabricius)	1	C	A
Gerridae	*Limnoporus canaliculatus* (Say)	1	C	A
Hebridae	*Hebrus consolidus* Uhler	128	H	A
Hydrometridae	*Hydrometra australis* Say	246	C	A
	Hydrometra hungerfordi Torre-Bueno	4	C	A

Table 5.1 continued

Mesoveliidae	*Mesovelia mulsanti* White	26	C	A
Naucoridae	*Pelocoris femoratus* (Palisot)	52	C	A
Nepidae	*Curicta scorpio* Stäl	6	C	A
Saldidae	*Micracanthia husseyi* Drake and Chapman	21	C	A
Veliidae	*Microvelia* sp.	1	C	A
HYMENOPTERA				
Braconidae	sp.1	1	P	--
	sp.2*	101	P	--
Formicidae	*Camponotus impressus* (Roger)	1	C	T
	Camponotus pennsylvanicus (DeGeer)	17	C	T
	Crematogaster sp.	103	C	T
	Crematogaster ashmeadi Mayr	12	C	T
Formicidae	*Crematogaster cerasi* Fitch	22	C	T
	Crematogaster vermiculata Emery	126	C	T
	Cyphomyrmex rimosus (Spinola)	1	C	T
	Hypoponera opacior (Forel)	23	C	T
	Pheidole sp.	13	C	T
	Pheidole dentata (Mayr)	32	C	T
	Pheidole metallescens Emery	3	C	T
	Pheidole moerens Wheeler	49	C	T
	Pseudomyrmex ejectus Smith	3	C	T
	Solenopsis sp.	39	C	T
	Solenopsis carolinensis Forel	1	C	T
	Solenopsis invicta Buren	80	C	T
	Solenopsis picta Emery	11	C	T
	Strumigenys louisianae Roger	3	C	T
	Strumigenys sylvestrii Roger	1	C	T
Ichneumonidae	Cryptinae: Hemigastrini sp.	741	P	--
	sp.2	187	P	--
	sp.3	19	P	--
Platygastridae	*Baeus* sp.	1	P	--
Pompilidae	*Anoplius depressipes* Banks	20	C	A
Sphecidae	sp.	1	C	T
ODONATA				
Libulellidae	sp.	1	C	A

Table 5.1 continued

Coenagrionidae	sp.	7	C	A
ORTHOPTERA		7		
Gryllidae	*Acheta* sp.	2	H	T
	Gryllodes sp.	10	H	T
Tetrigidae	*Tettigidea armata* Morse	106	H	T
	Tettigidea lateralis (Say)	36	H	T
PSOCOPTERA				
	sp.	1	F	T

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CHAPTER 6
SMALL SCALE SPATIAL HETEROGENEITY OF VEGETATION WITHIN A MAT OF *SALVINIA MINIMA* BAKER
AND IMPACTS ON THE COMMUNITY STRUCTURE OF INSECTS

INTRODUCTION

"Habitat heterogeneity hypothesis" is a fundamental concept in ecology that assumes that

structurally complex areas provide more niches or ways to exploit the habitat, thereby increasing

species diversity (Simpson 1949, MacArthur and Wilson 1967, Tews et al. 2004, Cramer and Willig 2005).

In most areas plants determine the structural complexity of the available microhabitats and impact

distributions and interactions among species (McCoy and Bell 1991). The habitat heterogeneity provided

by variation among aquatic macrophytes can influence both biomass and diversity of the

macroinvertebrates associated with them (Watkins et al. 1983, Thomaz and da Cunha 2010). The

preference of some taxa for specific macrophytes also influences community level patterns of

occurrence and abundance (Thomaz et al. 2008).

In Louisiana's aquatic systems the pleuston often consists of a unique habitat called a "flotant"

made of a combination of both floating aquatic vegetation and terrestrial rooted plants (Russell 1942).

Historically, the floating wetlands in the gulf south have been composed of marsh but with the

introduction of aquatic invasive weeds these mats now form in forested wetlands from *Salvinia* spp. and

a variety of other vegetation (Sasser et al. 1995, Jacono et al. 2001, Day et al. 2006). These forested

wetland swamps are integral parts of local ecosystems with high levels of primary productivity and

provide habitat, sources of food, and spawning/nursery areas (Conner and Day 1976, Conner et al.

1981).

Few prior studies have examined the invertebrate communities associated with the deep

swamp areas. Ziser (1978) and Sklar (1985) both previously sampled macroinvertebrates associated with

floating mats of *Lemna* sp. but did not work at sites heavily infested with *Salvinia* sp. While it was

reported in parts of Florida as early as 1928, *Salvinia minima* Baker was not reported in Louisiana until

1981 (Small 1931, Landry 1981). Our goal was to evaluate differences in insect communities between different types of secondary vegetation occurring with *S. minima*.

MATERIALS AND METHODS

This research was conducted on private property located near the junction of I-10 and US-61 in Gramercy, Louisiana (30.184519,-90.819144). The site is a perennially flooded Cypress-Tupelo backwater swamp with a solid mat of plant material covering the water's surface. The swamp vegetation in the area is dominated by baldcypress (*Taxodium distichum* L.), water tupelo (*Nyssa aquatica* L.), red maple (*Acer rubrum* L.) and Willow (*Salix spp.*). The pleuston flotant is primarily *S. minima* with interspersed other aquatic macrophytes including water hyacinth (*Eichornia crassipes* (Mart.) Solm.), alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.), and pennywort (*Hydrocotyle* spp.). Chinese tallow (*Triadica sebifera* (L.) Small) seedlings were also observed, rooting in the flotant toward the end of the year.

We sampled the area biweekly from May 18 to November 16 2009 using a 50 m x 50 m grid of 100 floating pitfall traps. All traps were serviced biweekly from the deployment date of May 18 for a total of 13 collection dates during 2009. Specimens of adult insects were identified using relevant literature and help from taxonomists. In addition to the insects collected at each trap location, on each collection date we measured plant height within 1 m^2 (cm), water depth (cm), and the presence of any plants other than *S. minima* within the 1 m^2 area.

Each of the floating pitfall traps was surrounded by a homogenous mat of *S. minima* in May of 2009 at the beginning of the field season, though as the field season progressed additional vegetation was observed within the 1 m^2 evaluation area at some of the trap sites. Each of the trap locations was coded for the presence/absence of each of the several observed species over the course of the year.

The resulting data set was analyzed using Proc Cluster by Ward's Methods in SAS 9.2 and the most appropriate tree selected.

Variation in insect community composition between resulting clusters was calculated using Jaccard's Similarity Index. This index is calculated using the following formula:

$$J = \frac{c}{(a+b+c)}$$

where c is the number of species common between clusters, and a and b are non-overlapping species that only occur in a single cluster (Jaccard 1908). We also calculated several community indices: Simpson's Index of Diversity, Shannon's Diversity Index, Pielou's Evenness Index, McIntosh's Evenness Index and Margalef's Adjusted Richness (Shannon 1948, Simpson 1949, Margalef 1958, Pielou 1966, McIntosh 1967). We calculated these indices for each trap location as a pooled sample for the year. Evaluation of possible relationships between measurements taken on site, calculated community indices and clusters were done using SAS 9.2 to perform a Multivariate Analysis of Variance and to establish Spearman's Rank Order Correlation Coefficients. Rarefied predicted species accumulation curves using *Mao Tao* were calculated following Colwell et al. (2004) using EstimateS (Colwell 2009). *Mao Tau* outputs were graphed and regression lines calculated using SigmaPlot 11.

RESULTS AND DISCUSSION

CLUSTER ANALYSIS

Each of the 100 floating pitfall traps used were surrounded by *S. minima* at the beginning of the 2009 field season, but as the year progressed additional vegetation was observed within the 1 m^2 evaluation area at some of the traps. Each of the traps was coded for the presence/absence of the

following species over the course of the year: *E. crassipes*, *Hydrocotyle* spp., *T. distichum* (both trees and knees), *A. philoxeroides*, *T. sebifera* (seedlings), sedges, and other trees (*A. rubrum*, *N. aquatica*, and *Salix* spp.). Data points were analyzed using Proc Cluster in SAS 9.2.Ward's Method of clustering provided the clearest tree with five distinct groups based on plant community (semi-partial R^2=0.75)(Figure 6.1). All variables except *A. philoxeroides* were significant in the model (P<0.001). All clusters contained *S. minima* as the predominant vegetation throughout the year. Cluster 1 (n=39) is the largest group of trap locations and is characterized by the presence of pennywort (*Hydrocotyle* spp) in addition to *S. minima*. Cluster 2 (n=18) is more structurally diverse of the clusters, including pennywort (*Hydrocotyle* spp.) and water hyacinth (*E. crassipes*) with a few of the trap locations having alligator weed (*A. philoxeroides*) and sedges (*Carex* sp.). Cluster 3 (n=13) includes the trees (*A. rubrum, N. aquatica, and T. distitchum*), chinese tallow seedlings (*T. sebifera*)*,* and pennywort *(Hydrocotyle* spp.). Cluster 4 (n=22) is characterized by *T. distichum* alone with *S. minima*. Cluster 5 (n=8) is locations that had *S. minima* alone over the course of the year (Figure 6.2, 6.3).

The vegetation clusters that resulted from the Ward's analysis was approximately what we expected to see, with each of the commonly observed macrophytes other than *S. minima* forming groups of trap locations. The 5 clustered vegetation groups roughly form a gradient from least structurally diverse (just *S. minima*) to complex (featuring large trees) and form irregular patches within the landscape (Figure 6.3). The patches fall such that no cluster is more than 10 m from a trap with a different vegetation cluster assignment.

VARIATION IN COMMUNITY STRUCTURE AMONG CLUSTERS

The community composition of adult insects within the five clusters of vegetation type is represented by 176 insect species (Table 6.1). Of the species collected, 46% were found in only one vegetation cluster while 23% (40/176) were found across all five clusters. Of the taxa found within all
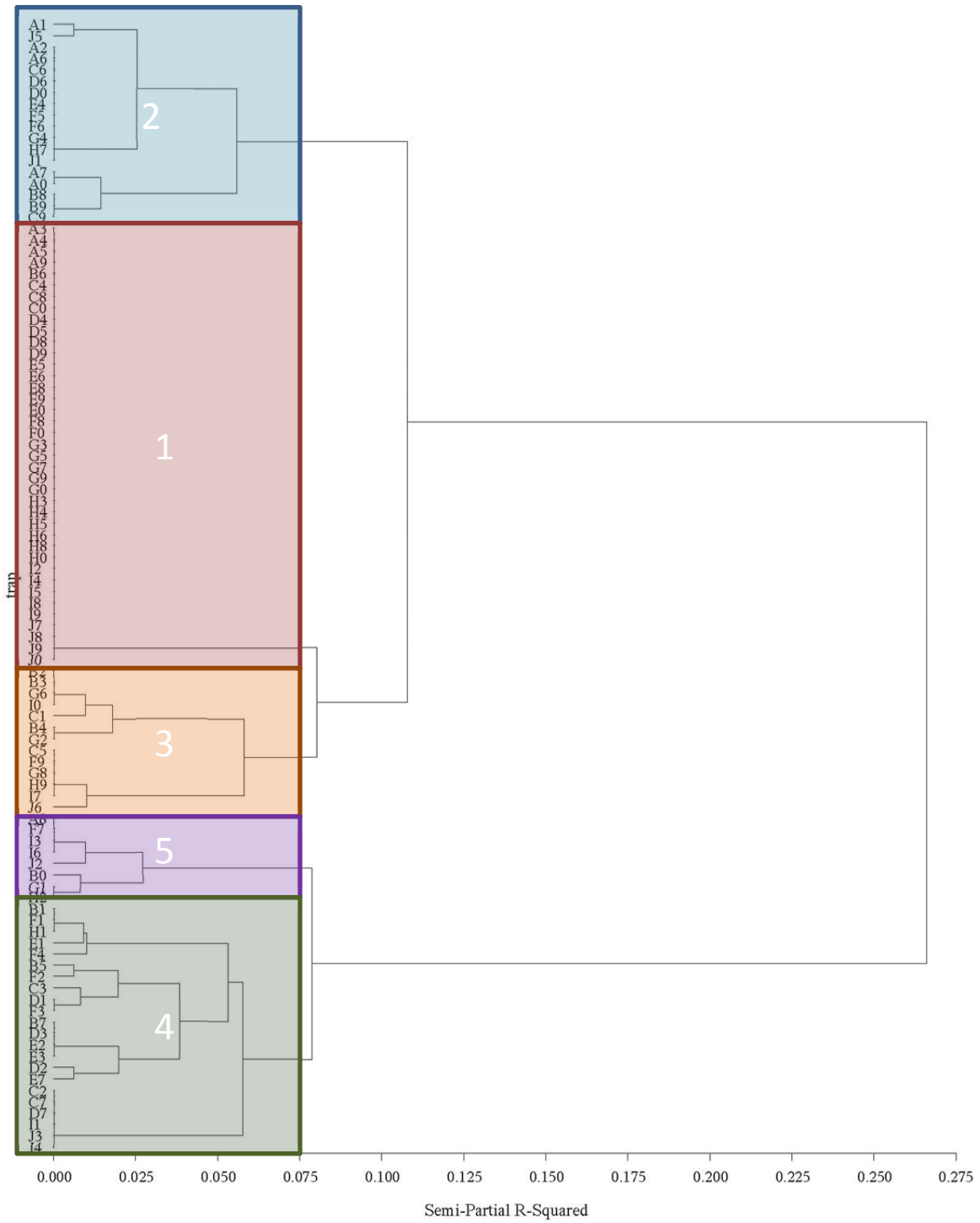
Figure 6.1. Plant community groups by Ward's Cluster Analysis. Colored boxes show the extent of each of the clusters.

Figure 6.2. (Clockwise from top left) Representative photographs of vegetation clusters 1-4 (Cluster 5 not shown, included no vegetation other than *S. minima*)

J10	J9	J8	J7	J6	J5	J4	J3	J2	J1
I10	I9	I8	I7	I6	I5	I4	I3	I2	I1
H10	H9	H8	H7	H6	H5	H4	H3	H2	H1
G10	G9	G8	G7	G6	G5	G4	G3	G2	G1
F10	F9	F8	F7	F6	F5	F4	F3	F2	F1
E10	E9	E8	E7	E6	E5	E4	E3	E2	E1
D10	D9	D8	D7	D6	D5	D4	D3	D2	D1
C10	C9	C8	C7	C6	C5	C4	C3	C2	C1
B10	B9	B8	B7	B6	B5	B4	B3	B2	B1
A10	A9	A8	A7	A6	A5	A4	A3	A2	A1

Figure 6.3. Trap grid with coordinate numbers. Colored blocks represent vegetation clusters.
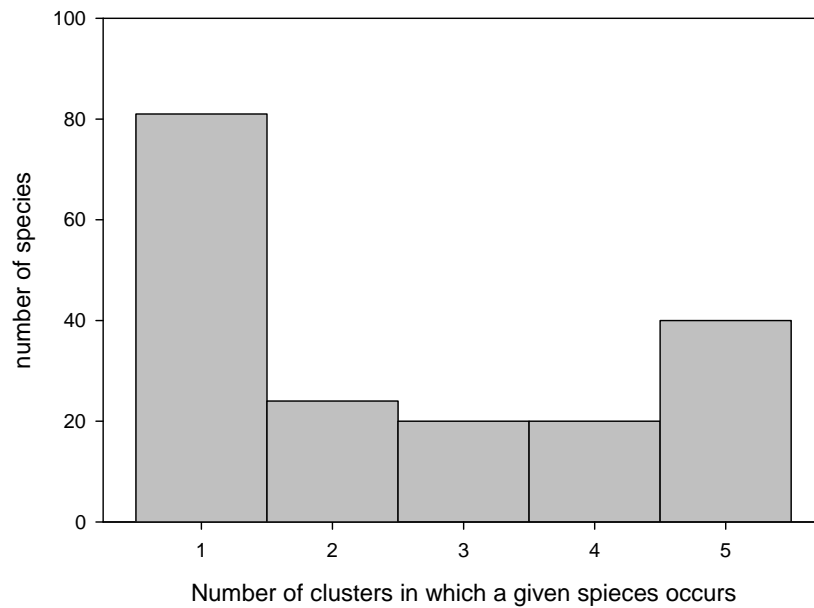
Figure 6.4. Frequency distribution of the number of taxa according to the number of clusters in which they occur.

Table 6.1 Comparative numbers of species of insects collected from each vegetation cluster (1-5).
(+ = present. - = absent)

Order/Family	Genus/Species	1	2	3	4	5
BLATTODEA						
Blattidae	sp.	+	-	-	-	-
COLEOPTERA						
Anobiidae	*Byrrhodes* sp.	+	-	+	-	-
Anthicidae	*Sapintus pubescens* Leferte	+	+	+	+	+
Carabidae	*Acupalpus (Philodes) rectangulus* Chadoir	+	-	-	+	-
	Agonum (s. str.) moerens Dejean	+	+	+	+	+
	Ardistomis obliquatus Putzeys	-	-	-	+	-
	Ardistomis schaumii LeConte	+	+	+	+	-
	Badister reflexus LeConte	-	-	-	+	-
	Bembidion (Furcacampa) affine Say	+	-	-	+	-
	Bembidion (Notaphus) sp.	+	-	-	-	-
	Bradycellus (Stenocellus) sp.	+	-	-	-	-
	Calleida viridipennis (Say)	+	-	-	-	-
	Chlaenius (Agostenus) impunctifrons Say	-	-	+	+	-
	Chlaenius (Agostenus) niger Randall	+	+	+	+	+
	Chlaenius (Agostenus) oxygonus Chaudoir	+	-	-	-	-
	Chlaenius (Agostenus) perplexus Say	-	-	-	+	-
	Chlaenius (s. str.) erythropus Say	+	-	-	-	-
	Chlaenius (s. str.) laticollis Say	+	-	+	+	+
	Clivina (Leucocara) americana Dejean	-	-	-	+	-
	Clivina (Semiclivina) dentipes Dejean	-	+	-	-	-
	Diplocheila major LeConte	+	+	-	+	+
	Elaphropus sp.	+	+	+	+	+
	Loxandrus sp.1	-	-	+	-	-
	Loxandrus sp.2	+	-	-	-	+

Table 6.1 continued

Carabidae	*Loxandrus* sp.3	+	-	-	-	-
	Oodes amaroides Dejean	+	+	+	+	+
	Oodes americanus Dejean	+	+	+	+	+
	Paratachys sp.	+	+	+	+	+
	Pterostichus (Melanius) ebeninus (Dejean)	+	+	+	+	+
	Scarites quadriceps Chadoir	-	-	+	-	-
	Scarites subterraneus Fabricius	+	-	-	-	-
	Stenocrepis duodecimstriata (Chevrolat)	+	+	+	+	+
	Stenolophus ochropezus (Say)	-	+	+	-	+
Cerambycidae	*Elaphidion mucronatum* (Say)	-	-	+	-	-
	Parandra polita Say	-	-	-	+	-
	Styloleptus biustus (LeConte)	-	-	+	-	-
Chrysomelidae	*Chaetocnema* sp.	-	-	+	-	-
	Colaspis sp.	+	-	-	+	-
	Epitrix sp.	+	-	-	-	-
	Myochrous sp.	+	-	-	-	-
	Nesaecrepida infuscata (Schaeffer)	-	-	+	-	-
	Pseudolampis guttata (LeConte)	+	+	+	+	+
Coccinellidae	*Diomus terminatus* (Say)	+	+	-	-	-
Corylophidae	*Clypastraea* sp.	+	-	-	+	-
	Orthoperus sp.	+	-	-	+	-
Curculionidae	*Bagous hydrillae* O'Brien	+	+	-	-	-
	Chramesus sp.	-	-	-	-	+
	Cyrtobagous salviniae Calder and Sands	+	+	-	-	-
	Neochetina bruchi Hustache	+	+	-	+	-
	Neochetina eichorniae Warner	+	+	-	+	-
	Onychylis texanus Burke	+	-	-	-	-
	Platypus flavicornis (Fabricius)	+	-	-	-	-

Table 6.1 continued

Curculionidae	Scolytinae sp.	+	+	+	+	+
	Stenopelmus rufinasus Gyllenhall	+	+	+	+	+
	Tanysphyrus lemnae Fabricius	+	+	+	+	+
	Xyleborus sp.	+	-	+	+	-
	Xylosandrus sp.	+	-	-	-	+
Dytiscidae	*Prodaticus bimarginatus* (Say)	-	+	-	+	+
	Thermonectus sp.	+	+	-	-	+
Endomychidae	*Rhymbomicrus* sp.	+	-	-	+	-
Erotylidae	*Triplax flavicollis* Lacordaire	-	-	-	+	-
Eucinetidae	*Eucinetus morio* LeConte	-	+	+	+	+
Eucnemidae	*Dirrhagofarsus lewisii* (Fleutiaux)	+	-	-	-	-
Haliplidae	*Peltodytes* sp.	-	-	-	-	+
Heteroceridae	*Lanternarius mollinus* (Kiesenwetter)	+	-	-	-	-
	Peditatus texanus Pacheco	-	-	-	-	+
Histeridae	*Euspilotus assimilis* (Paykull)	-	-	-	+	+
Hydraenidae	*Hydraena* sp.	-	+	+	+	-
Hydrophilidae	*Cercyon* sp.	+	+	+	+	+
	Enochrus consortus Green	+	+	+	-	-
	Enochrus interruptus Gunderson	+	+	+	+	-
	Enochrus ochraceus (Melshimer)	+	+	+	+	-
	Hydrobiomorpha casta (Say)	+	+	+	+	+
	Hydrochus callosus LeConte	-	-	+	-	-
	Paracymus sp.	+	+	-	+	-
	Phaenonotum exstriatum (Say)	+	+	+	+	+
	Tropisternus blatchleyi Orchymont	+	+	-	-	+
Laemophloeidae	*Placonotus* sp.	-	-	-	+	-
Latridiidae	*Corticarina* sp.	-	-	+	-	-
	Cortinicara sp.	-	-	-	+	-

Table 6.1 continued

Latridiidae	*Enicmus* sp.	-	+	-	-	-
	Melanophthalma sp.	+	+	+	+	+
Limnichidae	*Eulimnichus* sp.	+	+	+	-	+
	Limnichites punctatus (LeConte)	-	-	-	+	-
	Limnichoderus sp.	+	+	+	+	-
Melandryidae	*Dircaea liturata* (LeConte)	+	-	-	-	-
	Microscapha clavicornis (LeConte)	+	-	+	+	-
Mordellidae	*Mordellistena* sp.	+	+	-	-	-
Nitidulidae	*Carpophilus dimidiatus* (Fabricius)	+	-	-	-	-
	Stelidota sp.	-	-	-	+	-
Noteridae	*Hydrocanthus* sp.	+	+	+	-	+
	Suphisellus bicolor (Say)	+	-	-	+	-
Phalacridae	*Stilbus* sp.	-	+	-	-	-
Ptiliidae	*Smicrus americanus* Casey	-	-	-	+	-
	Unidentified genus (near *Nephanes*) sp.*	-	-	-	+	-
Ptilodactylidae	*Ptilodactyla* sp.	-	-	-	+	-
Scarabaeidae	*Dyscinetus morator* Fabricius	+	+	+	+	+
	Euphoria sepulcralis (Fabricius)	-	+	-	-	-
Scirtidae	*Cyphon* sp.*	+	+	+	+	+
	Scirtes tibialis Guerin-Meneville	+	+	+	+	+
Sphindidae	*Sphindus* sp.	-	-	-	+	-
Staphylinidae	*Acylophorus* sp.	+	+	+	+	+
	Adinopsis sp.	+	+	-	+	-
	Aleocharinae sp.	+	-	-	+	+
	Anaquedius sp.	-	+	+	-	-
	Anotylus sp.	+	-	+	+	-
	Atanygnathus sp.	+	-	-	-	-
	Athetini sp.	-	-	-	+	-

Table 6.1 continued

Staphylinidae	*Baeocera* sp.	-	+	-	+	-
	Bibloplectus sp.	-	+	-	-	-
	Carpelimus sp.	+	+	+	+	+
	Coproporus sp.	+	-	-	+	-
	Euaesthetus sp.	+	+	+	+	+
	Euconnus (Psomophora) sp.	+	+	-	+	+
	Euconnus (s. str.) sp.	+	-	-	-	-
	Homaeotarsus sp.	-	-	-	+	-
	Hoplandria (*Genosema*) *pulchra* Kraatz	-	-	+	+	+
	Hoplandria (Genosema) sp.*	-	+	-	-	-
	Myllaena sp.	+	+	+	+	-
	Neobisnius sp.	+	-	+	+	-
	Philonthus sp.	-	-	-	+	-
	Pinophilus sp.	-	-	+	-	-
	Scopaeus sp.	-	+	+	+	+
	Scydmaeninae sp.	+	+	+	-	-
	Staphylininae sp.	+	-	-	-	-
	Stenus sp.	+	+	+	+	+
	Tachinus sp.	+	-	-	-	-
	Tachyporus sp.	+	-	-	-	-
	Thinobius sp.	-	-	+	-	-
	Thoracophorus sp.	-	-	-	+	-
Tenebrionidae	*Lobopoda* sp.	-	-	-	+	-
	Platydema sp.	-	+	-	-	-
Tetratomidae	*Eustrophopsis bicolor* (Fabricius)	-	+	-	-	-
Thanerocleridae	*Ababa tantilla* LeConte	+	-	-	-	-
Throscidae	*Trixagus horni* Blanchard	-	-	-	+	-

Table 6.1 continued

HEMIPTERA						
Anthocoridae	sp.	+	-	-	-	-
Belostomatidae	*Belostoma lutarium* (Stäl)	+	+	+	+	+
	Belostoma testaceum (Leidy)	+	+	-	-	-
	Lethocerus uhleri (Montandon)	+	-	-	-	-
Cicadellidae	*Draeculacephela* sp.	+	+	+	+	-
Delphacidae	sp.	-	-	-	-	+
Gelastocoridae	*Gelastocoris oculatus* (Fabricius)	-	-	-	+	-
Gerridae	*Limnoporus canaliculatus* (Say)	+	-	-	-	-
Hebridae	*Hebrus consolidus* Uhler	+	+	+	+	+
Hydrometridae	*Hydrometra australis* Say	+	+	+	+	+
	Hydrometra hungerfordi Torre-Bueno	+	-	-	+	-
Mesoveliidae	*Mesovelia mulsanti* White	+	+	+	-	-
Naucoridae	*Pelocoris femoratus* (Palisot)	+	+	+	+	+
Nepidae	*Curicta scorpio* Stäl	+	-	-	+	-
Saldidae	*Micracanthia husseyi* Drake and Chapman	+	+	+	+	+
Veliidae	*Microvelia* sp.	+	-	-	-	-
HYMENOPTERA						
Braconidae	sp.1	+	-	-	-	-
	sp.2*	+	+	+	+	+
Formicidae	*Camponotus impressus* (Roger)	-	-	-	+	-
	Camponotus pennsylvanicus (DeGeer)	+	+	+	-	-
	Crematogaster sp.	+	+	+	+	+
	Crematogaster ashmeadi Mayr	+	+	+	+	-
	Crematogaster cerasi Fitch	+	+	+	+	-
	Crematogaster vermiculata Emery	+	+	+	+	+
	Cyphomyrmex rimosus (Spinola)	-	-	-	+	-
	Hypoponera opacior (Forel)	+	-	+	+	+

Table 6.1 continued

Formicidae	Pheidole sp.	+	-	+	+	+
	Pheidole dentata (Mayr)	+	+	+	+	+
	Pheidole metallescens Emery	-	-	+	+	-
	Pheidole moerens Wheeler	-	+	+	+	+
	Pseudomyrmex ejectus Smith	-	-	+	-	-
	Solenopsis sp.	+	+	+	+	+
	Solenopsis carolinensis Forel	-	-	+	-	-
	Solenopsis invicta Buren	+	+	+	+	+
	Solenopsis picta Emery	+	+	+	+	+
	Strumigenys louisianae Roger	+	-	+	-	-
	Strumigenys sylvestrii Roger	-	-	-	-	+
Ichneumonidae	Cryptinae: Hemigastrini sp.	+	+	+	+	+
	sp.2	+	+	+	+	+
	sp.3	+	-	-	-	-
Platygastridae	Baeus sp.	-	+	-	-	-
Pompilidae	Anoplius depressipes Banks	+	+	+	-	+
Sphecidae	sp.	-	-	-	+	-
ODONATA						
Libulellidae	sp.	-	+	-	-	-
Coenagrionidae	sp.	+	-	+	+	-
ORTHOPTERA						
Gryllidae	Acheta sp.	-	-	-	+	-
	Gryllodes sp.	+	+	+	+	-
Tetrigidae	Tettigidea armata Morse	+	+	+	+	+
	Tettigidea lateralis (Say)	+	+	+	+	+
PSOCOPTERA						
	sp.	+	-	-	-	-
	Total Number of morphospecies	**121**	**87**	**87**	**109**	**65**

Table 6.2. Community similarity index (Jaccard's Index of Similarity and number of shared species) between 5 different vegetation clusters.

Site:	1	2	3	4	5
					Similarity Index
1		0.50	0.46	0.47	0.40
2	69		0.55	0.47	0.52
3	66	62		0.49	0.50
4	74	61	64		0.43
5	53	52	51	52	
	No. of Shared Species				

five clusters, 24 of 40 belong to the order Coleoptera. The frequency of distribution of species and the number of clusters that occur in is skewed and does not follow a normal distribution (Figure 6.4). The community similarity among clusters is calculated using Jaccard's Similarity Index (J) (Jaccard 1908) and ranges from 0.40 to 0.55 (Table 6.2).

While the overlap in community composition is low between vegetation clusters with between 52 and 74 shared species (J= 0.40-0.55, Table 6.2), it is similar to what others have found when looking at community structure associated with different types of macrophytes (Poi de Neiff and Neiff 2006). Additional studies have found much higher levels of overlap between community structures but included a wider variety of organisms (Dioni 1967). The 40 taxa collected that were found in all five vegetation clusters represent a wide variety of orders and several different life history strategies including herbivores, predators, and parasitoids (Merritt et al. 2008).

We used the EstimateS program to calculate rarefied sampling curve with replacement to compare diversity of clusters because the clusters were of uneven size. While sampling with replacement results in lower overall estimates of population, the method of calculation allows

comparison of unequal datasets (Colwell 2009). M*ao Tao* species accumulation curves were calculated for each of the 5 vegetation clusters and indicate differing predicated species richnesses (R^2>0.98). Cluster 1 was the most species rich cluster both in raw data and rarefied analysis, followed by cluster 4. Clusters 2 and 3 both collected 87 species in the raw data, but using the rarefied analysis, cluster 3 would collect more species than cluster 2. Cluster 5 was the least rich in both analyses (Figure 6.5).

CORRELATION ANALYSIS

We performed a multivariate analysis of variance to examine the possible interaction effects of vegetation cluster on the richness and abundance of the insects collected, community indices (Simpson's, Shannon's, Margalef's, Pielou's and McIntosh's Indices), and variables measured on site (plant height and water depth). None of the diversity indices or richness/abundance showed a significant interaction with vegetation cluster (Figures 6.6-6.12). Resulting P values for the variables are presented in Table 6.3. Both water depth and plant height had highly significant differences between vegetation clusters (Figures 6.13, 6.14).

Multivariate analysis revealed no significant differences in diversity, evenness, abundance, or richness between clusters regardless of differences in structural complexity of vegetation present (Figures 6.6-6.12). There were highly significant differences in water depth and plant height between clusters. Vegetation clusters 3 and 4 contained tree species and had significantly shallower water and taller vegetation (df=4,95, P<0.0001)(Figures 6.13, 6.14) than the other clusters.

To test non-linear relationships between variables, we completed pairwise comparisons of the same variables listed above using a calculated Spearman's Rank Order Coefficient. These also indicate additional significant interactions that were not visible in the MANOVA results above (see Correlation Coefficients and P values in Table 6.4). Vegetation clusters were negatively associated with plant height and water depth as seen in the MANOVA, but additionally showed partial weak positive correlations
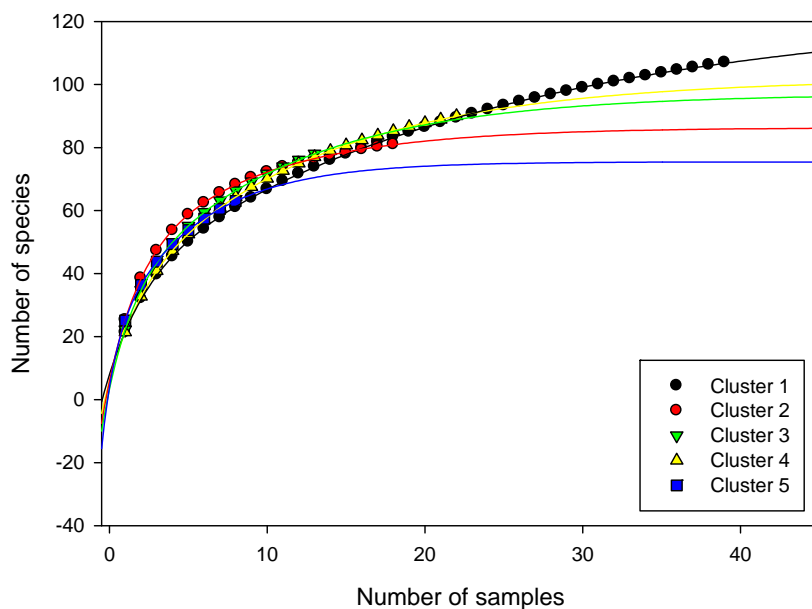
Figure 6.6. Comparison of randomized species (*Mao Tau*) accumulation curves for each vegetation cluster calculated with replacement.
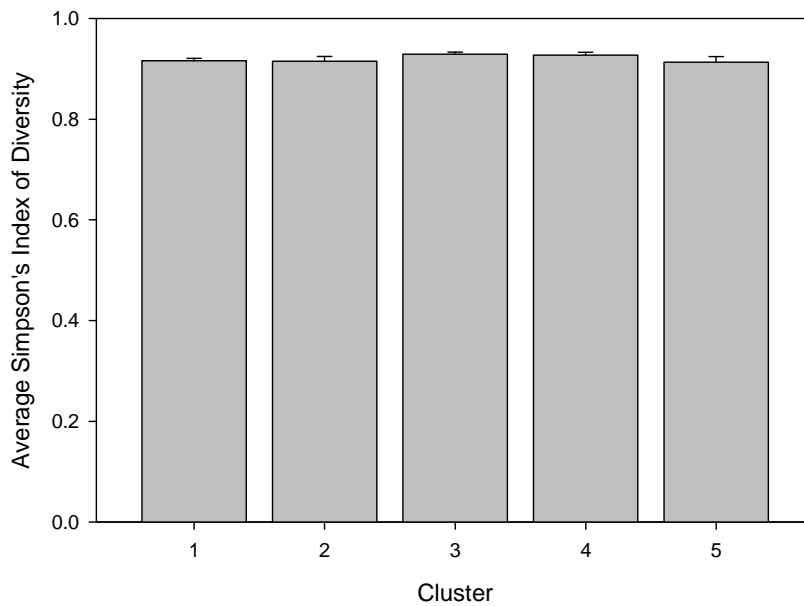

Figure 6.6. Average Simpson's Index of diversity for each vegetation cluster.
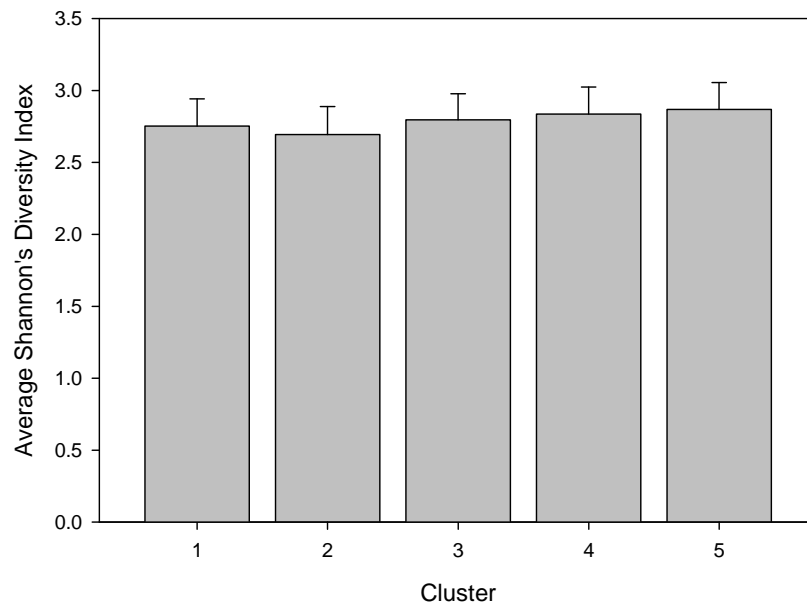
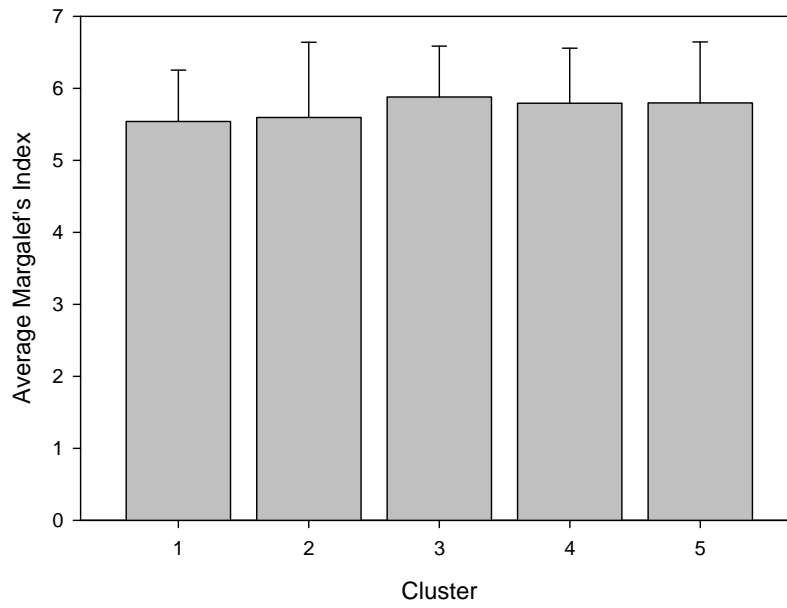Figure 6.7. Average Shannon's Diversity Index by vegetation cluster.

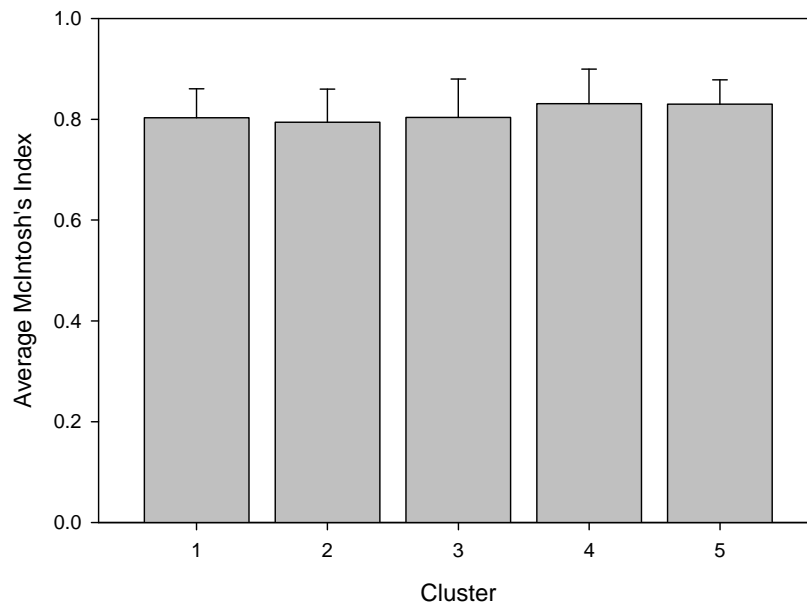Figure 6.8. Average Margalef's Index by vegetation cluster.

Figure 6.9. Average McIntosh's Index by vegetation cluster.
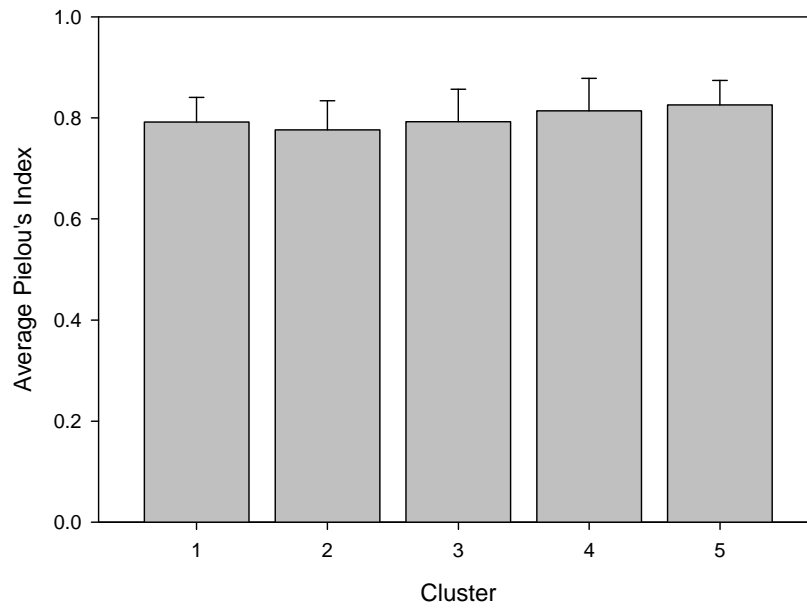

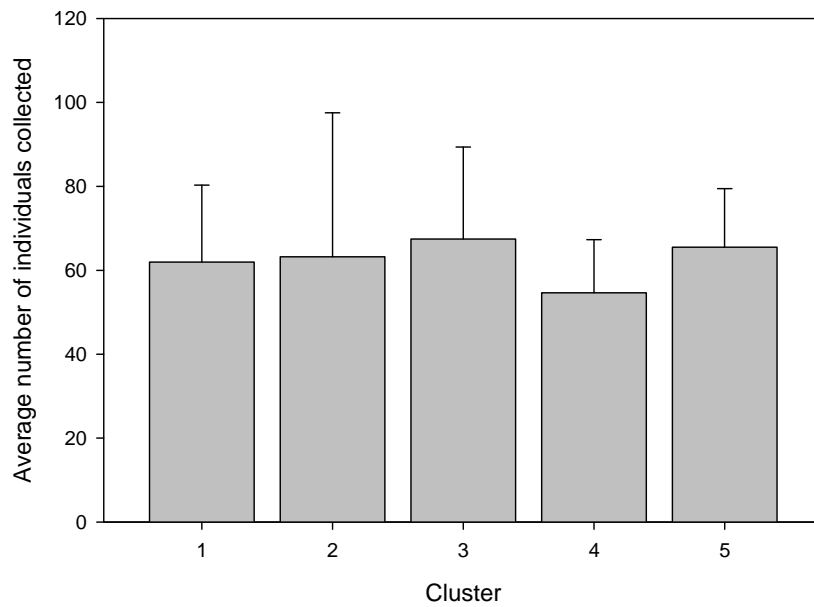Figure 6.10. Average Pielou's Index by vegetation cluster.

Figure 6.11. Average number of individual insects collected from each trap by vegetation cluster.
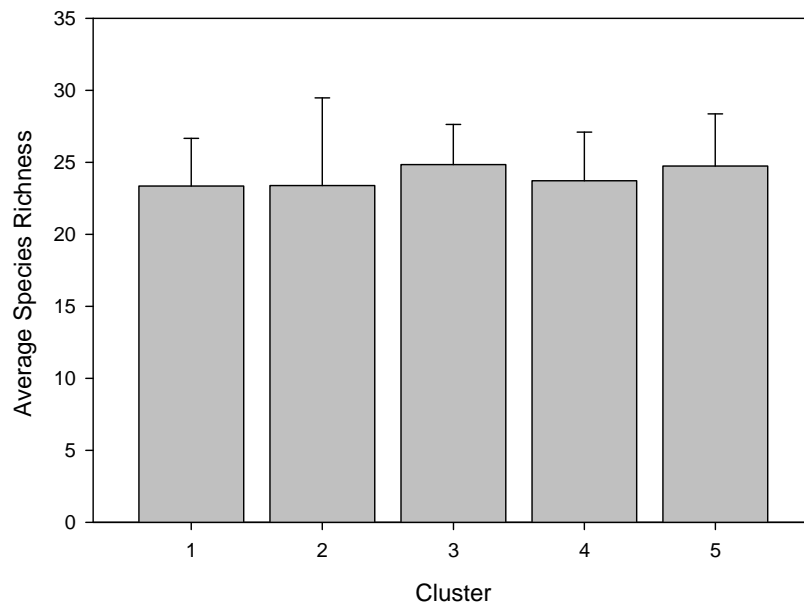

Figure 6.12. Average species richness for each vegetation cluster.
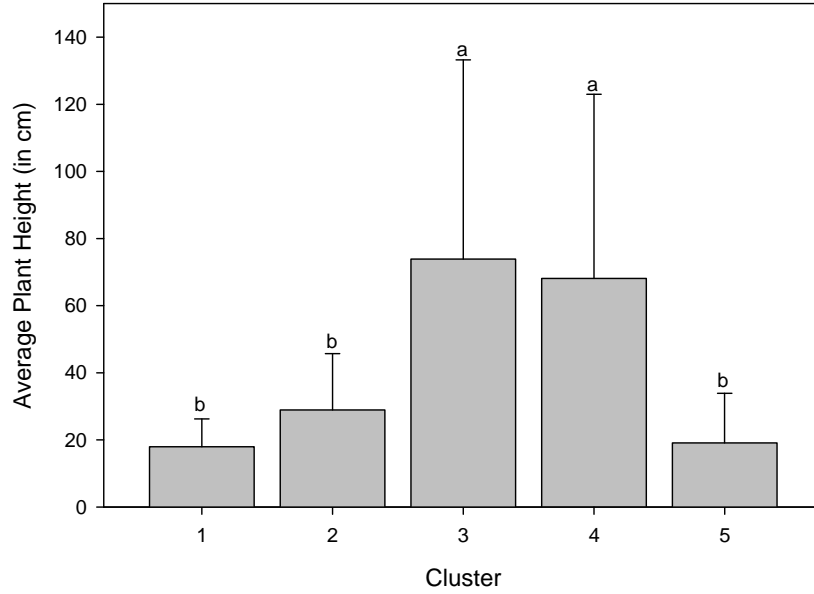
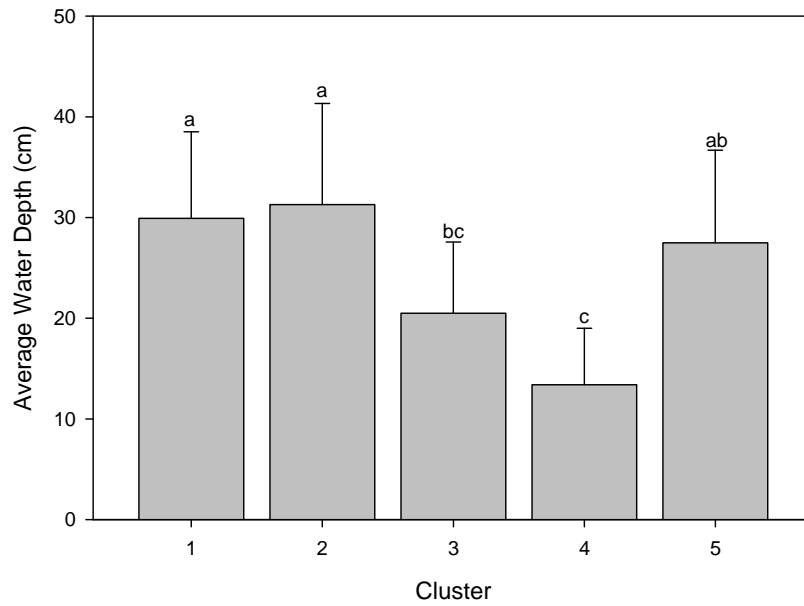Figure 6.13. Differences in average plant height between vegetation clusters.

Figure 6.14. Differences in the average water depth among vegetation clusters.

Table 6.3. MANOVA results analyzing differences among vegetation clusters.

Variable	Degrees of Freedom	F Value	P Value
Simpson's Index of Diversity	4, 95	1.22	0.3090
Shannon's Index of Diversity	4, 95	1.72	0.1519
Richness	4, 95	1.29	0.2778
Abundance	4, 95	0.9	0.4685
Margalef's	4, 95	0.70	0.5966
McIntosh	4, 95	1.22	0.3067
Pielou's	4, 95	1.74	0.1469
Plant Height (cm)	4, 95	11.78	**<0.0001**
Water Depth (cm)	4, 95	18.36	**<0.0001**

with Simpson's Diversity, Shannon's Diversity, McIntosh's Evenness, and Pielou's Evenness. Water depth was weakly partially positively correlated with abundance, but weakly partially negatively correlated with Simpson's Diversity, Shannon's Diversity, McIntosh's Evenness, and Margalef's Richness. Plant height was negatively correlated with water depth as in the MANOVA above. These additional weak interactions indicate through non-parametric correlation coefficients that other factors may be influencing associations between structural complexity and the diversity and evenness indices.

None of our analyses linked the more spatially complex vegetation clusters to higher levels of richness, abundance, or diversity. While positively linking diversity to structural complexity is commonly accepted (Tews et al. 2004), we found little support for the hypothesis in this system. Other studies have observed a lack of relationship between community variables and structural complexity (McAbendroth et al. 2005). Possibly, our gridded area of 1 m^2 plots every 5 m was the wrong scale to detect changes in community structure between habitat types. The scale of experiments examining spatial heterogeneity appear to be directly linked to other findings, and several studies have examined the differences in

Table 6.4. Spearman's Rank Order Coefficients and associated P values for community indices, vegetation cluster, and measured variables. Abbreviations are as follows: Simp= Simpson's Diversity, Shan= Shannon's Diversity, Rich= Richness, Abun= Abundance, Marg= Margalef's Richness, McIn= McIntosh's Evenness, Piel= Pielou's Evenness, H_2OD= Water Depth, Plant= plant height, Clus= Cluster. Variables and coefficients significant at the α=0.05 level are in bold.

	Simp.	Shan.	Rich.	Abun.	Marg.	McIn.	Piel.	H_2OD	Plant	Clus.
Simp.	1.00	**0.897**	0.116	**-0.471**	**0.509**	**0.989**	**0.936**	**-0.360**	0.159	**0.226**
		<0.0001	0.2526	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0002**	0.1145	**0.0236**
Shan.		1.00	**0.391**	-0.171	**0.782**	**0.848**	**0.913**	**-0.324**	0.153	**0.207**
			<0.0001	0.0880	**<0.0001**	**<0.0001**	**<0.0001**	**0.0010**	0.1295	**0.0308**
Rich.			1.00	**0.422**	**0.698**	0.041	0.058	-0.152	0.142	0.147
				<0.0001	**<0.0001**	0.6885	0.5672	0.1303	0.1579	0.1143
Abun.				1.00	0.105	**-0.585**	**-0.350**	**0.215**	0.024	0.015
					0.3006	**<0.0001**	**0.0004**	**0.0325**	0.8135	0.8855
Marg.					1.00	**0.455**	**0.591**	**-0.238**	0.106	0.119
						<0.0001	**<0.0001**	**0.0173**	0.2949	0.2391
McIn.						1.00	**0.915**	**-0.368**	0.139	**0.214**
							<0.0001	**0.0002**	0.1678	**0.0327**
Piel.							1.00	**-0.353**	0.114	**0.209**
								0.0003	0.2608	**0.0372**
H_2OD								1.00	**-0.351**	**-0.493**
									0.0003	**<0.0001**
Plant									1.00	**0.413**
										<0.0001
Clus.										1.00

micro- meso- and macro- studies and found differing interactions at each level (Tews et al. 2004). In addition, when our sampling grid for the study was established, the flotant was homogenous and composed entirely of *S. minima*. As the season progressed, the flotant's habitat structure grew more

fragmented and heterogeneous, possibly confounding results since vegetation was not consistent for the entire year.

While we were investigating non-native vegetation, many of the other studies looking at structural habitat complexity among aquatic macrophytes have taken place within the native range of the plants studied (Dioni 1967, Poi de Neiff and Neiff 2006). Several studies in Africa that examined invasions of *E. crassipes* versus native plant communities found a variety of effects on invertebrate communities, including positive effects (Brendonck et al. 2003), neutral effects (Midgley et al. 2006), and negative effects (Masifwa et al. 2001).

While we did not investigate water chemistry among vegetation clusters, variation in pH and dissolved oxygen can shape community structure (Heino 2000). Heavy stands of invasive aquatic vegetation like *Salvinia* spp. or *E. crassipes* can decrease dissolved oxygen levels (Begg 1970, Rai and Datta Munshi 1979, Flores and Carlson 2006), possibly changing the structure of the community surrounding the vegetation (Cheruvelil et al. 2002). While the spatial heterogeneity within the mat of *S. minima* did not visibly affect the diversity or abundance of the adult insects associated with the mat, rarefaction predicted differences in richness between clusters of vegetation. The cluster with the highest species richness was not among the more structurally diverse within the mat, suggesting that other (possibly abiotic) influences are driving variation in community structure.

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CHAPTER 7
CONCLUSIONS

SUMMARY

In this dissertation, field experiments were conducted to introduce and establish populations of the salvinia weevil, *Cyrtobagous salviniae* Calder and Sands, for the control of common salvinia, *Salvinia minima* Baker and to examine factors that may affect the success of the biological control program. These studies were necessary to establish a biological control program against *S. minima* in southern Louisiana and to address interactions that may affect the survival of *C. salviniae*. Chapter 2 details the history of introductions of *C. salviniae* on *S. minima* in southern Louisiana, and original locations of the populations of *C. salviniae* used to form colonies here at Louisiana State University. As part of this study, we released adult *C. salviniae* at 9 different locations across southern Louisiana between 2006 and 2010. We tracked the populations in Gramercy, LA and monitored their impacts on the biomass of *S. minima*, number of terminal buds damaged, percentage of the mat within the quadrat green, percentage of water within the quadrat covered, dissolved oxygen, pH, conductivity, and temperature of the water's surface. *Cyrtobagous salviniae* were found successfully established and having overwintered during 2008 at several field sites. The field populations that established significantly negatively impacted biomass and positively affected the number of terminals buds damaged. None of the other parameters measured differed between control sites and those where *C. salviniae* was released.

The study in Chapter 3 addresses the suspicion that the red imported fire ant, *Solenopsis invicta* Buren, may be interfering with the establishment of populations of *C. salviniae* in some locations. We constructed moated quadrats designed to exclude *S. invicta* from *S. minima* that had been infested with *C. salviniae* and monitored them for two years. The quadrats with moats had significantly fewer *S. invicta* and were successful in excluding most individuals from accessing the *C. salviniae* released. The moated quadrats also had significantly higher populations of *C. salviniae* at the end of the study, and a trend towards lower biomass. In conjunction with the suspicion that *S. invicta* is also preying on *Samea*

multiplicalis (Guneé), an additional native herbivore, we recommend controlling for populations of *S. invicta* where possible to increase the success of a biological control program with *C. salviniae* on *S. minima*.

Chapter 4 provides a brief overview of methods commonly used to sample insects associated with pleuston vegetation like *S. minima*. We wanted to be able to collect insects associated with the surface of the floating mat that may interact with *C. salviniae* and decided to attempt to find a design that worked in our habitat. We constructed several designs of floating surface traps found in the literature, and when none of them held up through testing we designed and constructed our own design.

In Chapter 5 we were interested in the biodiversity of the insects that we collected with the trap design from Chapter 4. We collected a total of 5,773 individuals between May 18 and November 16, 2009 that represent 176 species within 62 families and 7 orders. The order Coleoptera was both the most species rich and the most abundant and included three currently undescribed species. We also identified a previously unknown Braconid (Hymenoptera) wasp which is in the process of being described in conjunction with researchers at the University of Kentucky. Seven species of semi-aquatic Curculionidae (five of which have been used as biological control agents for aquatic plants) were also collected. We observed a single peak in both species richness and abundance during the month of July, and high diversity and evenness across the year. When we tried to evaluate β- diversity between our study and other studies of insects associated with floating mats of *Salvinia* sp., we had a much higher number of taxa collected. Since many of the taxa we collected have lifestyles that are nocturnal or crepuscular, we concluded that the large difference was due to differences in collection methodologies and strategies. To examine whether the taxa we collected were incidentally using *S. minima* as an extension of a terrestrial area, we looked at published habitat associations for the genera and species

that information. Most of the species we collected are indeed previously known as hydro- or hygrophilous suggesting that they are not present accidentally.

Chapter 6 examined whether the small scale spatial heterogeneity of plants within the flotant of *S. minima* affects the community structure of insects that are associated with it. Ward's cluster analysis broke the 100 traps into five groups based on secondary vegetation other than *S. minima* present with a gradient of structural complexity. Community similarity between clusters ranged from 0.40 to 0.55, and 40 species were found in all five vegetation clusters. We used estimates of *Mao Tau* to construct species accumulation curves with non- linear regression, to compare projected richness between clusters. Multivariate analysis revealed no significant differences in diversity, evenness, abundance, or richness between clusters regardless in the differences in structural complexity of vegetation present. Spearman's Rank Order Correlation was used to look for non-linear relationships between clusters and various calculated community indices. The spatial heterogeneity within the mat of *S. minima* did not affect the diversity or abundance of the insects associated with the mat but rarefaction predicted differences in species richness between clusters of vegetation. None of our analyses linked the more spatially complex vegetation clusters to higher levels of richness, abundance, or diversity. The most species rich cluster was one of the least spatially diverse, suggesting some other factor is driving differences among clusters.

In conclusion we successfully established *C. salviniae* on *S. minima* in southern Louisiana and monitored its impacts on both the mat and water quality measurements. We identified *S. invicta* as a negative factor in the biological control of *S. minima*, and recommended that *S. invicta* be controlled where possible to increase the chances of *C. salviniae*'s success. We designed an improved floating pitfall trap for collecting insects associated with floating vegetation and used it to collect a wide variety of adult insects. Community data was used to test the spatial heterogeneity hypothesis within our

experimental system and reject it as the driving force behind differences in community structure

between different groups of secondary vegetation. The findings here will be applicable to the biological

control programs of a variety of other floating aquatic weeds in addition to *S. minima*.

VITA

Katherine Ann Parys was born to Kenneth and Elizabeth Parys in Warrenton, Virginia, in August 1980. Katherine completed a Bachelor of Arts in biology with minors in studio art and entomology at the University of Rhode Island in 2002. She earned a Master of Science in Environmental Biology with Dr. Steven C. Harris at Clarion University of Pennsylvania in December of 2004. She moved to Boston in 2005 to complete an internship at the Butterfly Garden and Live Animal Center at the Boston Museum of Science. In the fall of 2005, she moved to Louisiana and began doctoral studies with Dr. Seth Johnson in January 2006.