THE IMPACT OF INVERTEBRATES TO FOUR AQUATIC MACROPHYTES:

Potamogeton nodosus, P. illinoensis, Vallisneria americana

AND *Nymphaea mexicana*

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This research investigated the impact of invertebrates to four species of native aquatic macrophytes: *V. americana, P. nodosus, P. illinoensis*, and *N. mexicana*. Two treatments were utilized on each plant species, an insecticide treatment to remove most invertebrates and a non-treated control. Ten herbivore taxa were collected during the duration of the study including; *Synclita*, *Paraponyx*, *Donacia*, *Rhopalosiphum*, and *Hydrellia*. Macrophyte biomass differences between treatments were not measured for *V. americana* or *N. mexicana*. The biomasses of *P. nodosus* and *P. illinoensis* in non-treated areas were reduced by 40% and 63% respectively. This indicated that herbivory, once thought to be insignificant to aquatic macrophytes, can cause substantial reductions in biomass.

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CHAPTER 1

INTRODUCTION

There is little information available that quantifies the impact of invertebrate herbivores on native macrophyte biomass in North America. Early research indicated that while macrophytes were useful as a substrate for invertebrates and epiphytic growth they provided little if any nutritive value (Shelford, 1918). However, additional studies have shown importance of macrophytes as a nutritive source for invertebrates. Among those, Soszka (1975) reported *Potamogeton* species can loose 50 to 90% of their leaf area by insect herbivory and non-consumptive destruction mostly from lepidopterans, trichopterans, and dipterans. Leaf area damage as high as 56%, depending on plant species and locality, was documented by Sand- Jensen and Madsen (1989) and attributed to herbivory mostly by trichopterans and dipterans. Newman (1991) later identified five insect orders, Trichoptera, Diptera, Lepidoptera, Coleoptera, and Homoptera, as containing most known herbivores to aquatic macrophytes. Live macrophytes were also found to be engaged in aquatic food webs, sometimes to the extent that macrophyte biomass, productivity, and relative species abundance is dramatically changed by grazers (Lodge, 1991). Finally, Cronin et al. (1998) determined that freshwater macrophyte herbivory is similar to that reported for terrestrial plants. This viewpoint differed widely from the idea that macrophytes offered surface substrates only (Shelford, 1918). Yet, herbivory is not the only source of interaction between invertebrates and aquatic plants.

Non-consumptive activities such as ovipositing and case making can also damage aquatic plants. Two anisopteran families, Aeschnidae and Petaluridae, as well

as most zygopterans, are known to oviposit in aquatic plant tissue, which can leave holes in plants once the larva emerge. This endophytic trait can result in excessive damage to plant tissue by large numbers of females (Westfall and Tennessen, 1996). The larvae of *Synclita obliteralis* (Walker), a lepidopteran that feeds on at least 60 different aquatic plants, construct a portable case of plant tissue (Center et al., 1999). As larvae grow they continually discard and build new cases. As with odonates mentioned above, the more numerous the larvae, the more detrimental this case making behavior can be. Yet unlike the odonates, *S. obliteralis* also feeds on plants, causing further damage.

The paucity of published accounts of invertebrate herbivory or non-consumptive damage to native macrophytes indicates more research is warranted. Previous studies quantified parameters such as percent herbivore damage to macrophytes, but without a comparison to ungrazed plants, the significance of this interaction is unknown. Exclusion studies are classified by Boavida et al. (1995) as the most efficient and straightforward method of evaluating the impact between relationships such as invertebrates and native macrophytes. A comparison between two different populations of macrophytes, one with invertebrates and one without, would add valuable information regarding the impact invertebrates have on aquatic plants by providing details to differences in plant quality and biomass when invertebrates are active in a system. This impact to native macrophytes is important for many reasons.

First, native plants are a valuable component of aquatic habitats. They provide important fish and wildlife habitat (Savino and Stein, 1982; Heitmeyer and Vohs, 1984; Dibble et al., 1996), improve water clarity and quality, and reduce rates of shoreline

erosion and sediment resuspension (Smart, 1995). Understanding the importance of native aquatic plants has prompted their use in an increasing number of re-vegetation projects. Yet, to better prepare plants to survive transplantation more information is needed that describes the impact of biological or environmental factors on their establishment and growth. One such challenge encountered when establishing native vegetation is herbivory. Turtles, crayfish, insect larva, muskrats, nutria, and beaver have been shown to pose a threat to establishment and growth of aquatic plant communities (Lodge, 1991; Dick et al., 1995; Doyle and Smart, 1995; Doyle et al., 1997). During re-vegetation, macrophytes are often planted within cages to reduce herbivory and biotic disturbance (Smart et al., 1998). Cages are constructed of various sizes and materials and are able to protect macrophytes from crayfish as well as larger herbivores. Yet invertebrates are not excluded using the current design and therefore substantial damage by invertebrate herbivores is commonly seen within cages (Dick, G.O., U.S. Army Engineer Research and Development Center, personal communication). Knowledge of the relationship between invertebrate herbivores and native macrophytes can aid in re-vegetation decisions such as appropriate plant species and locality of plantings.

Native plants have been shown to compete effectively against many invasive macrophytes thereby providing sustainable management of aquatic ecosystems. This provides a further reason to explore complex interactions between invertebrates and native macrophytes. Plants occurring in their native range generally grow below an economic threshold (level at which a pest starts to have an economic effect, i.e. incurs management costs) due to a series of natural enemies and several abiotic and biotic

factors, which limit their spread (Harley and Forno, 1992). These factors can include weather, climate, shelter availability, geographic barriers, and intraspecific and interspecific competition (U.S. Army Engineer Research and Development Center, 2005). Outside their native range, plants do not have these limiting factors and therefore have the capability of becoming invasive (Harley and Forno, 1992). Man-made reservoirs are particularly vulnerable to infestations by weedy species because they typically lack aquatic vegetation (Smart et al., 1998). Populating reservoirs with macrophytes can benefit the general health of the aquatic system and help prevent spread of nuisance exotic species (Smart, 1995). Some native plants commonly used in the southeastern U.S. for restoration efforts in preventing invasive species include *Vallisneria americana* Michx., *Potamogeton nodosus* Poir., *P. illinoensis* Morong, *Heteranthera dubia* (Jacq.) MacMill., *and Nymphaea odorata* Aiton. Native macrophytes such as *V. americana* have been noted as effective competitors with invasive plants under certain conditions (Smart et al., 1994). By establishing a diverse and hence stable community of native species, recurrence of aquatic plant problems might be slowed or even prevented.

Finally, it has also been shown that native plants can become problematic within their native range under certain conditions and even more importantly can become serious problems in other regions. Although native to North America, several species of *Nuphar*, *Nymphaea*, and *Potamogeton* are regarded as weeds in Holarctic countries (Sculthorpe, 1967). *Cabomba caroliniana* A. Gray, another native species, is becoming a problem in Australia where it forms monospecific stands that can cover an entire lake and is listed as one of Australia's 20 Weeds of National Significance (Schooler et al.,

2006). Wetlands in the United Kingdom, the Netherlands, and Australia are also being threatened by, *Hydrocotyle ranunculoides* L. f. This rooted aquatic plant can cause a wide range of environmental problems including; 1) dissolved oxygen reduction, 2) flooding due to clogged drainage systems, and 3) biodiversity reduction through competition (EPPO, 2006). By understanding the impact North American herbivores have on these macrophyte species new biological agents may be developed for use in regions facing these problems thereby providing another long term control strategy.

CHAPTER 2

OBJECTIVES

This research investigated the impact of invertebrates to four different species of native aquatic macrophytes: *V. americana, P. nodosus, P. illinoensis*, and *N. mexicana* Zucc. Two treatments were utilized on each plant species, an insecticide treatment to remove most invertebrates and a non-treated control. Invertebrates were collected, identified, and quantified to assess efficacy of the insecticide and to determine community structure. Invertebrate effects on aquatic plants were quantified by comparison of 1) plant dry biomass and 2) percent invertebrate leaf damage per plant species between treated and non-treated specimens. Hypotheses for the study are as follows:

H_0: There are no differences in number of invertebrates collected from the two treatments.

H_a: There are differences in number of invertebrates collected from the two treatments.

H_0: There are no differences in invertebrate communities due to; 1) pond, 2) plant species, or 3) treatment.

H_a: There are differences in invertebrate communities due to; 1) pond, 2) plant species, or 3) treatment.

H_0: There are no differences in percent invertebrate leaf damage from the two treatments.

H_a: There are differences in percent invertebrate leaf damage from the two treatments.

H_0: There are no differences in dry plant biomass from the two treatments at harvest III.

H_a: There are differences in dry plant biomass from the two treatments at harvest III.

CHAPTER 3

MATERIALS AND METHODS

Study Site and Design

The Lewisville Aquatic Ecosystem Research Facility (LAERF) is located in Lewisville, Texas, Denton County. The site includes 53 earthen ponds ranging from 0.2-0.81 ha in size and averaging 1 m in depth. Ponds were constructed in the 1950's with clay liners overlaid by sandy-loam topsoil, and were used as game fish production ponds until 1985. Native macrophytes and macroinvertebrates inhabit all ponds and water to ponds is gravity-fed from Lake Lewisville (Smart et al., 1995).

This study was conducted in three 0.3 ha ponds (ponds 50, 51, and 52) at the LAERF, measuring approximately 40 m by 60 m. Preparation of the study ponds included draining, mowing, rototilling, and installing a barrier to separate each pond lengthwise into two congruent sides. The barrier consisted of a fence covered by pond liner, creating two treatment areas per pond, an insecticide treatment and a non-treated control (Figure 1). The fence was placed in the center of each pond, lengthwise from the pond's kettle to the opposite bank. The kettle represents a concrete area where water supply and drain pipes are located in each pond. The fence was constructed of 1.5 m t-posts covered by galvanized pipe and set at 2.4 m increments along the center line of the pond. The height of each galvanized pipe was adjusted to fit the pond's contour. Each pipe was fitted with a cap, and a top rail was installed through the caps, perpendicular to the galvanized pipes (Figure 2). Mesh welded-wire fencing, 5 cm by 10 cm, was attached to the top rail parallel to the galvanized pipes. The fencing provided support for 45 Mil EPDM Firestone pond liner (AZPonds and Supplies, Inc.

Birdsboro, PA) which was hung from the top rail (Figure 3). The liner was fitted with 1 cm grommets every 61 cm and attached to the top rail by cable ties. The liner was measured to fit the height at each pipe, with 1 extra meter of liner left at the bottom to be buried in pond sediment. Water was supplied evenly to both sides of the pond. Upon completion of the fence, the three ponds were planted.

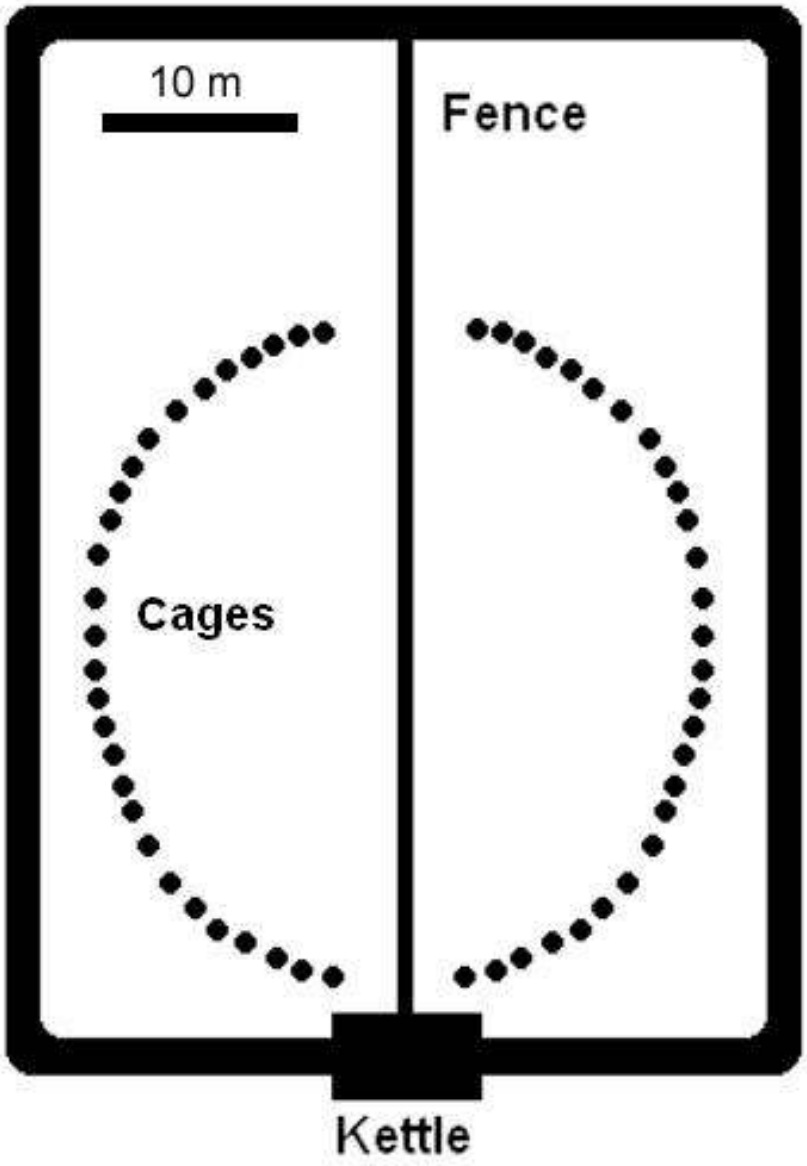
On May 27, 2005 each pond was planted with four native macrophytes; *V. americana, P. nodosus, P. illinoensis,* and *N. mexicana*. *Nymphaea odorata* was originally planned for this study because of its common use in re-vegetation projects in this region of Texas. Yet, *N. odorata* was unavailable in the quantity needed. Due to its similarities with *N. odorata*, *N. mexicana* was planted instead. Seven replicates of each species were planted in each treatment area. Each replicate was enclosed in a 91 cm diameter by 1.2 m tall cylinder (cage) and constructed from 5 cm by 10 cm mesh welded-wire fencing anchored with 1.2 m lengths of rebar. Cages were used for easy visibility of location of replicates and to protect plants from disturbances such as turtles or ducks. The cages were evenly spaced apart and positioned at equal depths by following the contour of each pond (Figure 4). Size and amount of pots determined to be suitable for a cage varied for each species due to plant size and growth rate. Each cage was planted with one of the following: three – 1 L pots of *P. nodosus* or *P. illinoensis*, one 6.7 L pot of *V. americana*, or one – 1 L pot of *N. mexicana*. Plants were removed from pots and planted directly into sediment. Placement of each plant species within each treatment area was randomly selected. Five triploid grass carp were also added per treatment area to help control vegetative growth outside of cages. Ponds were maintained at a depth of approximately 1 m.

Figure 1. View of an entire pond with separation fence spanning lengthwise from one bank to the other. Cages are visible in pond prior to planting.

Figure 2. The separation fence was constructed of 1.5 meter t-posts covered by galvanized pipe and set at 2.4 meter increments along the center line of each pond. Each pipe was fitted with a cap and top rail was installed through the caps perpendicular to the galvanized pipes.

Figure 3. Pond liner was attached to the top rail of the separation fence to form a barrier between the two treatment areas of each pond. Mesh welded wire-fencing was also hung from the top rail to provide fence stability and support for the pond liner.

Figure 4. Diagram of separation fence, kettle, and cages. The fence was placed in the center of each pond, lengthwise from the pond's kettle (location of water supply and drain pipes) to the opposite bank. Ponds measured approximately 40m by 60m. All plant replicates were enclosed in cages. Cages were evenly spaced apart along the pond's contour to equalize depth. Placement of each plant species was randomly selected.



An insecticide, Abate® 4-E (Abate) (Clarke Mosquito Control Products, Inc. Roselle, IL), was applied as an emulsifiable concentrate to one half of each pond weekly at 0.047 lbs a.i./ acre (1.5 fl. oz / acre) to remove most invertebrates. This treatment amount was chosen because it is the label rate suggested for applications in deep water or areas with dense surface cover such as macrophytes. Abate is a non-systemic organophosphate with 44.6% active ingredient temephos (O,O'-(thiodi-4, 1-phenylene) O,O,O',O',-tetramethyl phosphorothioate) and 55.4% inert ingredients including petroleum distillates. Abate acts as a cholinesterase inhibitor and is used for control of midge and mosquito larva. Half-life of abate photolysis in water is 15 days. The Abate application system was constructed of 1.3 cm diameter irrigation hose attached to the top of each cage within each Abate treatment area. One 2 gph drip emitter was attached to irrigation hose in the center of each cage so that Abate was directly applied to plants and water within the cage (Figure 5). Hose continued through each cage with one end capped shut and the other end left open (Figure 6). Abate was applied by attaching the open end of irrigation hose to a gas powered sprayer (FIMCO, No. Sioux City, SD) which forced Abate into the hose and out through the drip emitters.

Rhodamine WT Dye Test

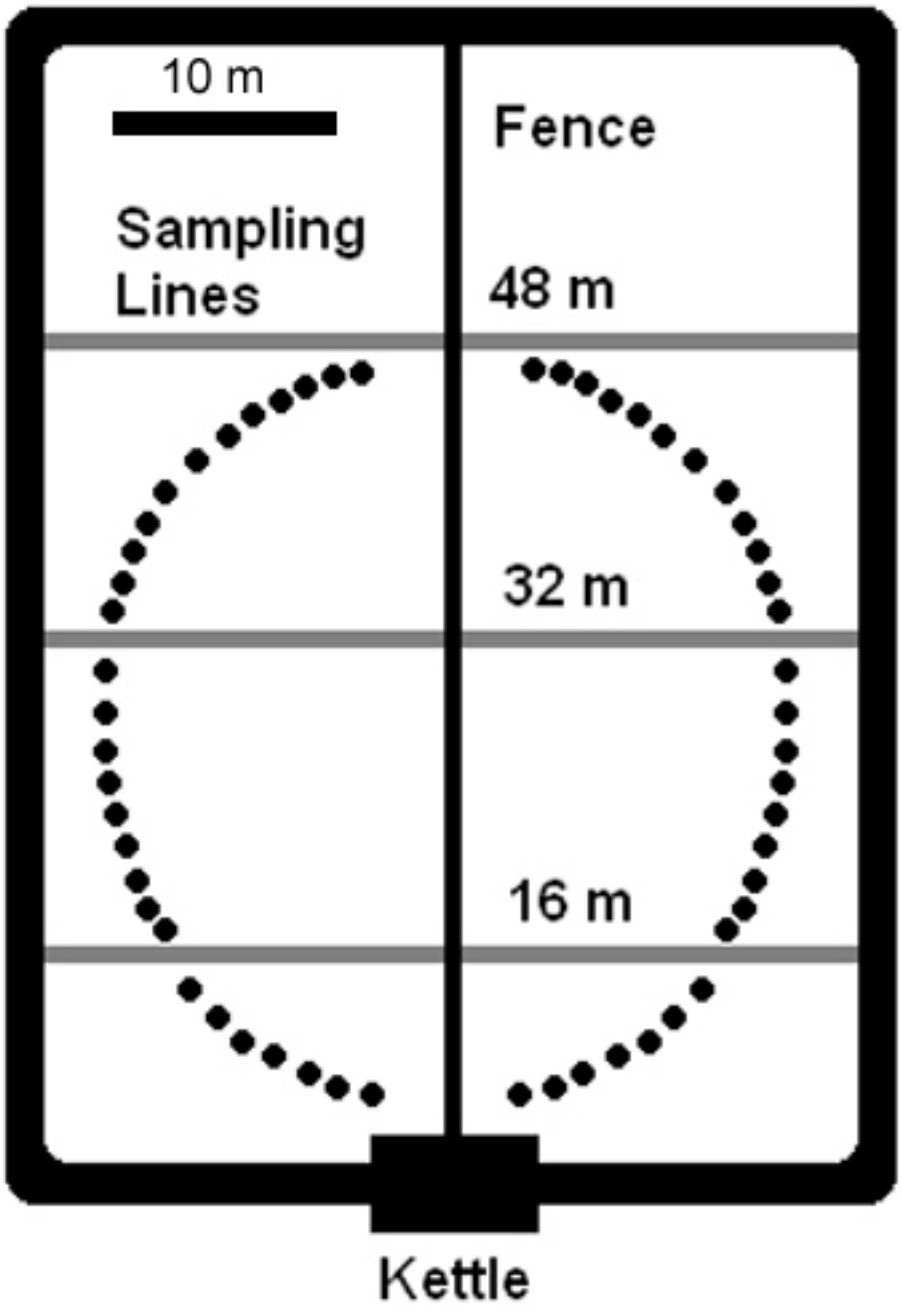
To evaluate efficacy of the separation fence to prevent mixing of water between treatment areas, rhodamine WT dye (rhodamine) was applied at 1.34 µg/L to each treated area prior to Abate application using the Abate application system. Water samples were taken across the width of each pond at transect lines at 16, 32, and 48 m along the length of the pond (Figure 7). Three water samples were taken on each side of the center separation fence at each transect line at a depth of 41 cm at 1, 5, 24, 48,

and 96 hours after treatment (HAT). Locations of transect lines were chosen in order to collect samples throughout the entire pond. One reference water sample was also taken from each pond area before application. All samples were stored in dark bottles for approximately 1 week until fluorometer (Turner Designs, Inc. Sunnyvale, CA) analysis for rhodamine concentration.

Figure 5. The abate application system was constructed of 1.3 centimeter diameter irrigation hose attached to the top of each cage. A drip emitter (2gph) was attached to the irrigation hose in the center of each cage so that Abate was directly applied to the water and plants within the cage.

Figure 6. One line of irrigation hose was inserted through all cages of a treatment area. One end of the hose was capped shut while the other remained open for attachment to a gas powered sprayer.

Figure 7. A diagram of rhodamine WT sampling sites. Water samples were taken across the width of each pond at transect lines at 16, 32, and 48 meters along the length of the pond. Three water samples were taken on each side of the separation fence at each transect line. Cages are represented by black circles.



Data Collection and Sample Processing

One replicate of each plant species per treatment area was randomly selected and harvested for invertebrates, plant biomass, and percent invertebrate damage at the first and second month after planting, June 20 (harvest I) and July 19 (harvest II) respectively. At the fourth month after planting, September 16 (harvest III), 5 replicates of each plant species per treatment area were randomly selected and harvested. As in previous harvests; invertebrates, plant biomass, and percent invertebrate damage were collected from 1 replicate while only plant biomass was collected from the remaining 4 replicates. Plant biomass was collected in low replication at harvest I & II to show general trends of plant growth. To evaluate end of growing season differences in plant biomass due to invertebrate / plant interactions, additional biomass replicates were collected at harvest III. The same methods were followed for each harvest.

Plants within each cage were severed at the top of sediment and immediately put into a plastic bag. Upon return to the lab all plant matter was rinsed with water to remove sediment or algae. Replicates harvested for invertebrates were rinsed with water over a bucket to dislodge any invertebrates. Buckets were emptied into 710 micron sieves and all invertebrates collected were preserved in 70% ethanol. After rinsing was complete, plants were physically examined and any remaining invertebrates were removed and preserved. Invertebrates were identified as follows: Annelids to class, Gastropoda & Insecta to genus (except for family Chironomidae to subfamily). Plant tissue was separated into species and if replicates were harvested for percent invertebrate leaf damage, three leaves were chosen at random and photographed. Plants were placed in brown paper bags, and dried in an oven at 55˚C for 48 hours to

obtain a dry weight. Upon removal from the oven, plant matter was weighed. A portion of plants were placed back into the oven for an additional 48 hours to verify that a constant weight had been obtained. Leaf photographs were analyzed using Image-Pro® Express 5.1 (Media Cybernetics, Inc. Bethesda, MD). Total area of each leaf as well as the total area of all invertebrate damage (consumptive as well as non-consumptive) was calculated to determine mean percentage of invertebrate damage to each plant replicate.

Statistical Analyses

Experimental data were analyzed using STATISTICA version 8.0 (StatSoft, Inc., 2007, Tulsa, OK) and include analysis of variance (ANOVA), Newman-Keuls (NK), Hartley F-max, Shapiro-Wilk's W test, power analysis, and correspondence analysis (CA). Statements of significance made throughout the text refer to alpha level 0.05.

A two-way ANOVA was performed to differentiate changes in total number of invertebrates due to: 1) harvest and 2) treatment. Nine invertebrate groups were analyzed separately including; Coleoptera, Diptera, Trichoptera, Hemiptera, Lepidoptera, Odonata, Ephemeroptera, Oligochaeta, and Gastropoda. This level of identification was considered taxonomically sufficient for this analysis due to low samples sizes when data were separated into family or genus. NK multiple range test differentiated statistically distinct means. Assumptions of normality and homogeneity of variances were evaluated with Shapiro-Wilk's W test and Hartley F-max respectively. All invertebrate groups failed to meet these assumptions. A two-way ANOVA was performed on ranked data as an acceptable non-parametric method for two factor and higher ANOVAs. Zar (1999) discusses comparing observed probabilities and statistical

decisions of parametric and non-parametric results. As in this case, if results are similar either test can be reported. For these analyses all factors deemed statistically significant were the same for both tests. Parametric two-way ANOVA results will be reported.

Invertebrate effects on aquatic plants were quantified by comparison of percent invertebrate leaf damage and plant dry biomass between treated and non-treated samples. A two-way ANOVA was performed for each plant species to differentiate changes in percent invertebrate leaf damage due to: 1) harvest and 2) treatment. Differences in plant biomass between treatments at Harvest III were analyzed with a one-way ANOVA for each plant species. Assumptions of normality and homogeneity of variances were met for both analyses.

Power analysis was evaluated for each factor of each ANOVA which did not gain statistical significance. Sample size required for statistically significant results was determined with standard deviation and means from original statistics at a power of 0.95.

Differences in invertebrate community structure based on plant species, pond, and treatment were analyzed by CA for harvests I-III. For this analysis invertebrate data was included at the lowest identified level: Annelids to class, Gastropoda & Insecta to genus (except for family Chironomidae to subfamily). CA results are presented as ordination biplots for each harvest of plant species per pond and treatment area.

CHAPTER 4

RESULTS AND DISCUSSION

Rhodamine WT Dye Test

Rhodamine concentrations were more variable in treated areas from 1 to 5 HAT than at subsequent samples times (Table 1). For instance, in pond 50 concentrations ranged from 0.20 µg/L to 4.40 µg/L (1 HAT) and 1.70 µg/L to 4.50 µg/L (5 HAT). This variability resulted from incomplete dye dispersal and possible 'hot spots' where dye was trapped by excess vegetation. The rhodamine dye in each treated area was uniformly distributed by 24 HAT (Table 1); therefore a 96 hour sampling period should have been sufficient to detect rhodamine in non-treated areas. Analysis of pre-treatment water from each pond area yielded 0.20 µg/L of background fluorescence. Therefore, the detectable level of rhodamine was greater than 0.20 µg/L. Concentrations in non-treated areas remained at 0.20 µg/L in pond 51 for all sampling sites and times. In ponds 50 and 52 concentrations of 0.30 µg/L and 0.40 µg/L respectively were recorded (Table 1). This could represent contamination of rhodamine (i.e. Abate) in the non-treated areas of ponds 50 and 52, but at low levels. Each separation fence was determined to be an effective barrier, with transfer of insecticide minimal at most.

Table 1. Rhodamine concentrations (µg/L) at transect lines at 16, 32 and 48 meters along the length of each pond at 1, 5, 24, 48, and 96 hours after treatment (HAT). Rhodamine was applied at the rate of 1.34 µg/L. Analysis of pre-treatment water from each pond area yielded 0.20 µg/L.

Pond	Treatment	Transect (m)	Rep	1 HAT µg/L	5 HAT µg/L	24 HAT µg/L	48 HAT µg/L	96 HAT µg/L
50	Control	16	1	0.20	0.30	0.20	0.20	0.20
50	Control	16	2	0.20	0.30	0.20	0.20	0.20
50	Control	16	3	0.20	0.20	0.20	0.20	0.20
50	Control	32	1	0.20	0.30	0.20	0.20	0.20
50	Control	32	2	0.20	0.20	0.20	0.20	0.20
50	Control	32	3	0.20	0.30	0.20	0.20	0.20
50	Control	48	1	0.20	0.30	0.20	0.20	0.20
50	Control	48	2	0.20	0.30	0.20	0.20	0.20
50	Control	48	3	0.20	0.30	0.30	0.20	0.20
50	Rhodamine	16	1	2.00	4.50	1.10	1.00	1.00
50	Rhodamine	16	2	0.60	2.20	1.10	1.00	1.00
50	Rhodamine	16	3	1.60	2.60	1.10	1.00	1.00
50	Rhodamine	32	1	4.40	2.80	1.20	1.00	1.00
50	Rhodamine	32	2	1.70	2.90	1.20	1.00	1.00
50	Rhodamine	32	3	1.70	2.70	1.20	1.00	1.00
50	Rhodamine	48	1	0.80	2.20	1.20	1.00	1.00
50	Rhodamine	48	2	0.20	2.20	1.20	1.00	1.00
50	Rhodamine	48	3	0.60	1.70	1.20	1.00	1.00
51	Control	16	1	0.20	0.20	0.20	0.20	0.20
51	Control	16	2	0.20	0.20	0.20	0.20	0.20
51	Control	16	3	0.20	0.20	0.20	0.20	0.20
51	Control	32	1	0.20	0.20	0.20	0.20	0.20
51	Control	32	2	0.20	0.20	0.20	0.20	0.20
51	Control	32	3	0.20	0.20	0.20	0.20	0.20
51	Control	48	1	0.20	0.20	0.20	0.20	0.20
51	Control	48	2	0.20	0.20	0.20	0.20	0.20
51	Control	48	3	0.20	0.20	0.20	0.20	0.20
51	Rhodamine	16	1	1.60	1.40	0.70	0.60	0.60
51	Rhodamine	16	2	1.60	1.40	0.70	0.60	0.60
51	Rhodamine	16	3	1.40	1.40	0.80	0.60	0.60
51	Rhodamine	32	1	2.00	1.40	0.70	0.60	0.60
51	Rhodamine	32	2	1.90	1.30	0.70	0.60	0.60
51	Rhodamine	32	3	1.90	1.40	0.70	0.60	0.60
51	Rhodamine	48	1	0.80	0.80	0.70	0.60	0.60
51	Rhodamine	48	2	0.70	0.80	0.80	0.60	0.60

51	Rhodamine	48	3	0.20	1.00	0.80	0.60	0.60
52	Control	16	1	0.30	0.20	0.30	0.20	0.30
52	Control	16	2	0.20	0.30	0.30	0.20	0.30
52	Control	16	3	0.30	0.30	0.30	0.20	0.30
52	Control	32	1	0.30	0.30	0.40	0.20	0.30
52	Control	32	2	0.30	0.30	0.40	0.20	0.30
52	Control	32	3	0.40	0.30	0.40	0.20	0.30
52	Control	48	1	0.40	0.40	0.40	0.20	0.30
52	Control	48	2	0.40	0.30	0.30	0.30	0.30
52	Control	48	3	0.30	0.30	0.30	0.30	0.30
52	Rhodamine	16	1	1.00	1.60	0.80	0.80	0.70
52	Rhodamine	16	2	3.50	1.20	0.80	0.80	0.70
52	Rhodamine	16	3	0.50	0.90	0.90	0.80	0.70
52	Rhodamine	32	1	2.50	2.00	0.80	0.70	0.70
52	Rhodamine	32	2	2.60	1.00	0.80	0.80	0.70
52	Rhodamine	32	3	3.00	2.00	0.90	0.80	0.70
52	Rhodamine	48	1	3.20	1.80	0.90	0.80	0.70
52	Rhodamine	48	2	3.00	1.80	0.90	0.80	0.70
52	Rhodamine	48	3	3.50	1.60	0.90	0.80	0.70

Invertebrate Collections

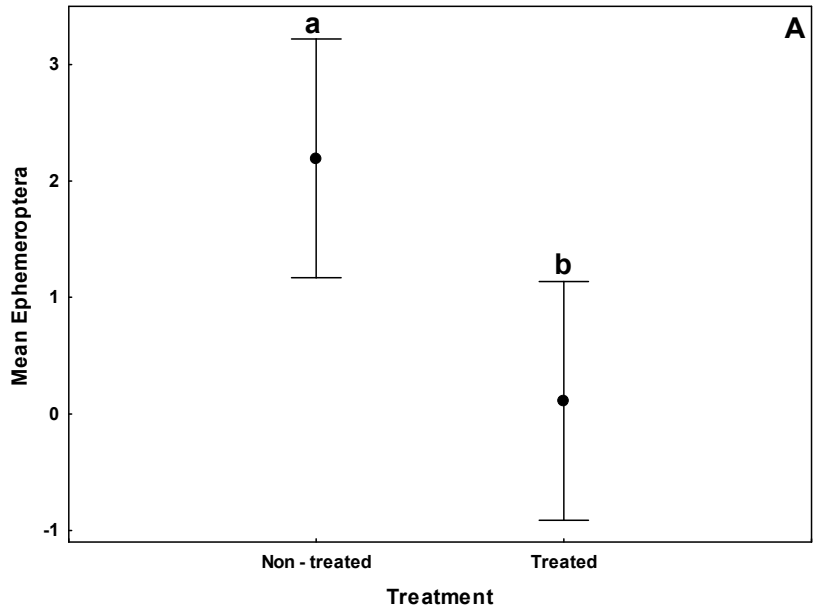
Harvest and Treatment Effects

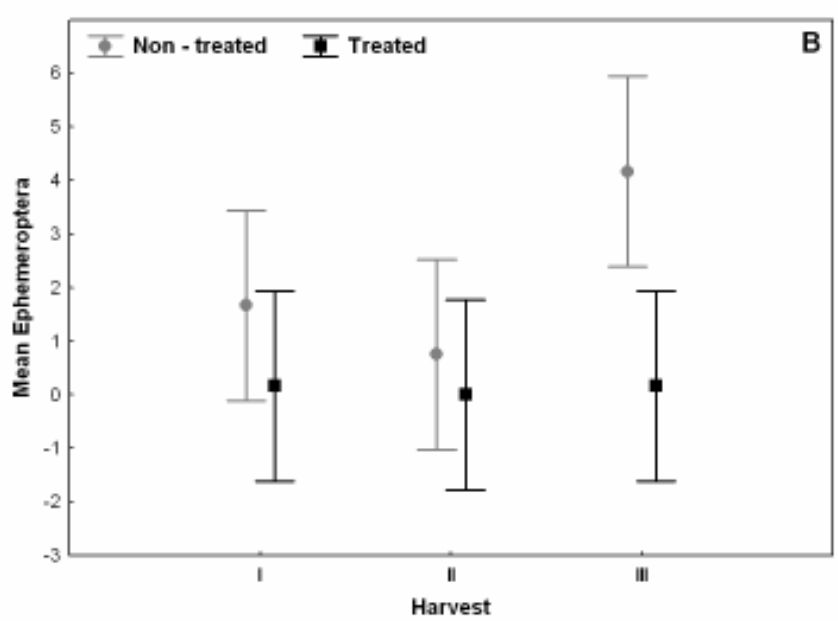
Ephemeropterans displayed statistically significant population reductions due to treatment only (Table 2 & Figure 8a). Power analysis determined that a sample size of 51 replicates would be required to achieve statistically significant results for harvest at a power of 0.95. Statistical significance was not attained for harvest differences, yet general trends were noted. Collections from treated areas averaged close to zero larvae per sample for the entire study. Mayfly collections from non-treated areas, while also low, peaked at a mean of about 4 larvae per sample at harvest III (Figure 8b).

Table 2. Results from two-way ANOVAs performed to identify changes in number of invertebrates due to harvest and treatment.

Invertebrate	Harvest		Treatment		Harvest * Treatment	
	F	p	F	p	F	p
Gastropoda	8.4356	0.0005	2.4117	0.1252	1.2381	0.2966
Coleoptera	5.1416	0.0084	44.5448	0.0000	8.5244	0.0005
Diptera	8.2512	0.0006	26.1916	0.0000	8.1864	0.0007
Oligochaeta	5.1801	0.0081	2.8124	0.0983	0.2760	0.7597
Trichoptera	3.0048	0.0564	11.8890	0.0010	3.0057	0.0563
Hemiptera	14.1597	0.0000	1.5998	0.2104	2.1962	0.1193
Hemiptera – Aphididae	6.2965	0.0031	10.6446	0.0018	6.4263	0.0028
Lepidoptera	7.9294	0.0008	33.9200	0.0000	6.2011	0.0034
Odonata	12.6545	0.0000	14.6409	0.0003	10.1269	0.0001
Ephemeroptera	2.1367	0.1261	8.2380	0.0055	1.8321	0.1681

Figure 8. Mean (± 0.95 confidence interval) Ephemeroptera larvae collected per treatment (A) and per treatment area at each harvest (B). Means with the same letter are not significantly different (NK multiple range test, α = 0.05). Two-way ANOVA, harvest: p = 0.126, F = 2.137, DF = 2, 66; treatment: p = 0.006, F = 8.238, DF = 1, 66; interaction: p = 0.168, F = 1.832, DF = 2, 66).





Three invertebrate groups, Gastropoda, Oligochaeta, and Hemiptera, had statistically significant differences at harvest only (Table 2). These invertebrate groups were not affected by the insecticide treatment. To achieve statistical significance differences between treatments at a power of 0.95, Gastropoda, Oligochaeta, and Hemiptera would require 80, 63, and 139 samples respectively. Gastropods significantly increased over 4 fold from harvest I - II, but did not significantly change at harvest III (Figure 9). Low numbers of oligochaetes were collected throughout the study, with means less than 3 individuals per sample (Figure 10). Hemipteras collected at harvest III were significantly greater than both harvest I & II (Figure 11). A closer look at the Hemiptera data reveals that while aphids (*Rhopalosiphum* sp.) were not collected at harvest I, aphids dominated Hemiptera collections by harvest II & III. Hemiptera samples consisted of 96 and 97 percent aphids at harvest II & III respectively. *Rhopalosiphum* aphids are known to overwinter as eggs on fruit trees and then as adults, migrate to aquatic vegetation (floating and emergent) in mid to late summer which would coincide with later harvests (Center et al., 1999). Aphids may not have been exposed to Abate while resting and feeding on top of plants, not in contact with water. When Hemiptera data were analyzed without aphids the insecticide was found to be effective (Figure 12).

Figure 9. Mean (± 0.95 confidence interval) Gastropoda collected per harvest. Means with the same letter are not significantly different (NK multiple range test, α = 0.05). Two-way ANOVA, harvest: $p < 0.001$, $F = 8.436$, $DF = 2, 66$; treatment: $p = 0.125$, $F = 2.412$, $DF = 1, 66$; interaction: $p = 0.296$, $F = 1.238$, $DF = 2, 66$).

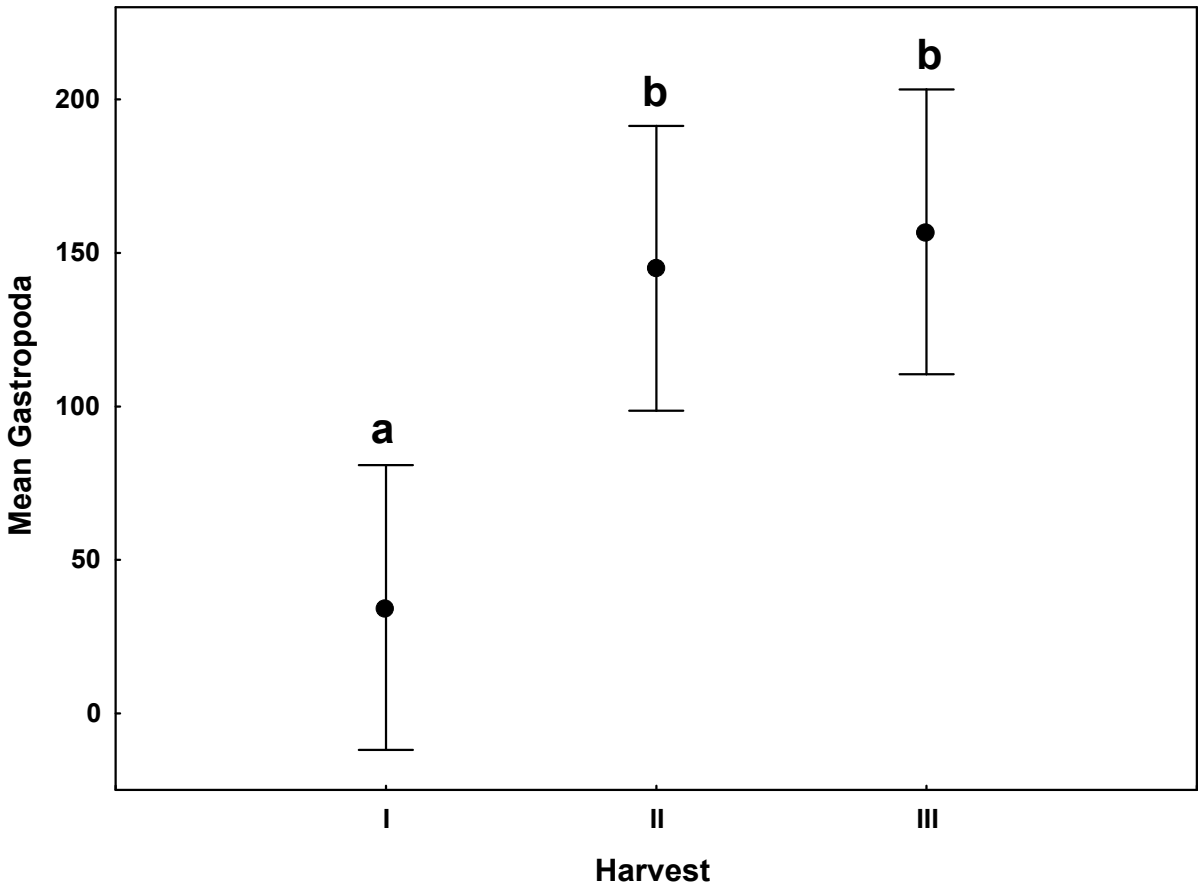


Figure 10. Mean (± 0.95 confidence interval) Oligochaeta collected per harvest. Means with the same letter are not significantly different (NK multiple range test, α = 0.05). Two-way ANOVA, harvest: p = 0.008, F = 5.180, DF = 2, 66; treatment: p = 0.098, F = 2.812, DF = 1, 66; interaction: p = 0.760, F = 0.276, DF = 2, 66).

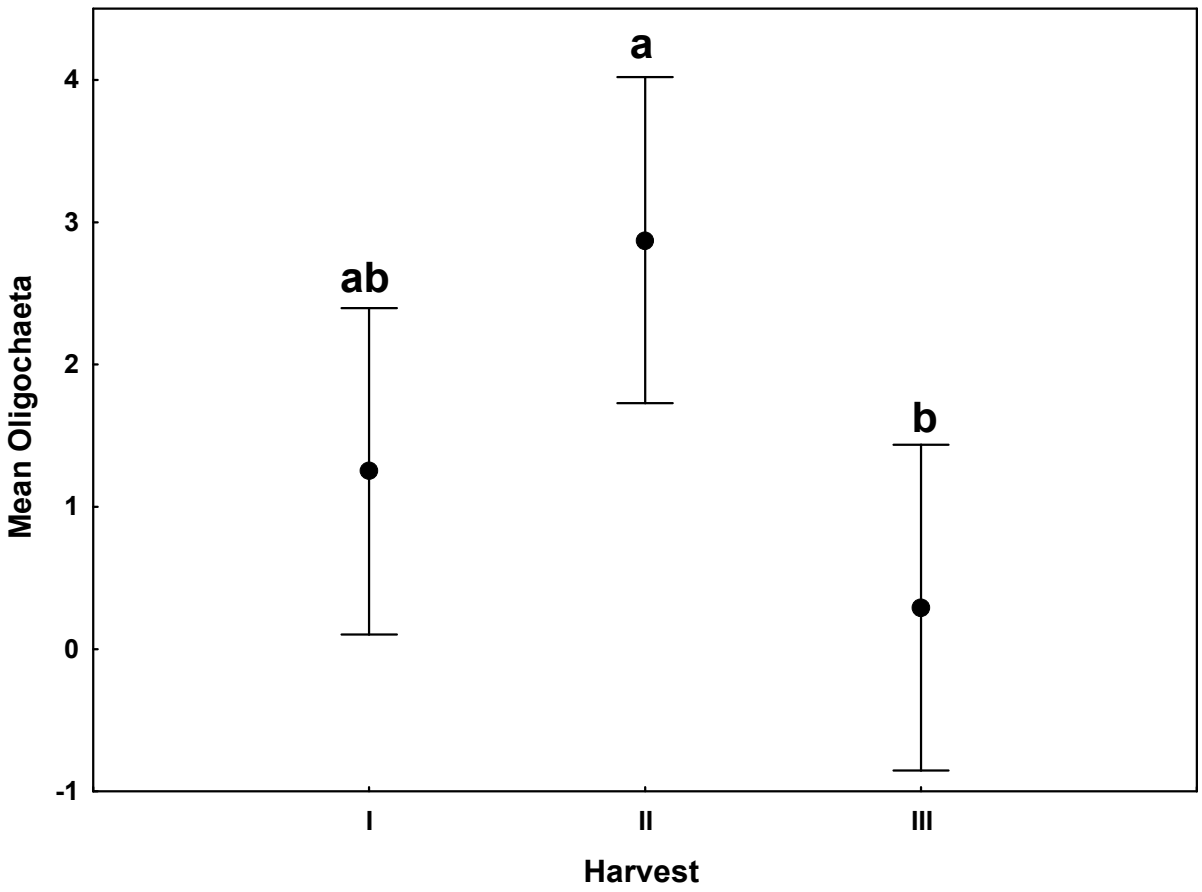


Figure 11. Mean (± 0.95 confidence interval) Hemiptera collected per harvest. Means with the same letter are not significantly different (NK multiple range test, α = 0.05). Two-way ANOVA, harvest: $p < 0.001$, $F = 14.160$, $DF = 2, 66$; treatment: $p = 0.210$, $F = 1.600$, $DF = 1, 66$; interaction: $p = 0.119$, $F = 2.196$, $DF = 2, 66$).

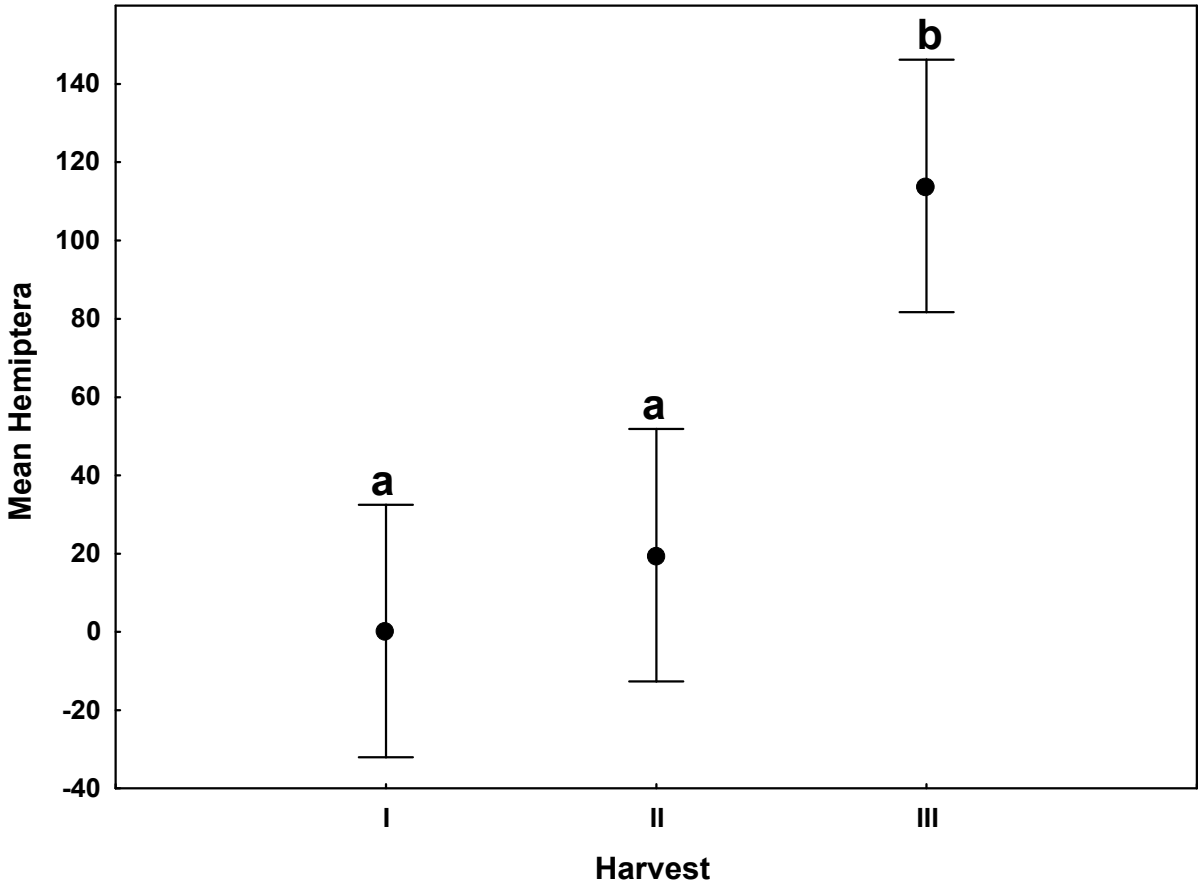
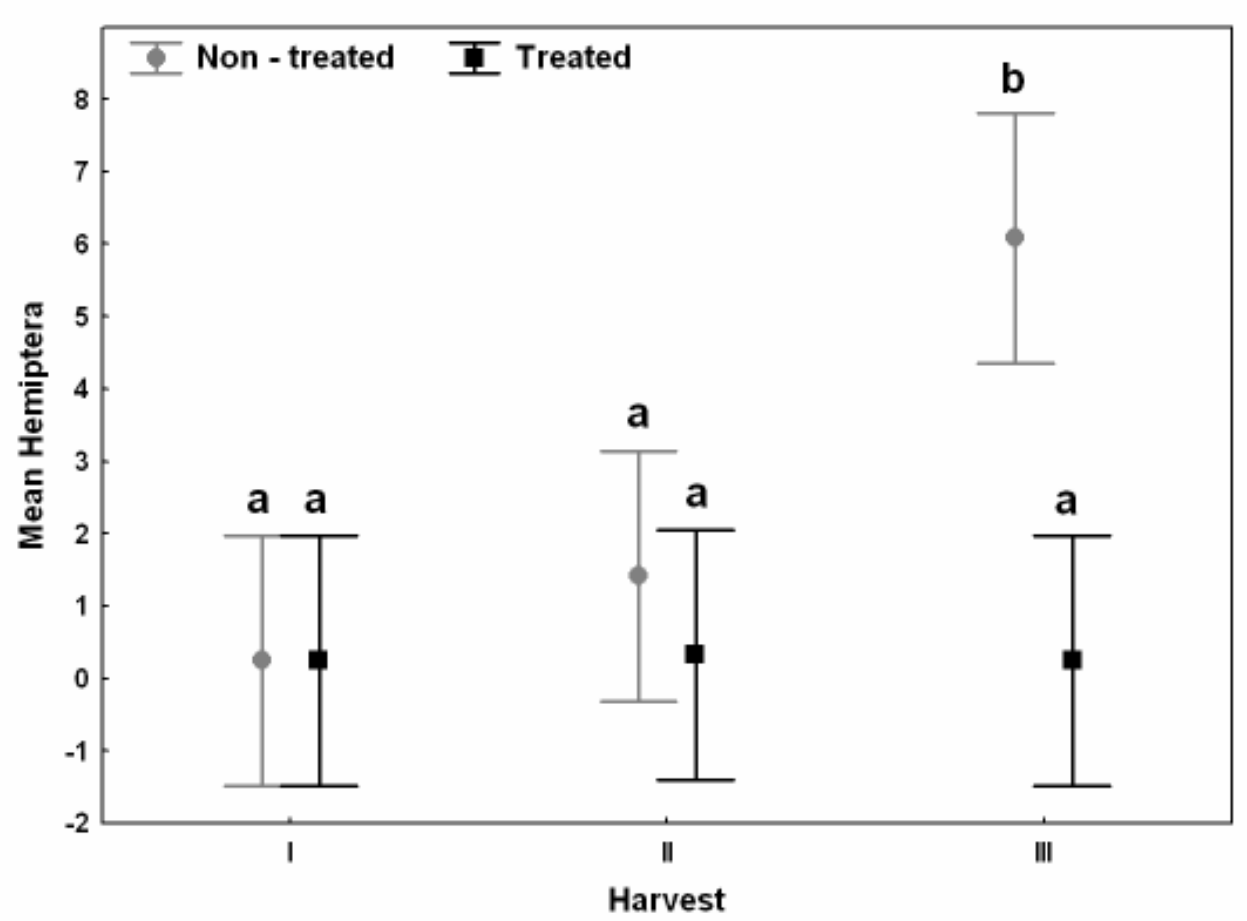


Figure 12. Mean (± 0.95 confidence interval) Hemiptera excluding aphids collected per treatment area at each harvest. Means with the same letter are not significantly different (NK multiple range test, α = 0.05). Two-way ANOVA, harvest: p = 0.003, F = 6.296, DF = 2, 66; treatment: p = 0.002, F = 10.645, DF = 1, 66; interaction: p = 0.003, F = 6.426, DF = 2, 66).



Analysis of six invertebrate groups (Coleoptera, Diptera, Trichoptera, Lepidoptera, Odonata, and Hemiptera - excluding aphids) resulted in statistical

significance for the interaction between harvest and treatment (Table 2). Three general trends were noted for each invertebrate group.

First, the number of invertebrates collected from treated areas did not significantly change throughout all harvests (Figures 12-17). Mean invertebrates collected remained less than 1 individual per sample for Hemiptera excluding aphids, Lepidoptera, Trichoptera, and Diptera (Figures 12-15), while Odonata and Coleoptera means were less than 6 individuals per sample (Figures 16-17).

Second, invertebrate populations collected from non-treated areas significantly increased over time (Figures 12-17). Increases in mean invertebrate numbers from harvest I-III ranged from a 3 fold increase in Coleoptera to a 41 fold increase in Odonata (Figure 16 & 17). These increases were most likely due to invertebrate colonization. All three ponds were dry prior to the start of the study and were therefore void of aquatic invertebrates and vegetation. Other ponds at the LAERF are maintained year round, therefore providing a 'stock' of invertebrates capable of colonization. In addition, invertebrates were probably introduced through the Lake Lewisville pond water used to fill all ponds at the LAERF.

Finally, statistically significant differences in invertebrate numbers between the two treatments were only attained at harvest II & III (Figs 12 – 17). Both treatment areas had few inhabitants at harvest I making it difficult to identify reductions in invertebrates due to abate application. By harvest II & III, invertebrates had colonized non-treated areas and treatment differences were evident. Abate applications reduced invertebrates numbers by 94 – 100 percent depending on harvest and invertebrate group. Lepidoptera, Diptera, and Coleoptera were significantly less in treated areas at

both harvest II & III (Figures 13, 15, and 17), while Hemiptera excluding aphids, Trichoptera, and Odonata attained statistically significant reductions at harvest III only (Figures 12,14, and 16).

Figure 13. Mean (± 0.95 confidence interval) Lepidoptera collected per treatment area at each harvest. Means with the same letter are not significantly different (NK multiple range test, α = 0.05). Two-way ANOVA, harvest: p = 0.001, F = 7.929, DF = 2, 66; treatment: p < 0.001, F = 33.920, DF = 1, 66; interaction: p = 0.003, F = 6.201, DF = 2, 66).

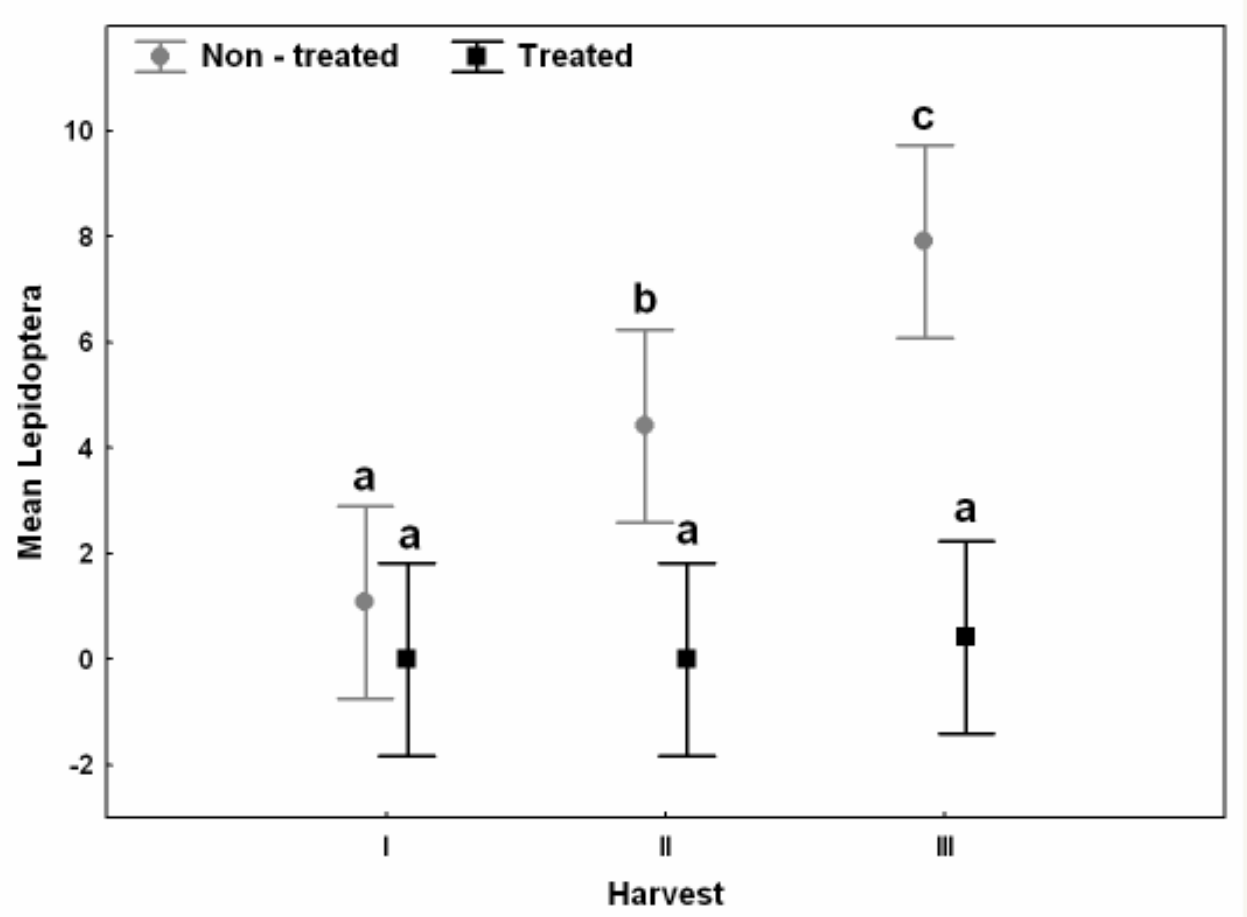


Figure 14. Mean (± 0.95 confidence interval) Trichoptera larvae and pupae collected per treatment area at each harvest. Means with the same letter are not significantly different (NK multiple range test, α = 0.05). Two-way ANOVA, harvest: p = 0.056, F = 3.005, DF = 2, 66; treatment: p = 0.001, F = 11.889, DF = 1, 66; interaction: p = 0.056, F = 3.006, DF = 2, 66).

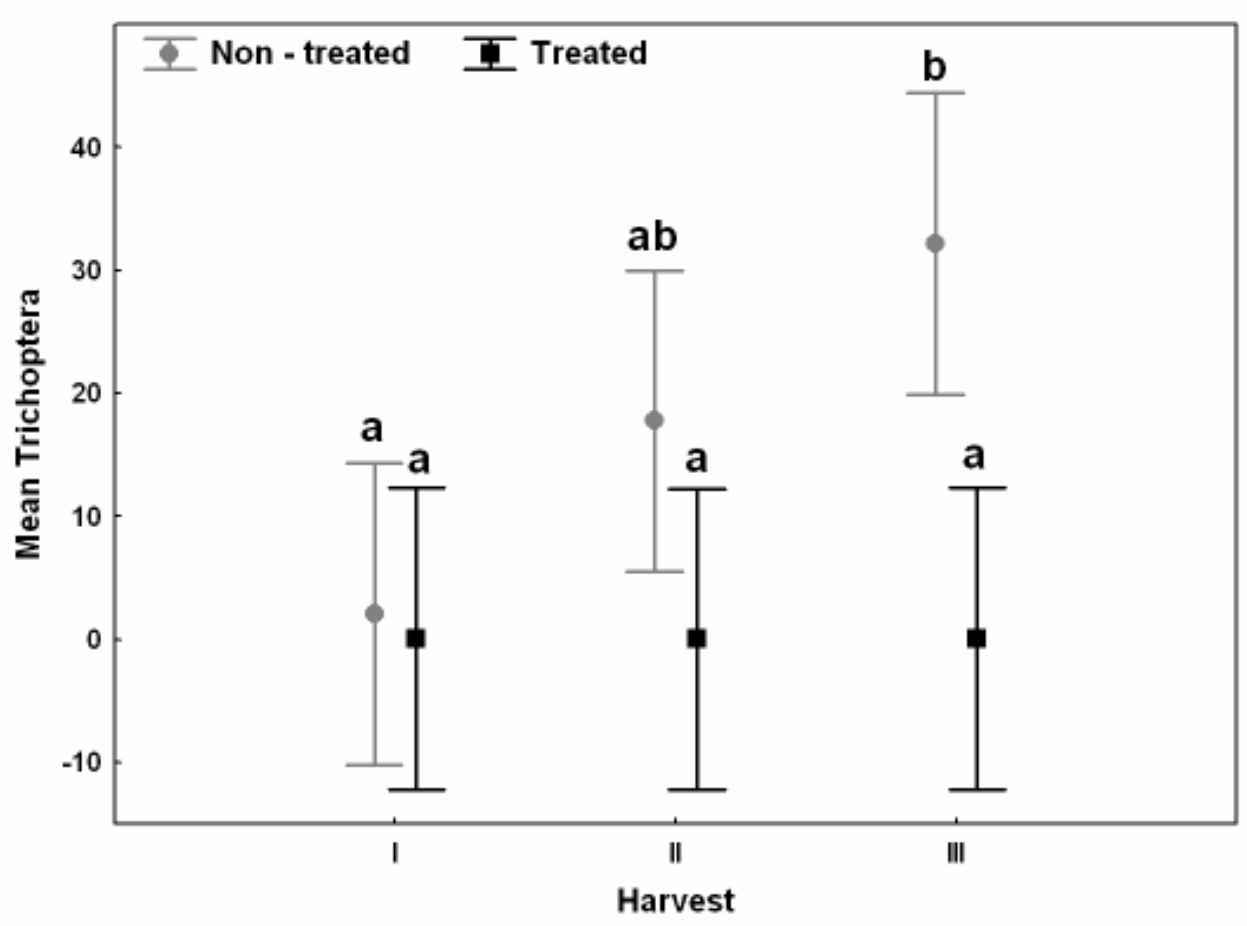


Figure 15. Mean (± 0.95 confidence interval) Diptera larvae and pupae collected per treatment area at each harvest. Means with the same letter are not significantly different (NK multiple range test, α = 0.05). Two-way ANOVA, harvest: p = 0.001, F = 8.251, DF = 2, 66; treatment: p < 0.001, F = 44.545, DF = 1, 66; interaction: p < 0.001, F = 8.524, DF = 2, 66).

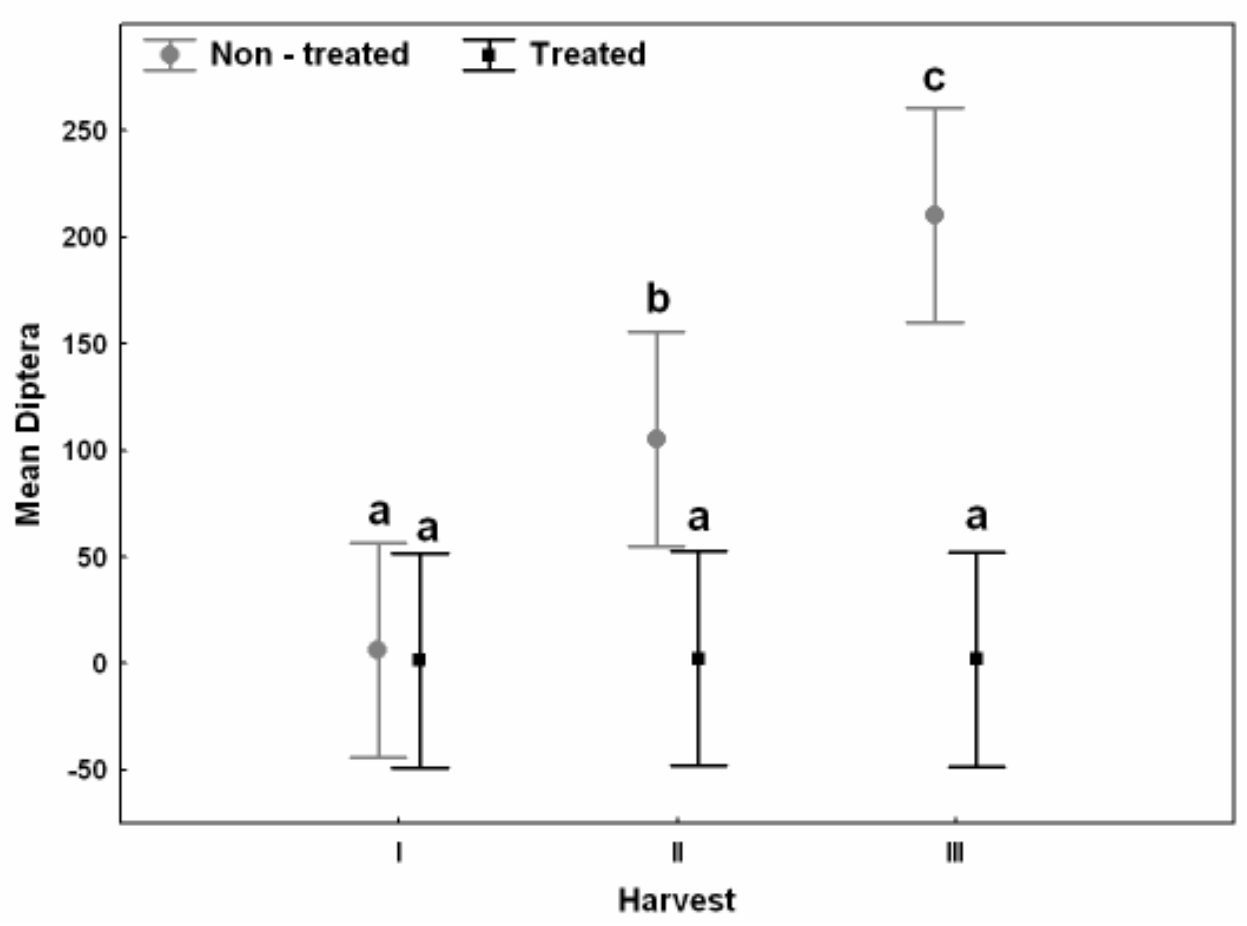


Figure 16. Mean (± 0.95 confidence interval) Odonata larvae collected per treatment area at each harvest. Means with the same letter are not significantly different (NK multiple range test, α = 0.05). Two-way ANOVA, harvest: $p < 0.001$, $F = 12.654$, $DF = 2, 66$; treatment: $p < 0.001$, $F = 14.641$, $DF = 1, 66$; interaction: $p < 0.001$, $F = 10.127$, $DF = 2, 66$).

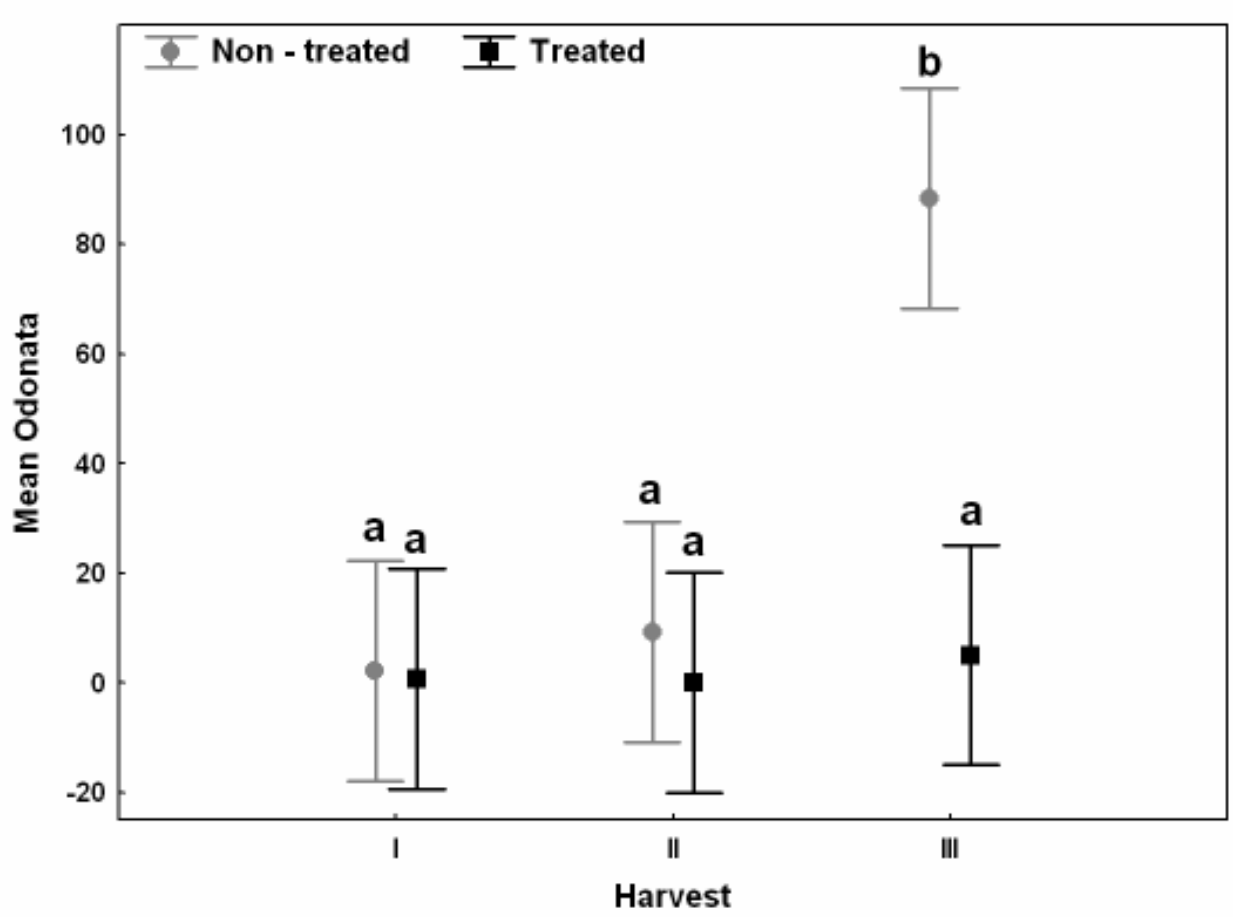
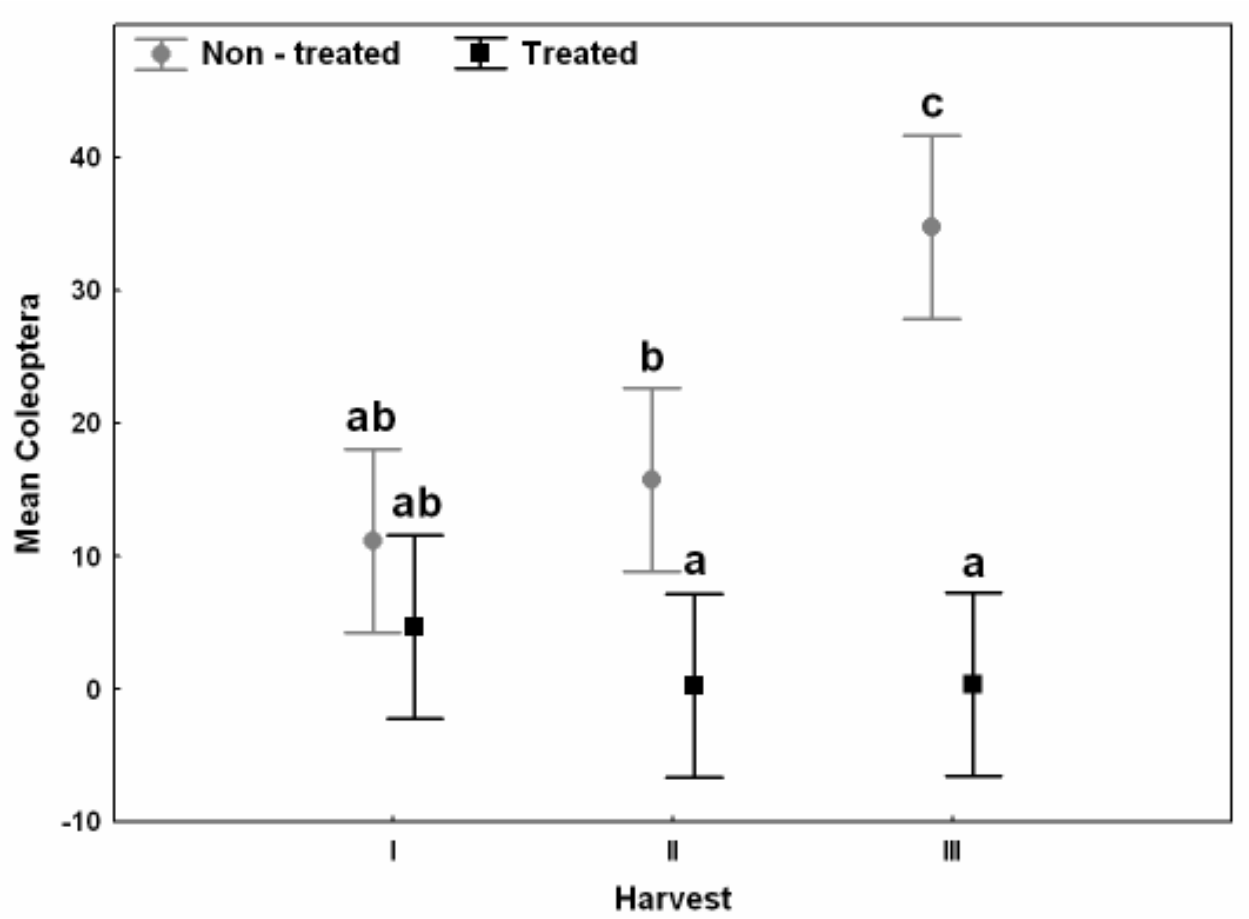


Figure 17. Mean (± 0.95 confidence interval) Coleoptera larvae collected per treatment area at each harvest. Means with the same letter are not significantly different (NK multiple range test, α = 0.05). Two-way ANOVA, harvest: p = 0.008, F = 5.142, DF = 2, 66; treatment: p < 0.001, F = 44.545, DF = 1, 66; interaction: p = 0.001, F = 8.524, DF = 2, 66).



Differences in number of invertebrates due to treatment varied based on invertebrate group (Table 2). The null hypothesis that numbers of invertebrates collected from the two treatments would not differ was accepted for Gastropoda, Oligochaeta, and Hemiptera when aphids were included in the counts. Significant reductions in invertebrate numbers due to abate were seen for Ephemeroptera, Coleoptera, Diptera, Trichoptera, Lepidoptera, Odonata, and Hemiptera when aphid counts were excluded and therefore the null hypothesis was rejected.

Invertebrate Collections

Community Analysis

Each CA biplot is represented by the first two axis deemed significant by the CA. The percent of total inertia (variance) explained collectively by the two axis increased overtime from harvest I (36%) to harvest III (57%) (Table 3). The location of replicates within CA biplots appeared random at harvest I, but by harvest II & III treatment differences defined the groupings (Figures 18–21).

Table 3. Correspondence Analysis results for harvests I-III including: 1) eigen values for each axis, 2) total inertia (variance), 3) percent of total inertia described by each axis, and 4) total percent of inertia describe by both axis together.

Harvest	Eigen Values			Percent of Total Inertia		
	Axis 1	Axis 2	Total Inertia	Axis 1	Axis 2	Total Percent
I	0.49	0.26	2.09	24	12	36
II	0.59	0.37	1.74	34	21	55
III	0.48	0.25	1.28	38	19	57
III-excluding aphids	0.42	0.18	1.12	37	16	53

At harvest I, few invertebrates inhabited the ponds; therefore, it was difficult to identify community differences. Most replicates within the CA biplot were grouped together without any distinct assemblages based on plant species, pond, or treatment (Figure 18). Four replicates (*N. mexicana* 51 treated (T), *N. mexicana* 52 Non-treated (NT), *P. illinoensis* 52 NT, and *P. nodosus* 52 NT) were separated from the main grouping due to an abundance or scarcity of one or more invertebrates (Figure 18). *Nymphaea mexicana* 52 NT was associated with an abundance of two different larval coleopterans, *Cybister* sp. and *Tropisternus* sp. while *Physa* sp., a gastropod, was absent from *N. mexicana* 51 T and present in all other samples. *Callibaetis* sp., an ephemeropteran, was present in *P. illinoensis* 52 NT and *P. nodosus* 52 NT in quantities 5-8 fold greater than any other sample. The remaining replicates did not assemble in any distinct order based on plant species, pond, or treatment (Figure 18).

At harvest II replicates began to separate into two different groups based on treatment, but no trends were observed for pond or plant species (Figure 19). Treated replicates were separated from non-treated because of their lack of invertebrates other than those not affected by the insecticide treatment; aphids, gastropods, and oligochaetes. Non-treated replicates contained much more diverse invertebrate communities and therefore had a broader range on the CA biplot (Figure 19). Three treated and 2 non-treated replicates appear to be detached from the remaining samples (Figure 19). Four of these replicates (*N. mexicana* 51 T, *P. illinoensis* 51 T, *P. illinoensis* 51 NT, and *V. americana* 51 NT) had an abundance of *Rhopalosiphum* sp. aphids ranging from 62 – 123 aphids, while all remaining samples had a maximum of 22

aphids. *Potamogeton nodosus* 51 T was separated from other treated replicates due to low populations of *Physa* sp.

At harvest III two distinct treatment groups were identified on the CA biplot, while no evidence of plant species or pond groupings were noted (Figure 20). The non-treated group contained two replicates (*P. nodosus* 51 NT and *P. nodosus* 52 NT) that were spaced apart from the remaining group due to an abundance of aphids. These two samples had quantities of aphids greater than 200 while all other non-treated replicates had a maximum of 73. Treated replicates had a much broader range on the biplot, than non-treated replicates. This was unexpected since treated replicates should have few invertebrates due to the insecticide treatment. A closer look at the data revealed that treated replicates were vertically spaced apart by aphid quantity. The topmost sample, *N. mexicana* 50 T had the least aphids, 0, while the bottommost sample, *N. mexicana* 51 T had the most aphids, 423 (Figure 20). To illustrate the degree of influence aphids had over treated community structure, aphids were removed from data and the CA biplot was regenerated. As expected, the treated replicates formed a tight group, distinct from non-treated replicates (Figure 21). Treated replicates contained few invertebrates other than aphids, gastropods, and oligochaetes. Of these invertebrates, the aphid's distribution among replicates was less uniform and therefore greatly influential to the CA outcome.

Figure 18. Correspondence Analysis ordination of macrophyte and macroinvertebrate taxa from harvest I. Points are labeled as to the plant species, pond number, and treatment (T = treated, NT = non-treated) where: *V. americana* (V.ame), *P. nodosus* (P.nod), *P. illinoensis* (P.ill), and *N. mexicana* (N.mex). Axis 1 & 2 explained a total of 36% of total inertia (variance).

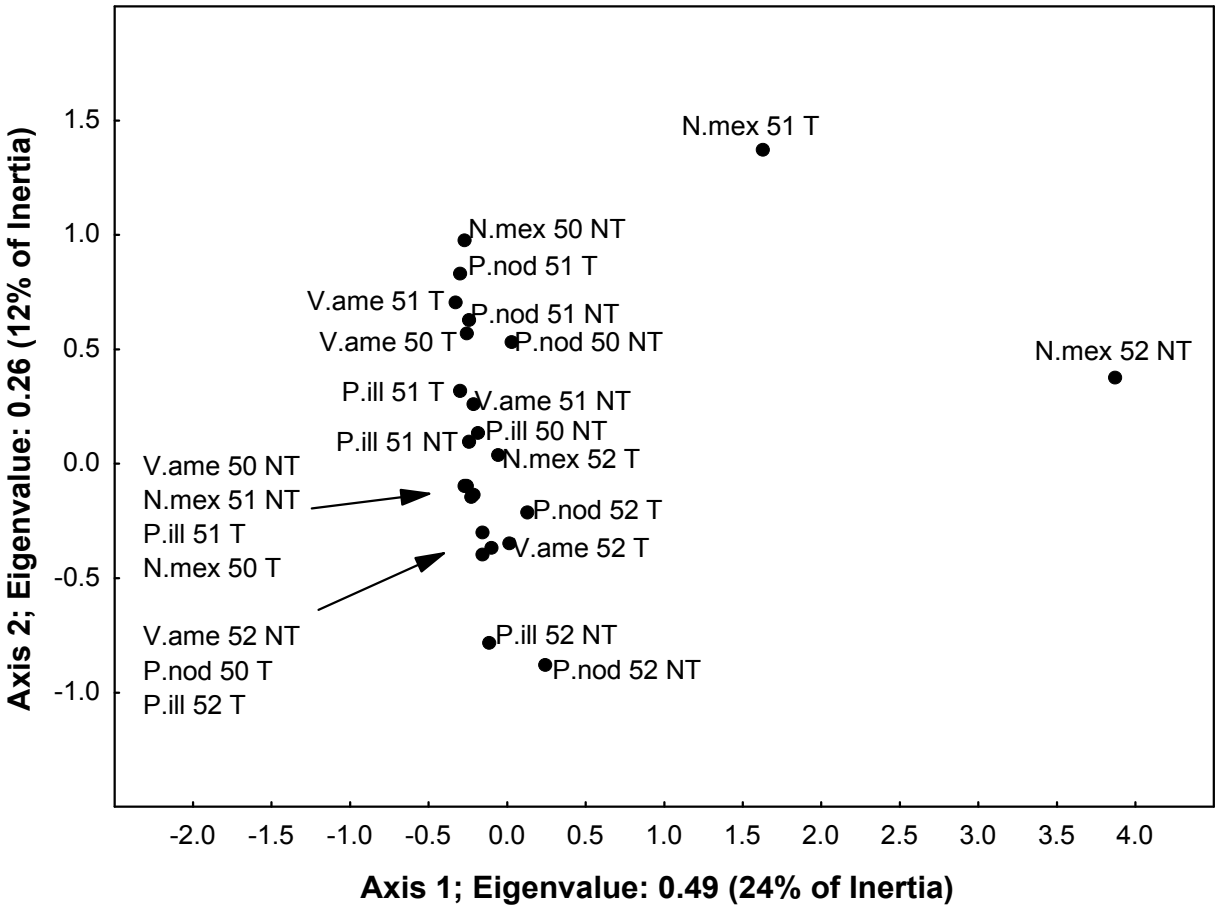


Figure 19. Correspondence Analysis ordination of macrophyte and macroinvertebrate taxa from harvest II. Points are labeled as to the plant species, pond number, and treatment (T = treated, NT = non-treated) where: *V. americana* (V.ame), *P. nodosus* (P.nod), *P. illinoensis* (P.ill), and *N. mexicana* (N.mex). Axis 1 & 2 explained a total of 55% of total inertia (variance).

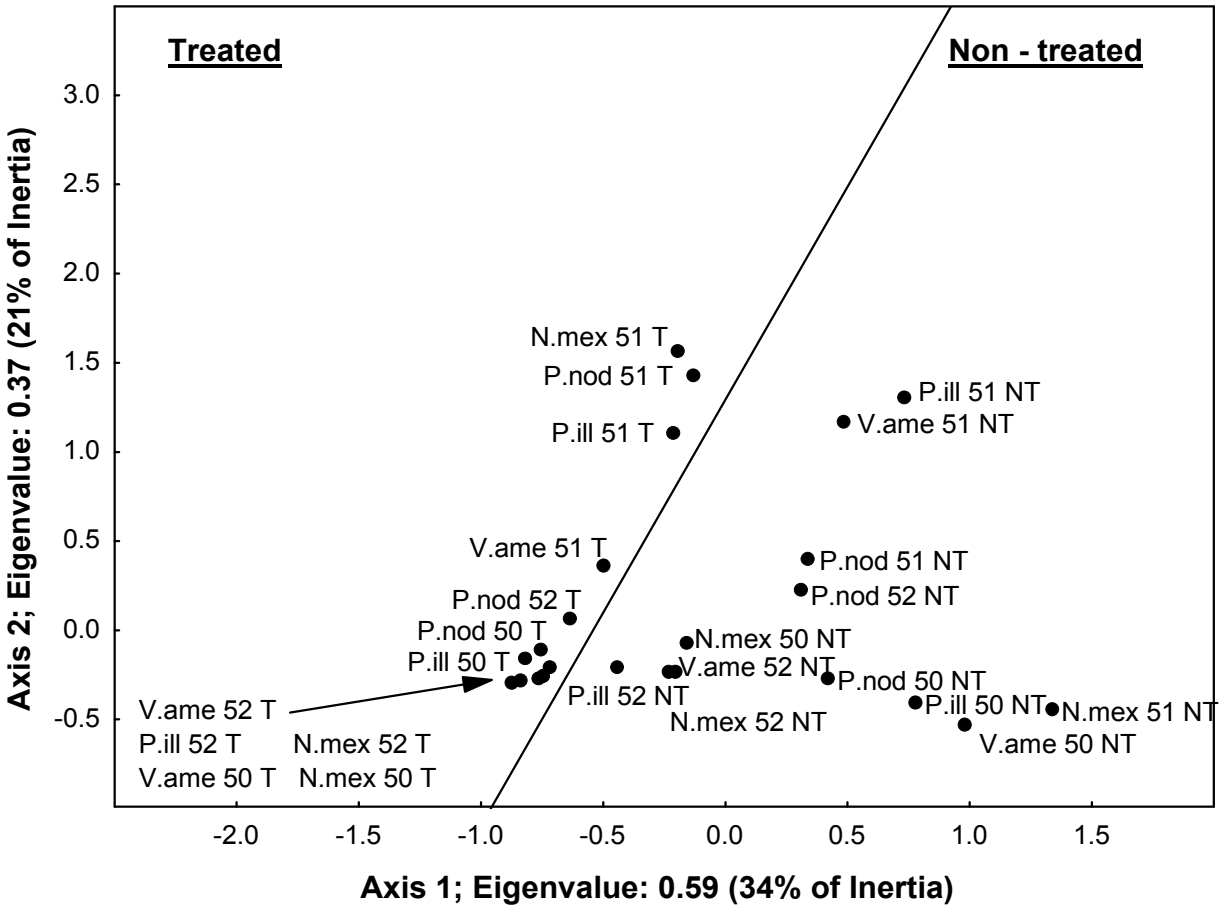


Figure 20. Correspondence Analysis ordination of macrophyte and macroinvertebrate taxa from harvest III. Points are labeled as to the plant species, pond number, and treatment (T = treated, NT = non-treated) where: *V. americana* (V.ame), *P. nodosus* (P.nod), *P. illinoensis* (P.ill), and *N. mexicana* (N.mex). Axis 1 & 2 explained a total of 57% of total inertia (variance).

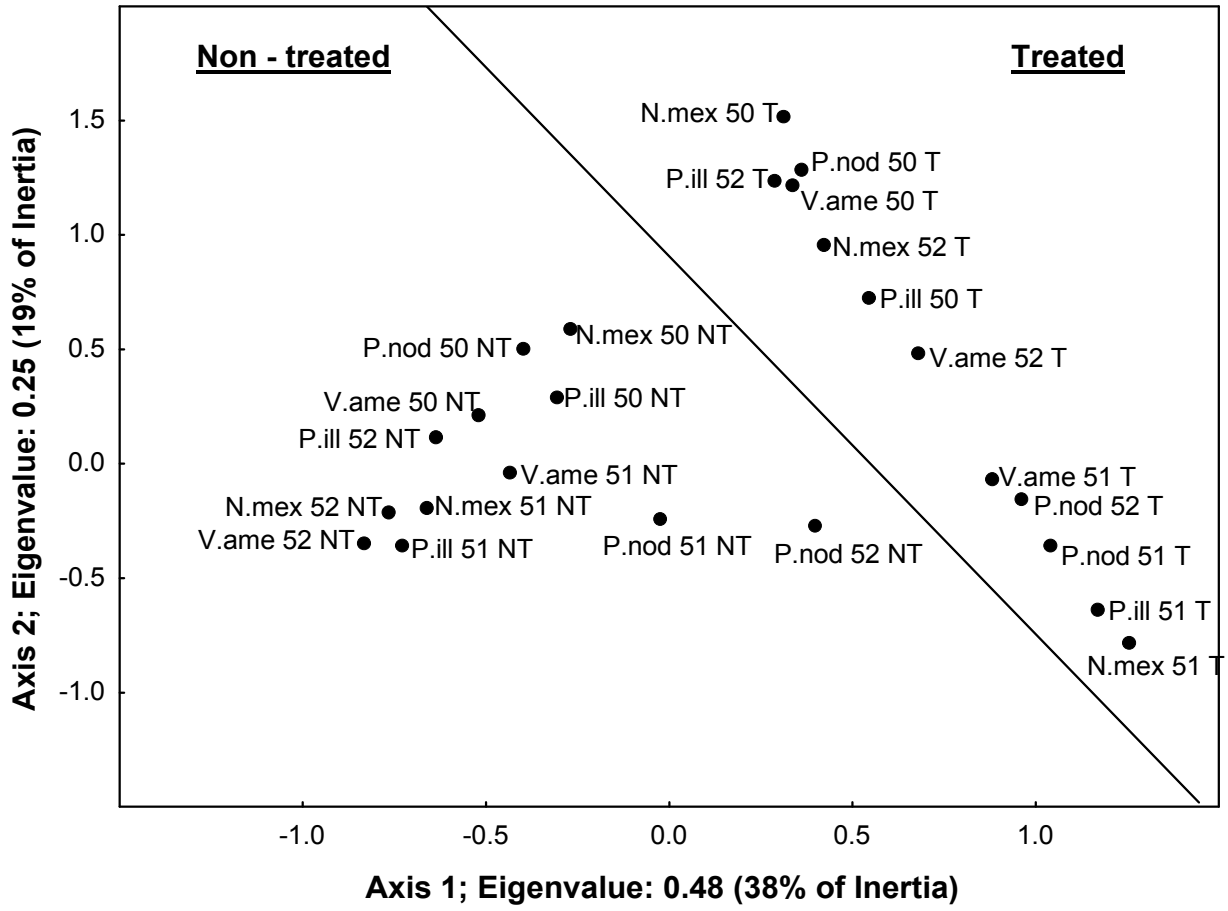
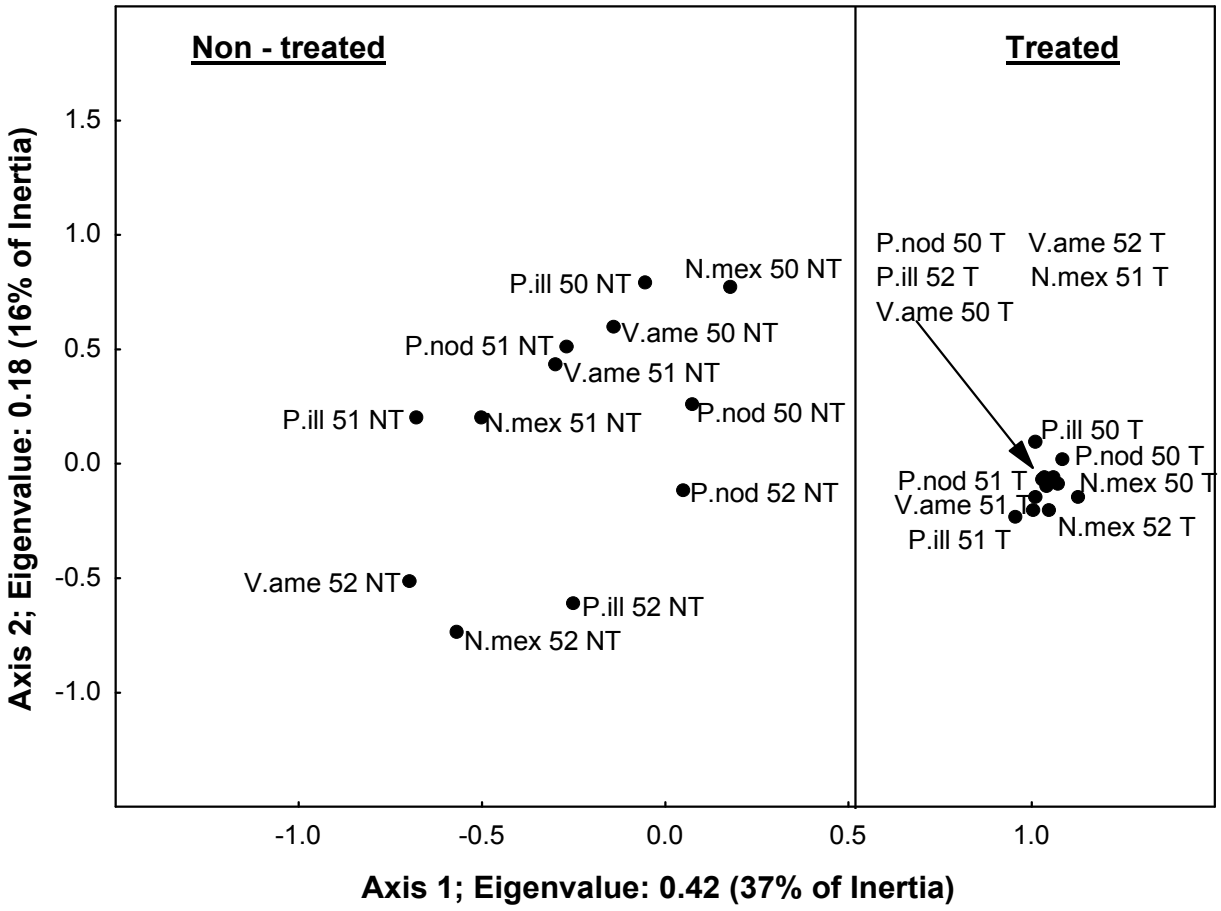


Figure 21. Correspondence Analysis ordination of macrophyte and macroinvertebrate taxa from harvest III (excluding aphids). Points are labeled as to the plant species, pond number, and treatment (T = treated, NT = non-treated) where: *V. americana* (V.ame), *P. nodosus* (P.nod), *P. illinoensis* (P.ill), and *N. mexicana* (N.mex). Axis 1 & 2 explained a total of 53% of total inertia (variance).



The null hypothesis that there would be no differences in invertebrate communities based on treatment was rejected. At harvest II & III invertebrate communities separated into two groups based on treatment. Treated community structure was predominately composed of invertebrates not affected by the insecticide treatment; aphids, gastropods, and oligochaetes. In contrast, non-treated samples

contained a more diverse assemblage of invertebrates. At harvest III, the CA biplot for treated samples was highly influenced by the distribution of aphids among samples.

The null hypothesis that there would be no differences in invertebrate communities based on pond and plant species was accepted. The invertebrate communities present in each pond were expected to be similar because the ponds were located in close proximity, received water from the same source, and had access to the same 'stock' invertebrate populations from nearby ponds at the LAERF. Community differences due to plant species were probably disrupted by the mixed plantings in each pond. Placement of four different plant species within the same pond and in close proximity could increase the chances of invertebrate emigration and immigration among plants and therefore homogenize invertebrate communities (Chilton, 1990).

Invertebrate Collections

Herbivores and Non-consumptive Invertebrate Damage

All invertebrates collected were grouped into functional feeding groups and consequently nine herbivores were identified (Table 4). Of these, five were most prevalent; *Synclita*, *Paraponyx*, *Donacia*, *Rhopalosiphum*, and *Hydrellia*.

Table 4. Invertebrates collected and identified as herbivores.

Order	Family	Genus	Life Stage
Coleoptera	Chrysomelidae	*Donacia*	Larvae and Adults
Coleoptera	Haliplidae	*Haliplus*	Larvae and Adults
Coleoptera	Hydrophilidae	*Berosus*	Adults
Diptera	Ephydridae	*Hydrellia*	Larvae
Hemiptera	Aphidiae	*Rhopalosiphum*	Larvae and Adults
Lepidoptera	Noctuidae	*Archanara*	Larvae
Lepidoptera	Pyralidae	*Synclita*	Larvae
Lepidoptera	Pyralidae	*Paraponyx*	Larvae
Trichoptera	Leptoceridae	*Nectopsyche*	Larvae

Larvae from the genera *Synclita* and *Paraponyx* (Lepidoptera: Pyralidae) not only feed on aquatic macrophytes, but also use plant matter to construct portable cases (Figure 22). *Synclita* larvae were observed living in free-floating portable cases from and feeding on floating leaves of *P. nodosus*, *P. illinoensis*, and *N. mexicana*. In contrast, *Paraponyx* larvae were observed below the water's surface in cases made from submersed leaves of *P. illinoensis* and *V. americana*, but were not observed actively feeding. Plants utilized for case construction are not necessarily food plants (Habeck, 1974), but based on observations, *Paraponyx* feeding probably occurred under the water's surface on submersed leaves. In Florida, *Paraponyx* have been associated with 25 different plant species in 17 families (Habeck, 1974) and in general, pyralids are considered to have the highest levels of polyphagy of insects that feed on aquatic plants (Stoops et al., 1998).

Figure 22. *Synclita* larvae cut pieces of plant matter from floating leaves in half circle shapes to construct portable cases. A floating leaf of P. nodosus is pictured here.

The genus *Donacia* (Coleoptera: Chrysomelidae) encompasses two subgenera *Donacia* and *Donaciomima* which include 10 and 21 Nearctic species respectively (Riley et al., 2002). Chrysomelids of the genus *Donacia* are typically found on floating or emergent vegetation and can exhibit host-specific feeding as well as polyphagy (Marx, 1957; Cronin et al., 1998). All known host plants of the subgenus *Donacia* are dicotyledons including *Brasenia*, *Nuphar*, and *Nymphaea*, while *Donaciomima* prefer monocotyledons such as *Scirpus* and *Typha*, but also host on *Nymphaea* a dicotyledon (Riley et al., 2002). During this study, *Donacia* adults, larvae, and eggs were commonly found on *N. mexicana*, but were not observed on any other macrophyte species. Adults fed on floating leaves of *N. mexicana* and remained on vegetation above the water's surface (Figure 23). Eggs were oviposited in concentric rows on the underside of floating leaves, through a hole chewed in the leaf (Figure 24). *Donacia* larvae live and feed near sediment on roots, rhizomes, or stems (Hoffman, 1940) for the entire larval stage which can be for 2 or more years (White and Brigham, 1996). Without species identification I can not be sure of feeding selectivity of *Donacia* encountered, yet during this and previous studies at the LAERF, *Donacia* exhibited a feeding preference for plants of the Nymphaeaceae family; including *N. odorata* and *N. mexicana* (Nachtrieb et al., 2007).

Figure 23. An adult *Donacia* is shown resting on *N. mexicana*. The holes on the leaf were created by *Donacia* adults for feeding and ovipositing.

Figure 24. Donacia eggs are laid in concentric rows on the underside of leaves, through a hole chewed in the leaf.

Rhopalosiphum (Hemiptera: Aphididae) were observed on all three floating leaved plants: *P. nodosus*, *P. illinoensis* and *N. mexicana*. As mentioned previously, these aphids over-winter as eggs on trees then migrate to aquatic environments during mid to late summer. During the colonizing phase females are ovoviviparous and can give birth to 2-4 young a day (Center et al., 1999). Aphids feed on plant nutrients by piercing through plant tissue directly into phloem tubes (Blackman, 1974). While not problematic in small numbers, large aphid colonies are capable of removing enough of

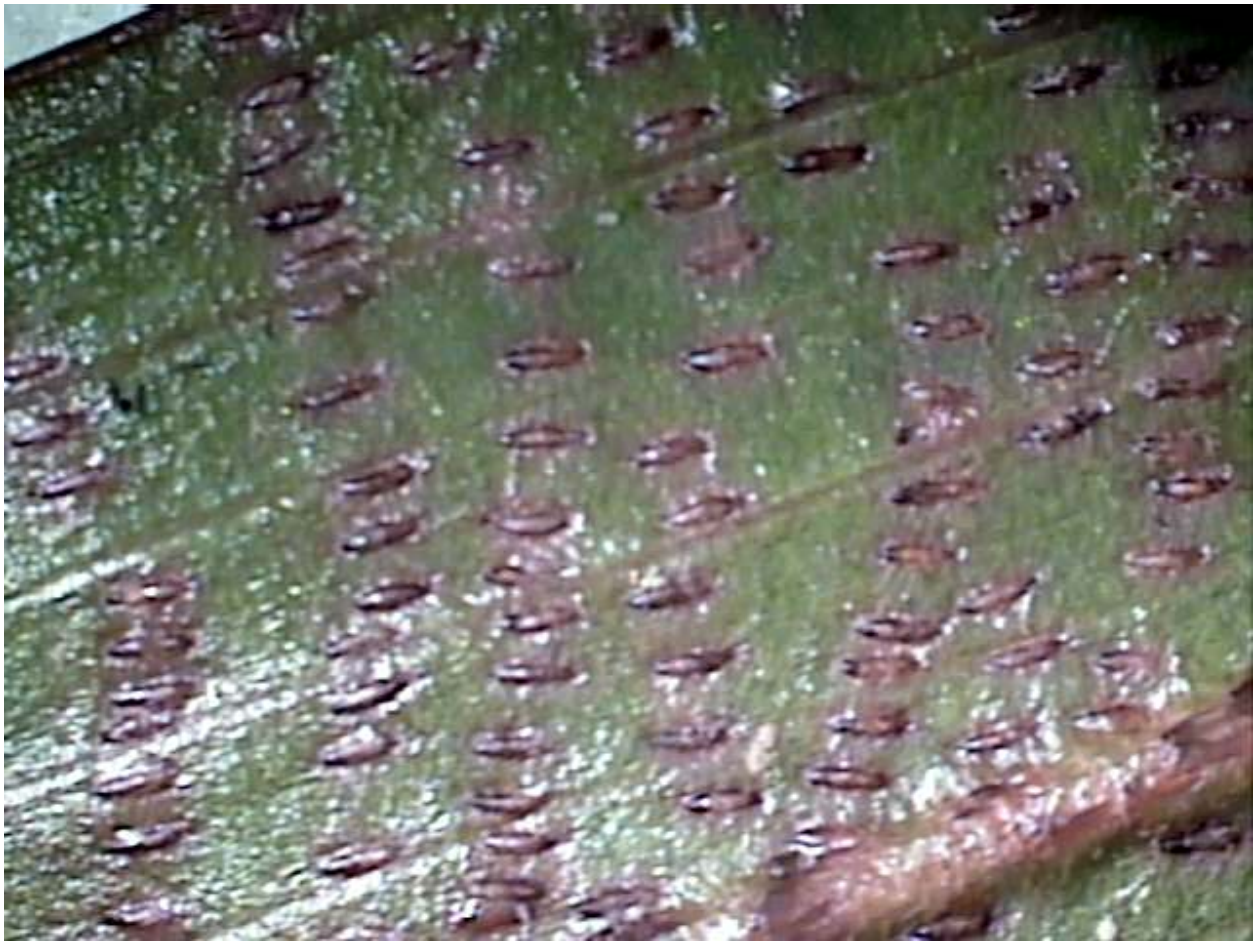
the plant's nutrients so that the plant prematurely breaks down plant tissue to replenish its nutrient supply. This directly halts plant growth and can ultimately cause death (Blackman, 1974). Aphid colonies were present in large enough numbers to completely cover floating leaves of both *Potamogeton* species as well as the larger leaves of *N. mexicana*.

The diet of adults of genus *Hydrellia* (Diptera: Ephydridae) is not well understood, but larvae are known to feed on the mesophyll of plants by mining leaves (Deonier, 1971). In aquatic environments, *Hydrellia* larvae are most commonly encountered on plants from family Potamogetonaceae (Deonier, 1971). *Hydrellia* mines were observed on the floating and submersed leaves of both potamogetons used in this study, *P. illinoensis and P. nodosus* (Figure 25). Three different *Hydrellia* species were collected from the study ponds, including two native species, *H. bilobifera* Cresson and *H. discursa* Deonier, and one introduced species, *H. pakistanae* Deonier. *Hydrellia bilobifera* and *H. discursa* adults are commonly observed at the LAERF perching on floating leaves of a wide range of plants, and larvae are known to feed on potamogetons as well as *Hydrilla verticillata* (L.f.) Royle, an exotic, submersed plant (Center et al., 1999). Native *Hydrellia* have been well described by Deonier (1971) yet few accounts exist documenting the effects that leaf mines have on aquatic plants. *Hydrellia pakistanae*, an Asian native, was introduced to the United States for biological control of *H. verticillata* (Center et al., 1999) and is currently reared in outdoor ponds at the LAERF. *Hydrellia pakistanae* is considered host specific to *H. verticillata* and therefore more research is warranted to determine if adults were emerging from native plants in the study pond.

Figure 25. A *Hydrellia* larvae is shown mining in a floating leaf of *P. nodosus*.

Non-consumptive damage was not restricted to invertebrate herbivores. Various eggs were observed on macrophytes in the study ponds, yet eggs laid by odonates were most prominent (Figure 26). Odonate eggs were found in floating leaves and stems of *P. nodosus, P. illinoensis, and N. mexicana*. Eggs were deposited in shallow indentions which left a hole in the plant tissue once larvae emerged. These holes were numerous and sometimes completely covered a leaf. One anisopteran and one zygopteran family, Aeshnidae and Coenagrionidae respectively, were reared from egg infested leaves.

Figure 26. Odonate eggs imbedded in a floating leaf of *P. illinoensis*.



Percent Invertebrate Leaf Damage

Statistically significant differences were not attained for any plant species based on harvest and only one plant, *P. nodosus*, displayed statistical significance due to treatment (Table 5). The null hypothesis that there would be no differences in percent invertebrate leaf damage from the two treatments was rejected for *P. nodosus* and accepted for *V. americana*, *P. illinoensis* and *N. mexicana*.

Table 5. Results from two-way ANOVAs performed to identify changes in percent invertebrate leaf damage due to harvest and treatment.

Plant Species	Harvest		Treatment		Harvest * Treatment	
	F	p	F	p	F	p
N. mexicana	0.0650	0.9374	0.5742	0.4632	0.1428	0.8684
V. americana	0.7731	0.4899	0.1223	0.7346	0.5496	0.5954
P. illinoensis	1.7336	0.1939	2.7600	0.1071	0.0395	0.9613
P. nodosus	0.2506	0.7800	9.6060	0.0042	0.1664	0.8475

Consumptive as well as non-consumptive invertebrate damage were rare on *V. americana* in both treated and non-treated areas. Mean invertebrate damage was 0.99% in treated and 1.09% in non-treated samples. Similar results of reduced herbivory to *V. americana* were found in a previous pond study (Nachtrieb et al., 2007), yet herbivore damage has been observed on plants in culture at the LAERF. It appears that herbivores feeding on this species did not colonize the ponds during the experimental time frame of four months.

For the remaining three plant species (*P. nodosus*, *P. illinoensis*, and *N. mexicana*) tissue damage in treated areas was rare, while plants within non-treated areas exhibited substantial levels of damage due to insects, primarily feeding, ovipositing, and case making (Figure 27, 28, and 29). Yet, these observations do not agree with the quantitative data in which *P. nodosus* was the only plant species to attain statistical significance (Table 4). Mean invertebrate damage between treatments (treated: non-treated) for each plant species were as follows; *P. nodosus* 1.1%:9.8%, *P. illinoensis* 3.5%:8.6%, and *N. mexicana* 11.2%:15.0%. For *P. illinoensis* and *N. mexicana* non-treated damage levels were not significantly different than treated.

Various authors have made note of similar difficulties in measuring invertebrate damage levels.

Figure 27. Treated (a) and non-treated (b) *N. mexicana* at harvest III.

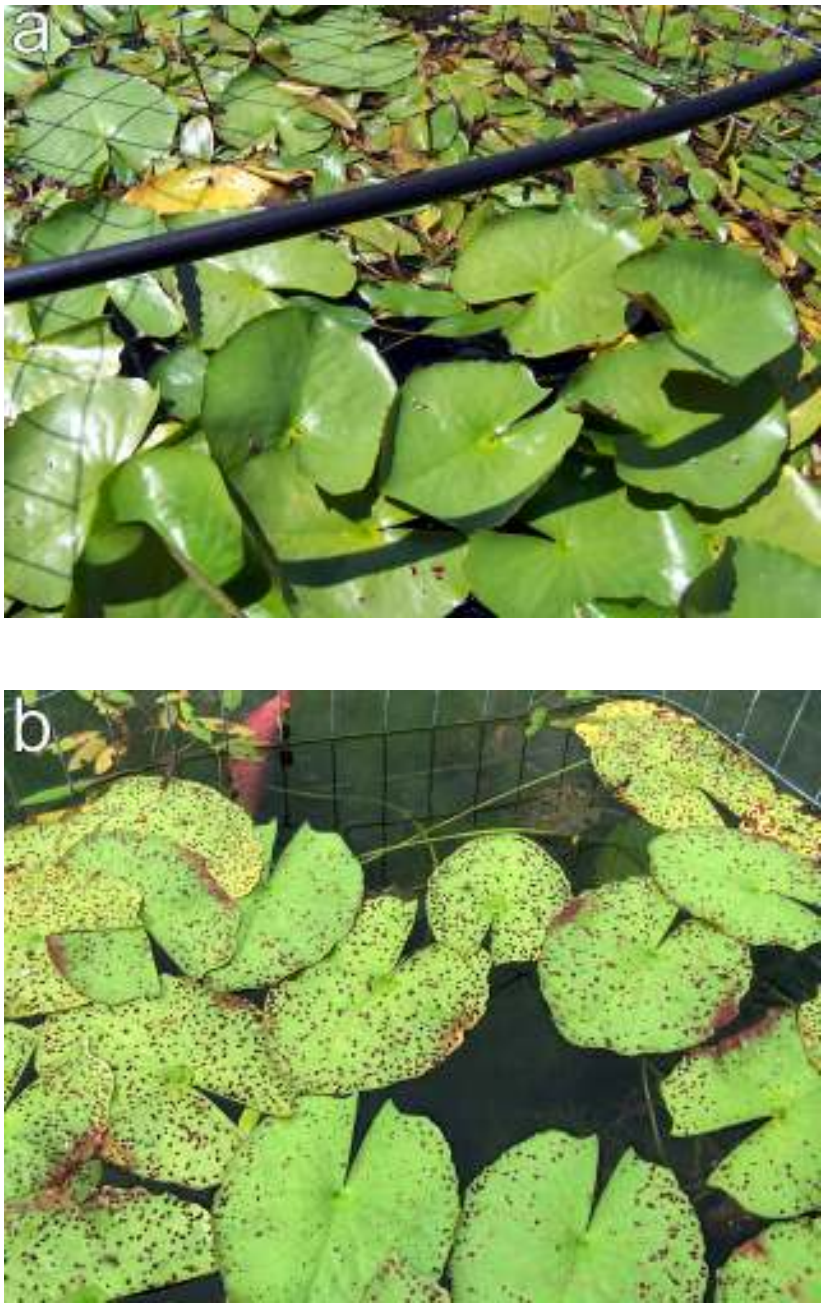


Figure 28. Treated (a) and non-treated (b) *P. nodosus* at harvest III.

Figure 29. Treated (a) and non-treated (b) P*. illinoensis* at harvest III.

While studying *Nuphar luteum* (L.) Sibth & Sm., a water lily, Wallace and O'Hop (1985) documented that leaf turnover rate was higher at a site that experienced herbivory by *Pyrrhalta nymphaeae* (L.), the waterlily leaf beetle, as opposed to a site where the beetles were absent. At the herbivore site leaves died faster, but were replaced quickly as if plant growth was compensating for herbivory losses. If herbivory causes increased leaf turnover rates, when randomly selecting leaves to measure invertebrate damage there is a higher chance that new, less damaged leaves will be present since older leaves with higher damage levels have probably decomposed. Jacobsen and Sand-Jensen (1992) agreed that herbivory could be underestimated unless leaf turnover was taken into account. To correct for this problem, new undamaged leaves can be marked so that invertebrate damage can be measured throughout the life of the leaf. This gives a higher chance that maximum levels of herbivory can be recorded. Invertebrate damage levels measured during this study were probably underestimated since leaves were randomly selected and only measured at harvest times.

Macrophyte Biomass

Dry weights of *V. americana* from both treatment areas decreased throughout the study and biomass between treatments was not significantly different at the final harvest (harvest III) (Figure 30). At harvest III mean biomasses between treatments were approximately 0.60 g apart. To achieve statistical significance between treatments at a power of 0.95 with the same standard deviation and means, a sample size of 1,161 would be required. Biomass decreases were not attributed to herbivory or non-consumptive damage which were rare. *Vallisneria americana* is a submersed

macrophyte and typically distributes its biomass uniformly throughout the water column. Macrophytes which inhabit water closer to the surface or those that have floating leaves can have a competitive advantage for light. Titus and Stephens (1983) documented reduced growth of *V. americana* while growing in the presence of *Chara vulgaris* L. (a macroalga) and *P. amplifolius* Tuckerman. Smart (1991) also documented establishment difficulty in ponds at the LAERF in the presence of *C. vulgaris* and *Najas guadalupensis* (Sprengel) Magnus. Furthermore, biomass of *V. americana* was negatively correlated to biomass of *C. vulgaris* in a previous pond study at the LAERF (Nachtrieb et al., 2007). During this study *C. vulgaris* and *N. guadalupensis*, species endemic to the LAERF, commonly inhabited *V. americana* cages. The floating leaves of *P. nodosus*, *P. illinoensis*, and *N. mexicana* were also capable of shading as each species commonly migrated into nearby *V. americana* cages (Figure 31). Dry weights of all plants harvested from *V. americana* replicates were quantified, but a significant correlation was not attained. Regardless, invertebrate damage does not explain biomass decreases and the presence of highly mixed plant communities leads to the conclusion that during the four month duration of this study, *V. americana* was unable to overcome competitive pressures of nearby macrophytes.

Figure 30. Mean (± 0.95 confidence interval) dry biomass (g) of *V. americana* collected per treatment area at each harvest. A one-way ANOVA was performed at harvest III, treatment: p = 0.689, F = 0.163, DF = 1, 28.

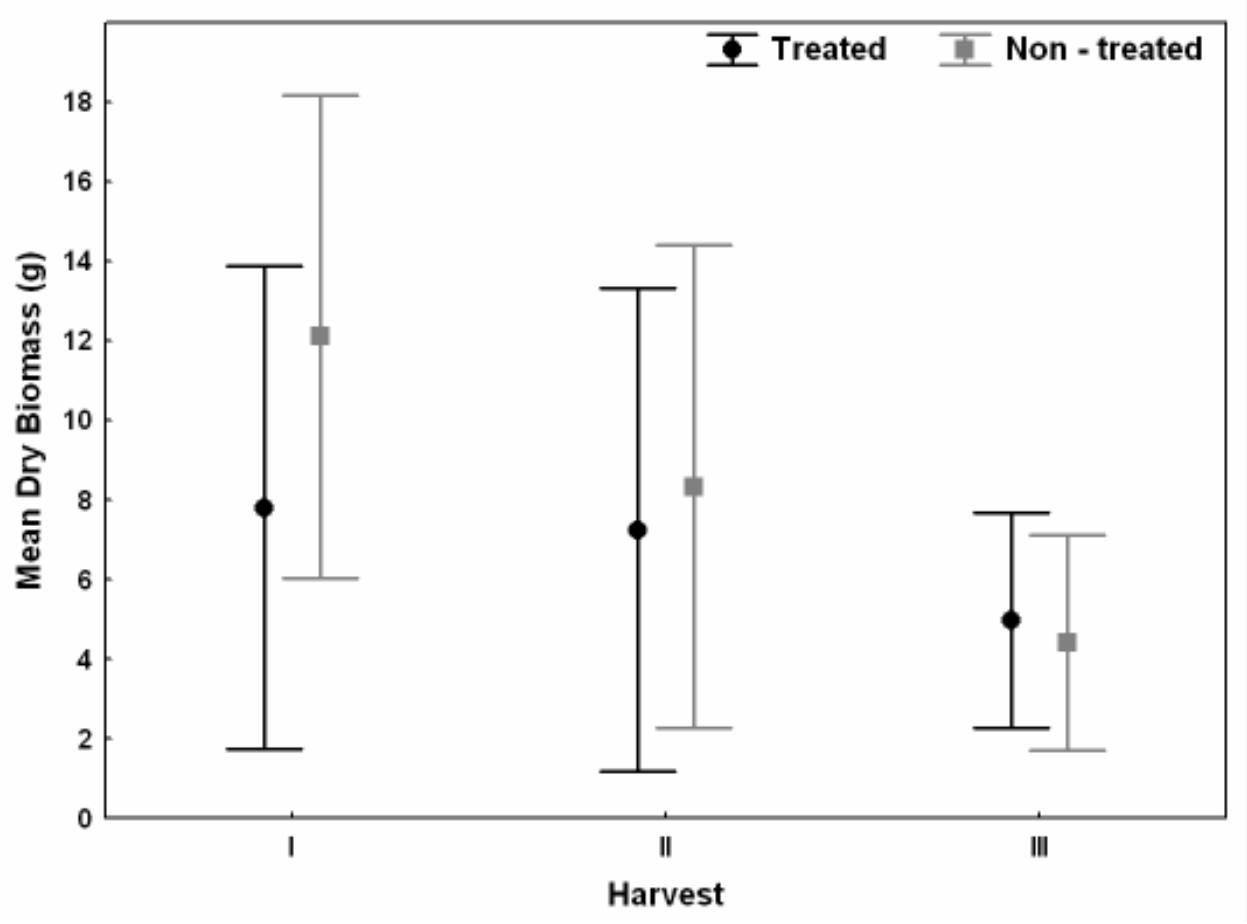
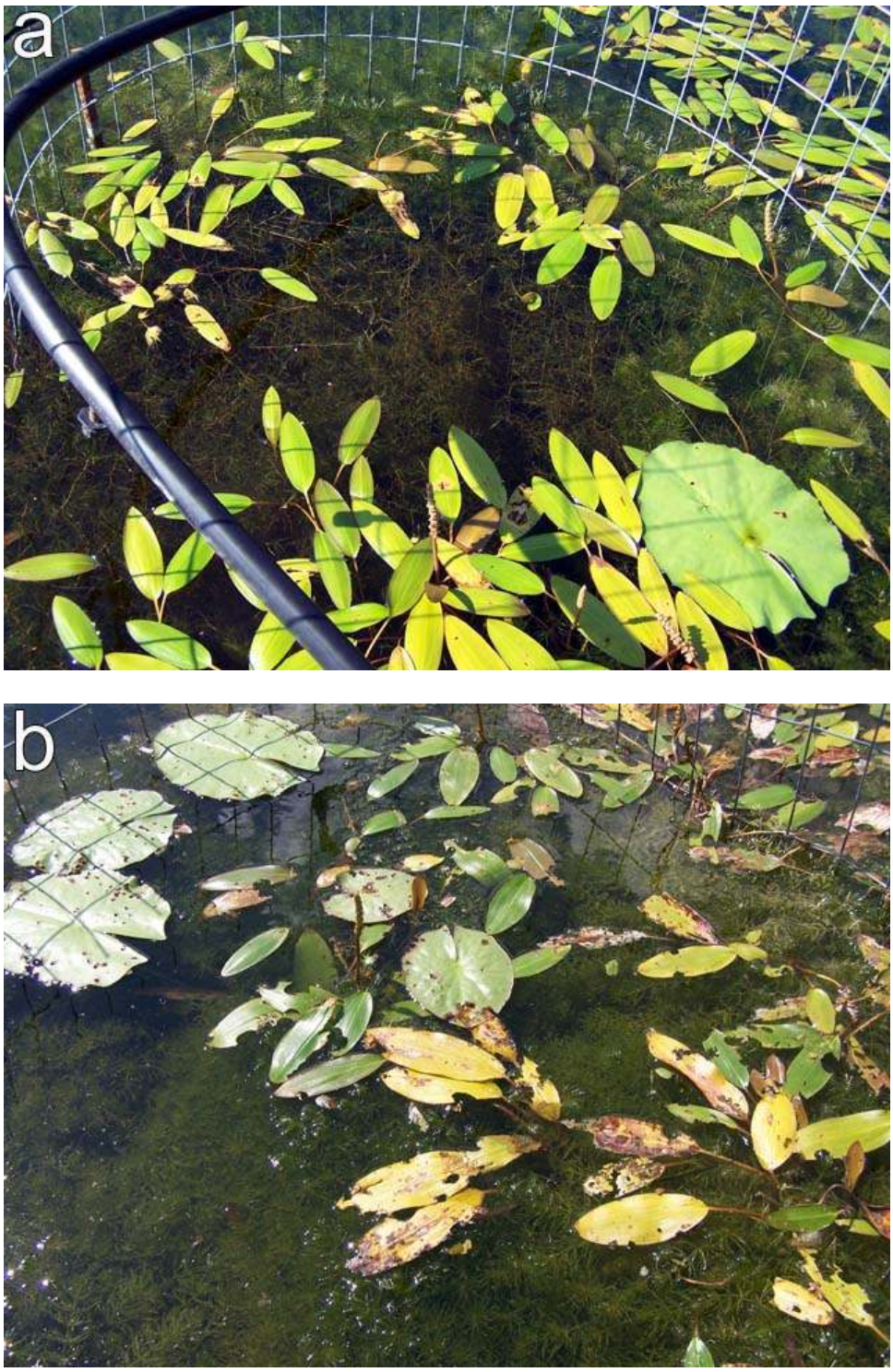
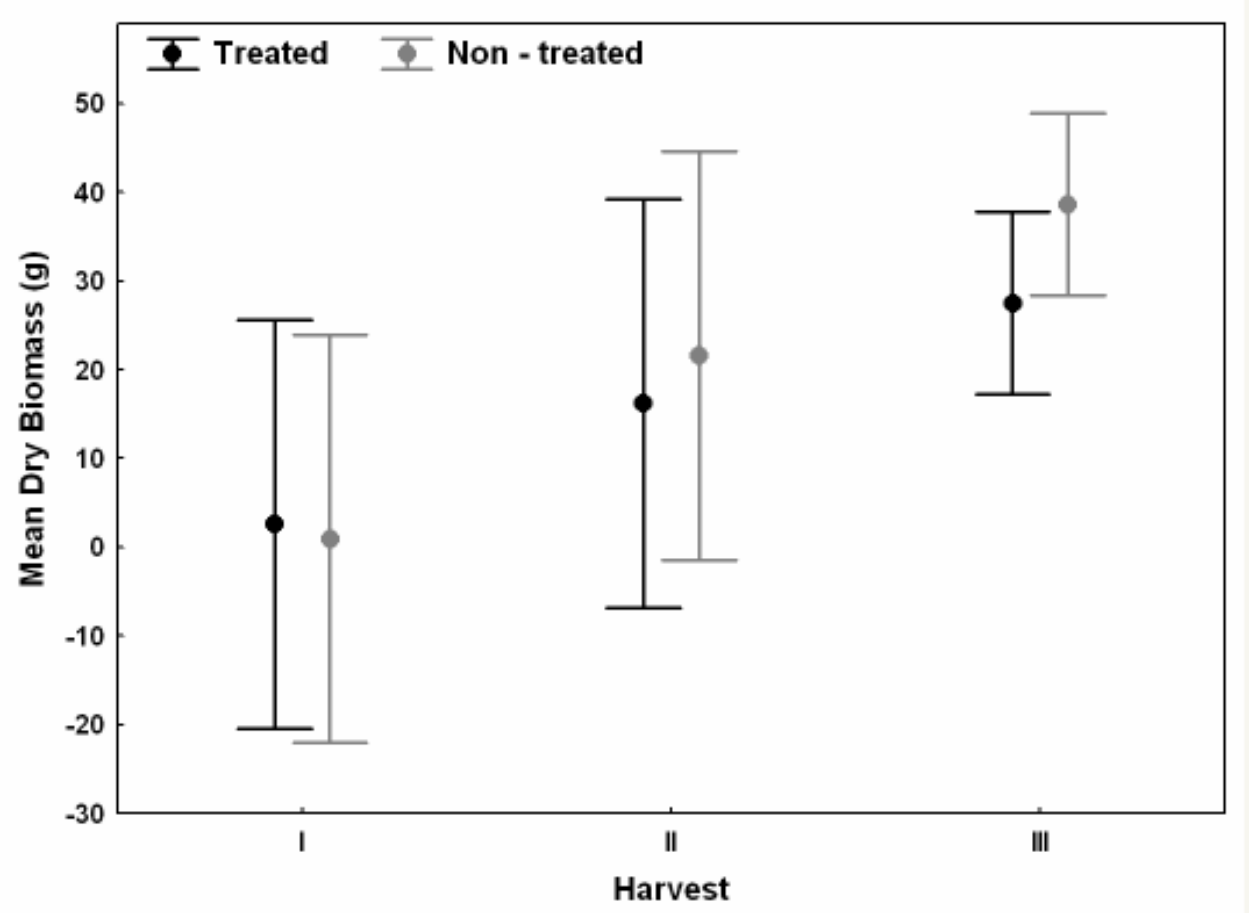


Figure 31. Treated (a) and non-treated (b) *V. americana* at harvest III.



Dry weights of *N. mexicana* from both treatment areas increased throughout the study and biomass between treatments was not significantly different at the final harvest (harvest III) (Figure 32). At harvest III mean biomasses between the two treatments were approximately 11 g apart. To achieve statistical significance between treatments at a power of 0.95 with the same standard deviation and means, a sample size of 92 would be required. High levels of herbivory and non-consumptive damage from *Donacia* adults, *Synclita* larvae*, Rhopalosiphum* aphids, and odonate eggs were apparent on non-treated *N. mexicana*, but changes in leaf density within cages were less obvious since new leaves were continuously emerging while highly damaged leaves were decaying. As mentioned previously, Wallace and O'Hop (1985) documented increased leaf turnover rates in *Nuphar luteum* in the presence of herbivores. Observations from this study imply that leaf turnover rate increased in non-treated *N. mexicana* plants which were subject to various types of invertebrate damage. This would make it difficult to determine biomass differences between treatments and could result in underestimates of the impact of invertebrates to *N. mexicana*. In contrast, treated *N. mexicana* plants were mostly void of any signs of invertebrate damage other than *Rhopalosiphum* aphids. Aphids were present in both treatment areas in large quantities at harvest II & III and possibly slowed plant growth by draining nutrients. Without aphids in treated samples biomass may have increased at a rate greater than non-treated plants. Therefore even though plant conditions from the two treatments were widely different, combined effects of increased leaf turnover rate in non-treated plants and aphid herbivory in treated plants made it difficult to identify biomass difference due to the impact of invertebrates on *N. mexicana*.

Figure 32. Mean (± 0.95 confidence interval) dry biomass (g) of *N. mexicana* collected per treatment area at each harvest. A one-way ANOVA was performed at harvest III, treatment: p = 0.144, F = 2.258, DF = 1, 28.



Both *Potamogeton* species followed similar trends throughout the study and biomass between treatments was significantly different at the final harvest (harvest III) (Figures 33 & 34). Biomass from both treatment areas increased from harvest I – II as plants became established in the ponds and invertebrates were not present in high enough quantities to greatly impact plant growth. Treated plant biomass remained stable into the final harvest and growth may have been slowed by the increased presence of *Rhopalosiphum* aphids. In contrast, non-treated biomass decreased

following harvest II as plants sustained high levels of invertebrate damage mostly from *Rhopalosiphum* aphids, *Synclita*, *Paraponyx*, and *Hydrellia* larvae. Non-treated biomasses of *P. nodosus* and *P. illinoensis* were reduced by 40% and 63% respectively when compared to treated dry weights at the final harvest. Invertebrate herbivory and non-consumptive damage were shown to significantly impact both *Potamogeton* species.

Differences in plant biomass throughout the study and between treatments at the final harvest (harvest III) varied based on plant species. The null hypothesis that there would be no differences in dry biomass between treatments at harvest III was accepted for *V. americana* and *N. mexicana*. *Vallisneria americana* was difficult to establish in both treatment areas, possibly due to light competition from other species, and signs of invertebrate damage were rare. In contrast, *N. mexicana* exhibited high levels of invertebrate damage, primarily herbivory, case making, and ovipositing. Yet biomass differences were hard to perceive possibly due to increased leaf turnover rate in non-treated replicates and slowed growth due to aphids in treated replicates. The null hypothesis was rejected for *P. nodosus* and *P. illinoensis*. Non-treated biomasses of both *Potamogeton* species were significantly less than treated at harvest III.

Figure 33. Mean (± 0.95 confidence interval) dry biomass (g) of *P. nodosus* collected per treatment area at each harvest. A one-way ANOVA was performed at harvest III, treatment: p = 0.003, F = 10.568, DF = 1, 28. Means with the same letter are not significantly different.

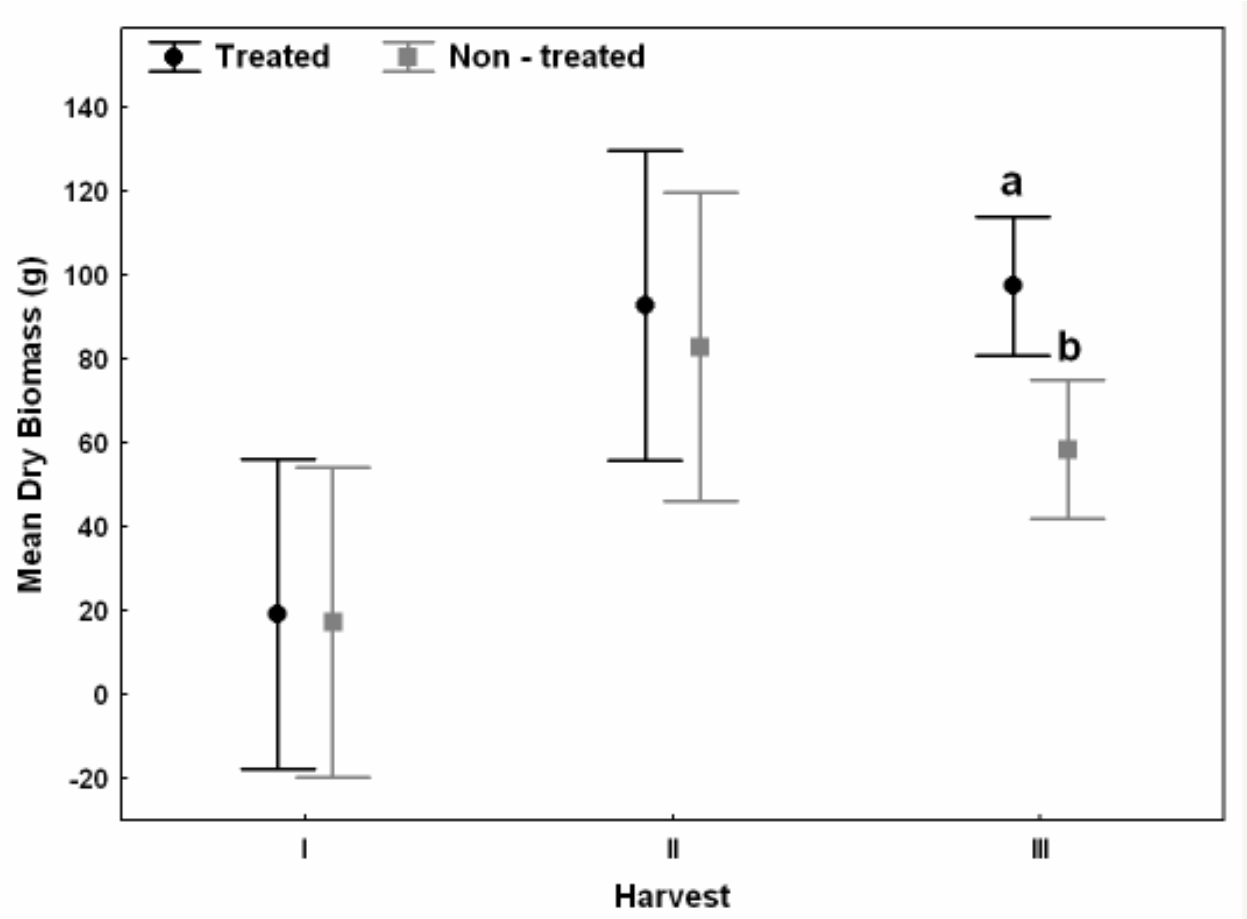
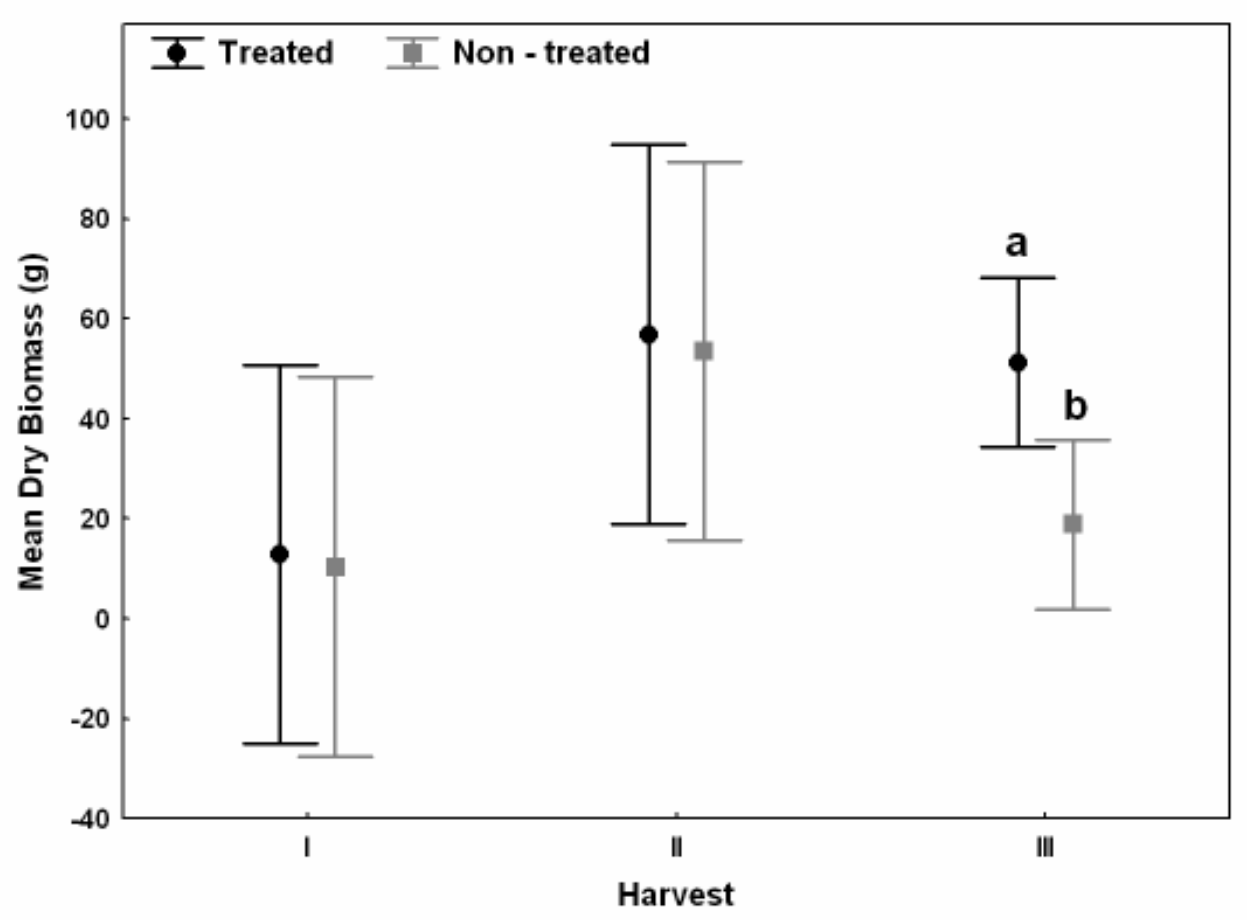


Figure 34. Mean (± 0.95 confidence interval) dry biomass (g) of *P. illinoensis* collected per treatment area at each harvest. A one-way ANOVA was performed at harvest III, treatment: p = 0.010, F = 7.668, DF = 1, 28. Means with the same letter are not significantly different.



CHAPTER 5

CONCLUSIONS

The main objective of this study was to investigate the impact of invertebrates to four native macrophytes, *V. americana*, *P. nodosus*, *P. illinoensis*, and *N. mexicana*. Two treatment areas, a non-treated control and an insecticide treatment, were created in three ponds so that comparisons could be made between plants with and without invertebrate interactions. The insecticide effectively removed most invertebrates including Ephemeroptera, Coleoptera, Diptera, Trichoptera, Lepidoptera, Odonata, and Hemiptera when aphid counts were excluded. Ten herbivore taxa were collected during the duration of the study including; *Synclita*, *Paraponyx*, *Donacia*, *Rhopalosiphum*, and *Hydrellia*. Invertebrate communities collected from the four macrophytes did not show similarities based on plant or pond. Instead, communities were separated into two main groupings based on treatment. Non-treated invertebrate communities were more diverse than treated in this particular study since insecticide applications removed most invertebrates from treated samples.

Few signs of invertebrate damage were observed on *V. americana* and consequently statistical differences between treatment areas in percent invertebrate leaf damage and biomass were not attained. More research should be conducted to see if *V. americana* possesses properties that make it an undesirable food source for invertebrates. Substantial levels of invertebrate leaf damage to non-treated samples were observed for *N. mexicana* and both *Potamogeton* species, yet statistical significance was only reached for *P. nodosus*. In order to document maximum herbivory levels in future studies, leaves should be marked prior to damage and

damage levels should be recorded throughout the life of the leaf. Statistically significant differences in biomass were not measured for *N. mexicana*. Observations of plant growth throughout the study suggest that leaf turnover rate may have increased in plants subject to herbivory and *Rhopalosiphum* aphids may have slowed the growth of treated plants. In combination, these two effects would make it difficult to identify changes in biomass for *N. mexicana*. Statistically significant reductions in biomass due to invertebrate herbivory and non-consumptive damage were achieved for both *Potamogeton* species. The biomasses of *P. nodosus* and *P. illinoensis* were reduced by 40% and 63% respectively. Herbivory, once thought to be insignificant to aquatic macrophytes, was shown to cause substantial reduction in biomass in two of the plant species studied.

APPENDIX

TAXA LIST OF ALL INVERTEBRATES COLLECTED

Life stages are abbreviated as follows: larvae (L), pupae (P), adult (A).

Class / Order	Family	Subfamily / Genus	Life Stage(s)
Gastropoda	Planorbidae	Helisoma	
Gastropoda	Physidae	Physa	
Oligochaeta			
Coleoptera	Chrysomelidae	Donacia	L, A
Coleoptera	Dytiscidae	Celina	L
Coleoptera	Dytiscidae	Cybister	L
Coleoptera	Dytiscidae	Dytiscus	L
Coleoptera	Gyrinidae	Dineutus	L
Coleoptera	Haliplidae	Haliplus	L, A
Coleoptera	Hydrophilidae	Berosus	L, A
Coleoptera	Hydrophilidae	Tropisternus	L
Coleoptera	Noteridae	Hydrocanthus	L, A
Diptera	Ceratopogonidae	Bezzia, Palypomyia	L, P
Diptera	Ceratopogonidae	Dasyhelea	L
Diptera	Chironomidae	Chironominae	L, P
Diptera	Chironomidae	Orthocladiinae	L, P
Diptera	Chironomidae	Tanypodinae	L, P
Diptera	Ephydridae	Hydrellia	L
Diptera	Stratiomyidae	Odontomyia	L, A
Diptera	Tabanidae	Chrysops	L
Ephemeroptera	Baetidae	Callibaetis	L
Ephemeroptera	Caenidae	Caenis	L
Hemiptera	Aphidiae	Rhopalosiphum	L, A
Hemiptera	Belostomatidae	Belostoma	L
Hemiptera	Hebridae	Merragata	L, A
Hemiptera	Mesoveliidae	Mesovelia	L, A
Hemiptera	Naucoridae	Limnocoris	L
Hemiptera	Naucoridae	Pelocoris	L, A
Hemiptera	Notonectidae	Notonecta	L, A
Hemiptera	Veliidae	Microvelia	A
Lepidoptera	Noctuidae	Archanara	L
Lepidoptera	Pyralidae	Synclita	L, P
Lepidoptera	Pyralidae	Paraponyx	L, P, A
Odonata	Aeshnidae	Anax	L

Odonata	Coenagrionidae	Coenagrion, Enallagma	L
Odonata	Coenagrionidae	Telebasis	L
Odonata	Gomphidae	Erpetogomphys	L
Odonata	Libellulidae	Libellula	L
Odonata	Libellulidae	Orthemis	L
Odonata	Libellulidae	Cordulia	L
Odonata	Libellulidae	Somatochlora	L
Trichoptera	Hydroptilidae	Oxythira	L, P
Trichoptera	Hydroptilidae	Hydroptila	L, P
Trichoptera	Leptoceridae	Nectopsyche	L
Trichoptera	Leptoceridae	Oecetis	L, P

REFERENCES

Boavida, P., Neuenschwander, P., Herren, H.R., 1995. Experimental assessment of the impact of the introduced parasitoid *Gyranusoidea tebygi* Noyes on the mango mealybug *Rastrococcus invadens* Williams, by Physical Exclusion. Biological Control 5, 99-103.

Blackman, R., 1974. Aphids. Ginn & Company Limited, London and Aylesbury.

Center, T.D., Dray, F.A. Jr., Jubinsky, G.P., Grodowitz, M.J., 1999. Insects and other arthropods that feed on aquatic and wetland plants. Technical Bulletin No. 1870. U.S. Department of Agriculture, Agricultural Research Service.

Chilton, E.W. II, 1990. Macroinvertebrate communities associated with three aquatic macrophytes (*Ceratophyllum demersum*, *Myriophyllum spicatum*, and *Vallisneria americana*) in Lake Onalaska, Wisconsin. Journal of Freshwater Ecology 5 (4), 455-466.

Cronin, G., Wissing, K.D., Lodge, D.M., 1998. Comparative feeding selectivity of herbivorous insects on water lilies: aquatic vs. semi-terrestrial insects and submersed vs. floating leaves. Freshwater Biology 39, 243-257.

Deonier, D.L., 1971. A systematic and ecological study of nearctic *Hydrellia* (Diptera: Ephydridae). Smithsonian Institution Press, Washington.

Dibble, E.D., Killgore, K.J., Harrel, S.L., 1996. Assessment of fish-plant interactions. In: Miranda, L.E., DeVries, D.R. (Eds.), Multidimensional approaches to reservoir fisheries management. American Fisheries Society Symposium 16, 347-356.

Dick, G.O., Smart, R.M., Keiser, E.D., 1995. Populations of turtles and their potential impacts on aquatic plants in Guntersville Reservoir, Alabama. Joint Agency

Guntersville Project Aquatic Plant Management, Tennessee Valley Authority Report.

Doyle, R.D., Smart, R.M., 1995. Competitive interactions of native plants with nuisance species in Guntersville Reservoir, Alabama. In: Proceedings, 29th Annual Meeting, Aquatic Plant Control Research Program. Miscellaneous Paper A-95-3. Vicksburg, MS: U.S. Army Engineer Waterways Experiment Station, pp. 237-242.

Doyle, R.D., Smart, R.M., Guest, C., Bickell, K., 1997. Establishment of native aquatic plants for fish habitat: test plantings in two north Texas reservoirs. Lake and Reservoir Management 13, 259-269.

European and Mediterranean Plant Protection Organization (EPPO), 2006. *Hydrocotyle ranunculoides*. EPPO Bulletin 36 (1), 3-6. doi:10.1111/j.1365-338.2006.00945.x.

Habeck, D.H., 1974. Caterpillars of *Paraponyx* in relation to aquatic plants in Florida. Hyacinth Control Journal 12, 15-17.

Harley, K.L.S., Forno, I. W., 1992. Biological control of weeds a handbook for practitioners and students. Inkata Press, Melbourne.

Heitmeyer, M.E., Vohs, P.A. Jr., 1984. Distribution and habitat use of waterfowl wintering in Oklahoma. Journal of Wildlife Management 48,51-62.

Hoffman, C.E., 1940. Limnological relationships of some northern Michigan *Donaciini* (Chrysomelidae; Coleoptera). Transactions of American Microscopical Society 59 (3), 259-274.

Jacobsen, D., Sand-Jensen, K., 1992. Herbivory of invertebrates on submerged macrophytes from Danish freshwaters. Freshwater Biology 28, 301-308.

Lodge, D.M., 1991. Herbivory on freshwater macrophytes. Aquatic Botany 41, 195-224.

Marx, E.J.F., 1957. A Review of the Subgenus *Donacia* in the Western Hemisphere (Coleoptera, Donaciidae). Bulletin of the American Museum of Natural History 112 (3).

Nachtrieb, J.G., Grodowitz, M.J., Smart, R.M., 2007. Impact of invertebrate herbivory on native aquatic macrophytes. APCRP-BC-09, U.S. Army Engineer Research and Development Center, Vicksburg, MS.

Newman, R.M., 1991. Herbivory and detritivory on freshwater macrophytes by invertebrates: A review. Journal of the North American Benthological Society 10, 89-114.

Riley, E.G., Clark, S.M., Flowers, R.W., Gilbert, A.J., 2002. Chrysomelidae Latreille 1802. In: Arnett, R.H. Jr., Thomas, M.C., Skelley, P.E., Frank, J.H. (Eds.), American Beetles Volume 2 Polyphaga: Scarabaeoidea through Curculionoidea. CRC Press, Boca Raton London New York Washington, D.C., pp. 617-691.

Sand-Jensen, K., Madsen, T.V., 1989. Invertebrates graze submerged rooted macrophytes in lowland streams. Oikos 55, 420-423.

Savino, J.F., Stein, R.A., 1982. Predator-prey interactions between largemouth bass and bluegills as influenced by simulated, submerged vegetation. Transactions of the American Fisheries Society 111, 225-266.

Schooler, S., Julien, M., Walsh, G.C., 2006. Predicting the response of *Cabomba caroliniana* populations to biological control agent damage. Australian Journal of Entomology 45, 327-330.

Sculthorpe, S.P., 1967. The biology of aquatic vascular plants. Edward Arnold, London.

Shelford, V.E., 1918. Conditions of existence. In: Ward, H.B., Whipple, G.C. (Eds.), Freshwater biology. John Wiley, New York, pp. 21-60.

Smart, R.M., 1991. Lewisville aquatic ecosystem research facility: progress report. In: Proceedings, 25th annual meeting, aquatic plant control research program. Miscellaneous Paper A-91-3, US Army Engineer Waterways Experiment Station, Vicksburg, MS, pp. 22-23.

Smart, R.M., 1995. Preemption: an important determinant of competitive success. In: Proceedings, 29th annual meeting, aquatic plant control research program. Miscellaneous Paper A-95-3, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. pp. 231-236.

Smart, R.M., Barko, J.W., McFarland, D.G., 1994. Competition between *Hydrilla verticillata* and *Vallisneria americana* under different environmental eonditions. Technical Report A-94-1, U.S. Army Engineer Research and Development Center, Vicksburg, MS.

Smart, R.M., Dick, G.O., Doyle, R.D., 1998. Techniques for establishing native aquatic plants. Journal of Aquatic Plant Management 36, 44-49.

Smart, R.M., Madsen, J.D., Snow, J.R., Dick, G.O., Honnell, D.R., 1995. Physical and environmental characteristics of experimental ponds at the Lewisville aquatic ecosystem research facility. Miscellaneous Paper A-95-2, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Soszka, G. J., 1975. Ecological relations between invertebrates and submerged macrophytes in the lake littoral. Ekologia Polska 23, 393-415.

Stoops, C.A., Adler, P.H., McCreadie, J.W., 1998. Ecology of aquatic Lepidoptera (Crambidae: Nymphulinae) in South Carolina, USA. Hydrobiologia 379, 33-40.

Titus, J.E., Stephens, M.D., 1983. Neighbor influences and seasonal growth patterns for *Vallisneria americana* in a Mesotrophic Lake. Oecologia 56, 23-29.

U.S. Army Engineer Research and Development Center (ERDC), 2005. Aquatic plant information system (APIS). http://el.erdc.usace.army.mil/aqua/apis/apishelp.html.

Wallace, J.B., O'Hop, J., 1985. Life on a fast pad: waterlily leaf beetle impact on water Lilies. Ecology 66(5), 1534-1544.

Westfall, M.J. Jr., Tennessen, K.J., 1996. Odonata. In: Merritt, R.W., Cummins, K.W. (Eds.), An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Company, Dubuque, Iowa, pp.164-216.

White, D.S., Brigham, W.U., 1996. Coleoptera. In: Merritt, R.W., Cummins, K.W. (Eds.), An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Company, Dubuque, Iowa, pp.399-473.

Zar, J.H., 1999. Biostatistical analysis fourth edition. Prentice Hall, Upper Saddle River, New Jersey.