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Herbaceous Population Response to *Galerucella* species - Biocontrol for Purple Loosestrife (*Lythrum salicaria*)

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EXECUTIVE SUMMARY

Over the past two centuries the natural areas of North America have been subjected to ever increasing pressures from immigrants. Colonization has undermined the integrity of both terrestrial and aquatic ecosystems through the destruction of habitat, the fragmentation of ecosystems and the introduction of invasive exotic species. Specifically, purple loosestrife (*Lythrum salicaria*) has altered aquatic systems by outcompeting native species and subsequently forming monocultures in wetland ecosystems (Blossey *et al*., 2001). Throughout the early 1900's various control methods including flooding, mowing, burning and herbicides were employed in an attempt to control the population (Blossey *et al.*, 2001). Limited success was observed from such management techniques and during the 1980's the need for a more effective course of action was identified. By the end of the decade *Galerucella* species, an Asian beetle that predates specifically on *L. salicaria*, were being released to target sites across North America. In 1993 two sites on the property of Royal Botanical Gardens were included among these (Corrigan *et al.*, 1998). Fifteen years after the initial release, a study was conducted at Royal Botanical Gardens to determine the success of the *L. salicaria* biocontrol. Three locations were selected, including two release sites and one control site. Vegetation in six 1 m^2 plots along a 20 m transect were sampled at each sampling station. Species richness, stem density, percent cover and average height were recorded. In addition to this, percent predation of *L. salicaria* was recorded. Presence of *L. salicaria* was documented at all stations however dominance of the species was not observed at any station indicating a clear reduction in the purple loosestrife population on RBG property. In addition to this, two of the three sites demonstrated less than 20% coverage of exotic species. Species richness ranged from six to fifteen and the Shannon-Weaver diversity index ranged from 0.413 to 1.604. One of the release sites had the highest score on the Shannon-Weaver index (1.604), the highest species richness (15) and the greatest *L. salicaria* stem density (12 stems/plot). The site showing the most predation (40-60% of *L. salicaria* plants in the plots) was the release site with the smallest *L. salicaria* stem density (average = 1.33 stems/plot). In the future, monitoring of the *Galerucella* population should be completed to ensure that *Galerucella* are not having a detrimental effect on native species. In addition to this, vegetation should continue to be monitored to prevent habitat domination by other exotic species

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ACKNOWLEDGEMENTS

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INTRODUCTION

Over the past two centuries the natural areas of North America have been subjected to ever increasing pressures from immigrants. Colonization has undermined the integrity of both terrestrial and aquatic ecosystems through the destruction of habitat, the fragmentation of ecosystems (Mbora and McPeek, 2009; Kolb, 2008) and the introduction of invasive exotic species (Hight *et al.,* 1995; Blossey *et al.,* 2001). Specifically, purple loosestrife (*Lythrum salicaria*) has altered aquatic systems by outcompeting native species, subsequently forming a monoculture and decreasing functionality of the wetland (Blossey *et al*., 2001; Hight *et al.,* 1995; Brown *et al.,* 2006). This is facilitated by *L. salicaria*'s ability to produce high volumes of seed, which subsequently germinate more quickly and successfully than those of its native counterparts (Blossey *et al.,* 2001). *L. salicaria* also disrupts the lifecycle of native species, acting as a contributing factor to the destruction of nesting habitat for waterfowl (Hickey and Malecki, 1997; Lor, 2000).

Throughout the 1900's various control methods including flooding, mowing, burning and herbicides were utilized in an attempt to control the population (Hight *et al.,* 1995; Blossey *et al.,* 2001), but during the 1980's the need for a more effective course of action was recognized. Three different insect species were identified as suitable biological controls for *L. salicaria*: a root-eating weevil, *Hylobius transversovittatus* and two leaf-eating beetles, *Galerucella pusilla* and *G. calmariensis,* jointly referred to as *Galerucella* (Hight *et al.,* 1995). By 1992 all three species were being released to target sites across North America (Hight *et al.,* 1995; Hight and Drea, 1991). On May 26, 1993 two sites on the property of Royal Botanical Gardens, Hamilton-Burlington, Ontario were included among these (Corrigan *et al.,* 1998). At Long Pond, 200 adult breeding pairs of *G. calmariensis* and 200 adult breeding pairs of *G. pusilla* were released (Figure 1). At Mercer's Glen 100 mated adult pairs of both *G. calmariensis* and *G. pusilla* were released (MacKenzie, D, unpublished data). Minimal monitoring has occurred since the initial release, and all existing monitoring has focused on the *Galerucella* population, with little regard to the changing vegetative community. This study provides the basis for a complete vegetative analysis of the release sites and determines the success, measured in terms of species composition, of the *L. salicaria* biocontrol used at the Royal Botanical Gardens.

METHODS

Transect Preparation

Three locations within Royal Botanical Gardens were selected for monitoring, based on physical characteristics of the wetland, station access and previous *Galerucella* releases (Corrigan *et al.*, 1998). The two locations of *Galerucella* release, Mercer's Glen (17T 589651m E 4793196m N) and Long Pond (17T 589735m E 4793593m N), were included in the study as well as a control station on RBG property, Pond 4 (17T 591574m E 4794071m N; Figure 1). Sites were sampled on September 2nd, 2008, September 12th, 2008, and September 29th, 2008 respectively. Each of the sites had fluctuating water levels, were surrounded by wooded areas and had predated *L. salicaria* plants present. A twenty metre transect was marked at each station, starting at the co-ordinates listed above and following the contour of the water line. Distance into each marsh area was standardized across all areas such that 8cm of water depth was present at each end point on the sampling date. Six one meter square plots were measured off each the transect line with 3 meter spacing and plots extending towards the marsh (Figure 2).

Vegetative Sampling

Species accumulation curves confirmed that six quadrats was sufficient to sample 90 percent of the species along the transect. All living plants rooted in the plot were identified to species level (??). For *L. salicaria*; stem density, percent cover, average height per plot and degree of herbivory per plot was determined. The degree of herbivory was rated on a scale of 1 to 5 with each number representing a range of 20 percent total leaf herbivory. For example, a plot rated 1 would have less than 20 percent of the *L. salicaria* leaves predated upon while a plot rated 5 would have more than 80 percent of the *L. salicaria* leaves consumed. For non *L. salicaria* species; the number of stems per species per plot and average height of each species per plot were measured.

Data Analysis

The average predation per quadrat, stem density per quadrat and average plant coverage per quadrat were calculated for *L. salicaria* at each site. Historic percent cover and stem density of *L. salicaria* (Corrigan *et al.*, 1998) were compared to the data collected in 2008. The

proportion of exotics to natives, species richness and species composition for each of the sites was determined. Exotic was defined as anything that is not naturally found in the north east of North America. The Shannon-Weaver diversity index was used to compare the diversity of each site.

Winged Loosestrife

Four 5 x 5 cm plugs of winged loosestrife (*Lythrum alatum*) from the Royal Botanical Gardens aquatic nursery were planted off shore from the location of the Mercer's Glen transect (Figure 1) to determine if *Galerucella* would feed upon the native loosestrife species. The plants were in good health before planting and showed no signs of predation. Observational data was recorded weekly during the month of July.

RESULTS

Vegetation

During the study it was found that all sites had *L. salicaria* present but were not dominated by the plant (Figure 3). In addition to this, two of the three sites (Pond 4 and Long Pond) were composed of less than 20% exotic species (Figure 4). Species richness ranged from six to fifteen (Table 1) and the Shannon-Weaver diversity index ranged from 0.413 to 1.604 (Table 1). Long Pond, a release site, had the highest score on the Shannon-Weaver index (1.604), the highest species richness (15) and the greatest *L. salicaria* stem density (12 stems/plot) (Table 1; Figure 5). The second release site, Mercer's Glen, showed the most predation (40-60% of *L. salicaria* plants in the plots) and the smallest *L. salicaria* stem density (1.33 stems/plot; Table 1, Figure 5). Percent coverage of purple loosestrife per quadrat has decreased at both release sites, compared to data collected in 1996 (D. Mackenzie pers. comm.; Figure 6). The stem density of *L. salicaria* has also decreased for both sites since 1996 (Figure 5).

Winged Loosestrife

The plants quickly showed signs of herbivory. Within the first week of observation the plants had leaf damage and presence of *Galerucella* upon the plants was visually confirmed (Figure 7, Figure 8). In subsequent weeks the plants continued to experience predation. Holes in the leaves appeared, brown spots became abundant and the plants began to develop chlorosis. During the time of observation there was fluctuation in water levels of more than 30 cm. In the third week of observation, the plants were completely underwater and further observations were not carried out.

DISCUSSION

The results of this study demonstrate decreased plant biomass of the invasive *Lythrum salicaria* on RBG property since *Galerucella* release in 1996. Both stem density and percent coverage have improved (Figure 5, Figure 6) and none of the three sampling stations demonstrated dominance of *L. salicaria* (Figure 3).

Whether this is a direct result of *Galerucella* upon the population, or a collective result of several factors is unknown. It is possible that the observed population collapse is a result of competition, specifically at the Mercer's Glen site. Ninety-one percent of the stems sampled at this site were reed canary grass (*Phalaris arundinacea*) (Figure 3). Domination of *P. arundinacea* in former *L. salicaria* stands has also been recorded in other locations (Blossey *et al.,* 2001) and it has been shown that *P. arundinacea* can outcompete native species (Houlahan and Findlay, 2004). Alternatively, it is possible that *P. arundinacea* took advantage of the niche vacated by *L. salicaria* once the population was weakened by the *Galerucella*. Long Pond and Pond 4 do not show such a dominance of exotic plant species, likely a result of their isolation from major roadways. Mercer's Glen is located adjacent to the 403, a major highway in Southern Ontario. While Pond 4 is located near Plains Road, there is a wooded slope separating the water body and the roadway. Long Pond has a high level bridge (x m elevation) that spans the northern tip of the water body but here the road is separated from the water body by 100 m of open air.

Both the native and exotic loosestrife showed evidence of predation, a trend observed by others as well. Blossey *et al.* (2001) have shown that *Galerucella* will feed on native plants, specifically *L. alatum* and waterwillow (*Decodon verticillatus*) if there is not sufficient *L. salicaria* available or if *Galerucella* are present in high densities. Albright *et al.* (2004) have also observed feeding upon red osier dogwood (*Cornus stolonifera*) and speckled alder (*Alnus rugosa*) and noted both dispersal and aestivation (summer hibernation) of *Galerucella* when *L. salicaria* was rare. During the study, native plants sampled were not subject to predation quantification and so only *L. alatum* was observed to have *Galerucella* predation. In the future, one should look specifically for evidence of *Galerucella* predation upon both native and exotic species. This evidence includes the presence of larva, eggs, adults, shot gun holes (holes through

the leaf created by feeding of the adult form) and windows (the consumption of the lower epidermis of the leaf, created by the larval form) (Dech and Nosko, 2002; Hight and Drea, 1991). It should be noted that the predation observed on both the native and exotic loosestrife species cannot entirely be attributed to *Galerucella.* There are many native insect species that also consume these marsh plants although not at a level sufficient to control the population (Blossey *et al.,* 2001; Dech and Nosko, 2002).

Of note is the fact that the *L. salicaria* population at Long Pond showed late season regrowth. This in turn would affect the rank assigned for predation. According to Environmental Monitoring and Assessment Network (EMAN) protocols, a complete vegetative study should include three surveys over a year, once in the spring, summer, and fall (Roberts-Pichette and Gillespie, 1999). This will make certain that season regrowth is accounted for. It is also possible that the variation in predation between sites was a result of sampling over a longer time period. In the future, all sampling should be completed over a period of two weeks.

Another consideration that should be addressed is the potential for the hybridization and competition of *L. salicaria* with the native species *L. alatum,* as RBG has an exposed nursery population of the species. Although Houghton-Thompson *et al.* (2005) determined that the hybridization rate was low and therefore a minor threat, it is still a possibility. This is especially true in light of the fact that Houghton-Thompson *et al.* (2005) predict that multiple introductions of *L. salicaria* must occur if a new genotype is to be expressed. In regards to competition, Blossey *et al.* (2001) demonstrated that *L. salicaria* outcompetes *L. alatum* by more effectively attracting pollinators, therefore reducing the pollination of *L. alatum*. In the future, the *L. alatum* population should be carefully monitored to protect the integrity of the population's genetics.

FUTURE DIRECTIONS

In the future, a more complete assessment of the *Galerucella* population should be completed. Egg masses, larvae and adults should be enumerated using methods developed during the initial study completed by Corrigan *et al.* (1998). Marsh areas surrounding release sites should be assessed to determine if and how far the population has dispersed. This information will facilitate the comparison of historic and current *Galerucella* populations. Additionally, *Galerucella* predation upon native species should be quantified, to develop a better understanding of the predation that is occurring and to ultimately protect the native plant populations. Once the *Galerucella* population is better understood, one can then determine further actions for the management of the population.

In addition to this, vegetation should continue to be monitored to prevent domination by other invasive plants that could occupy the niche vacated by *L. salicaria*. *L. alatum* requires special attention as it could hybridize with and be out-competed by *L. salicaria*. Vegetation should be monitored three times a year to obtain a more complete list of vegetation present along each transect.

Additional shores of the marsh should be included in the survey to account for variation in fetch and exposure. Transects perpendicular to the shoreline would help to characterize the transition zone from land to water. Permanent transects should be considered for improved comparison between years, however fluctuation in water levels may make this challenging.

It is apparent that *L. salicaria* populations have been weakened, however, it seems that the sensitive wetland habitats are still falling victim to other exotic species. In light of this, management of wetland plant communities should continue and control methods of *P. arundinacea* should be investigated. It is thought that removal coupled with intensive native revegetation efforts may be successful (Reinhardt Adams and Galatowitsch, 2008).

TABLES AND FIGURES

Figure 1. Location of Transects and Release Sites. The red dot represents transect location. The yellow dot represents release sites.

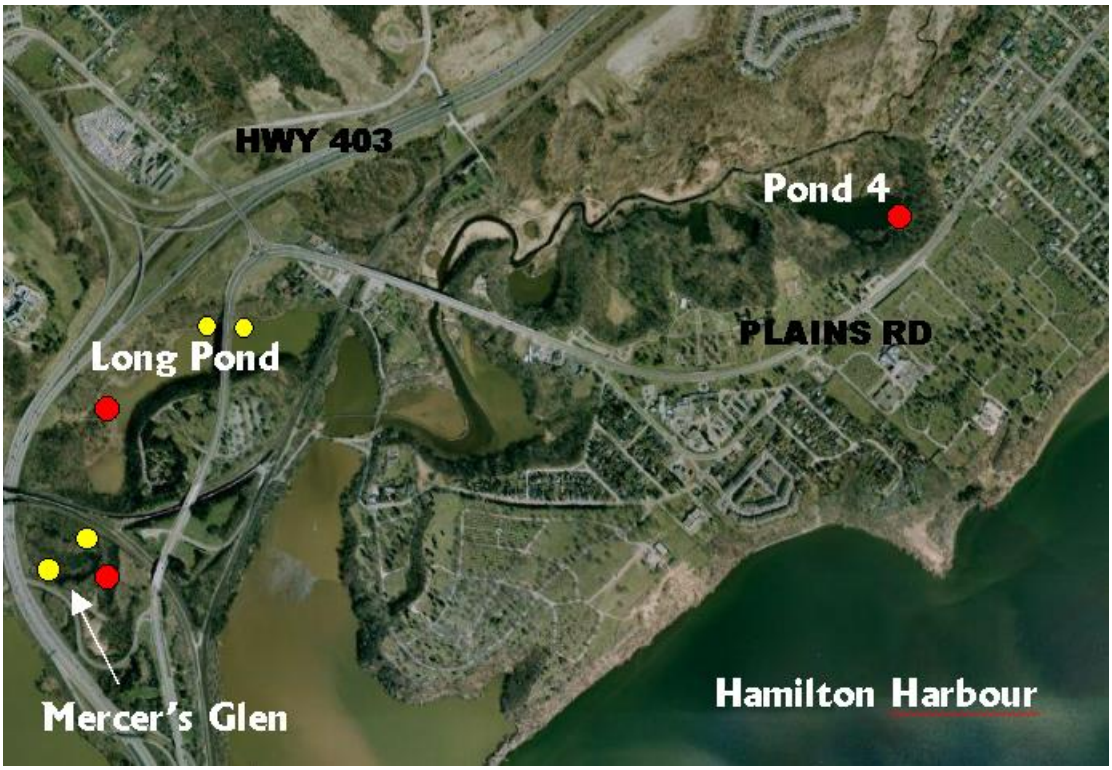


Figure 2. Standard Transect

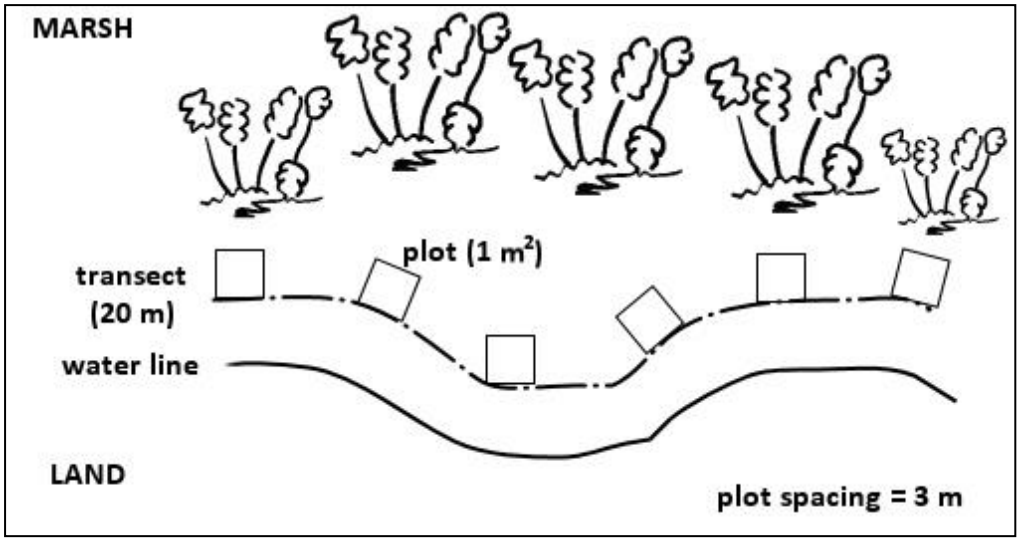
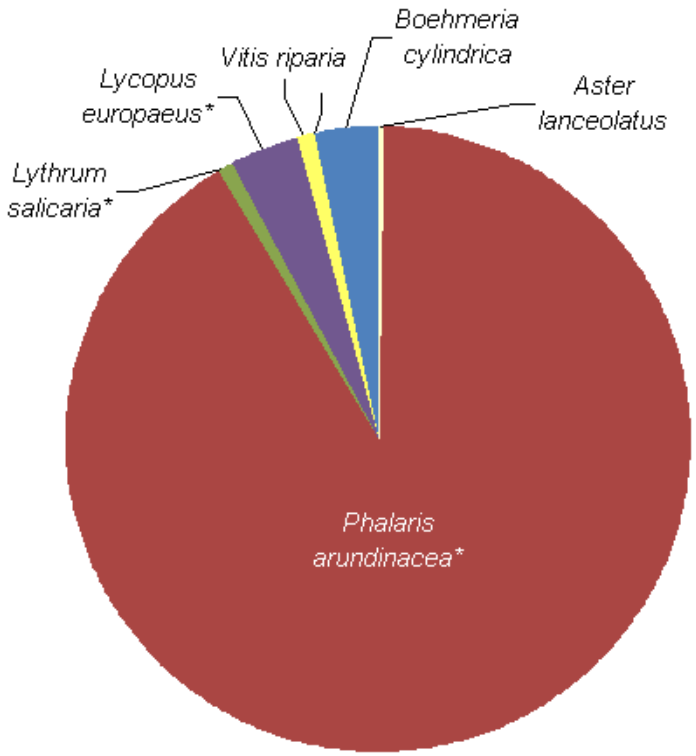


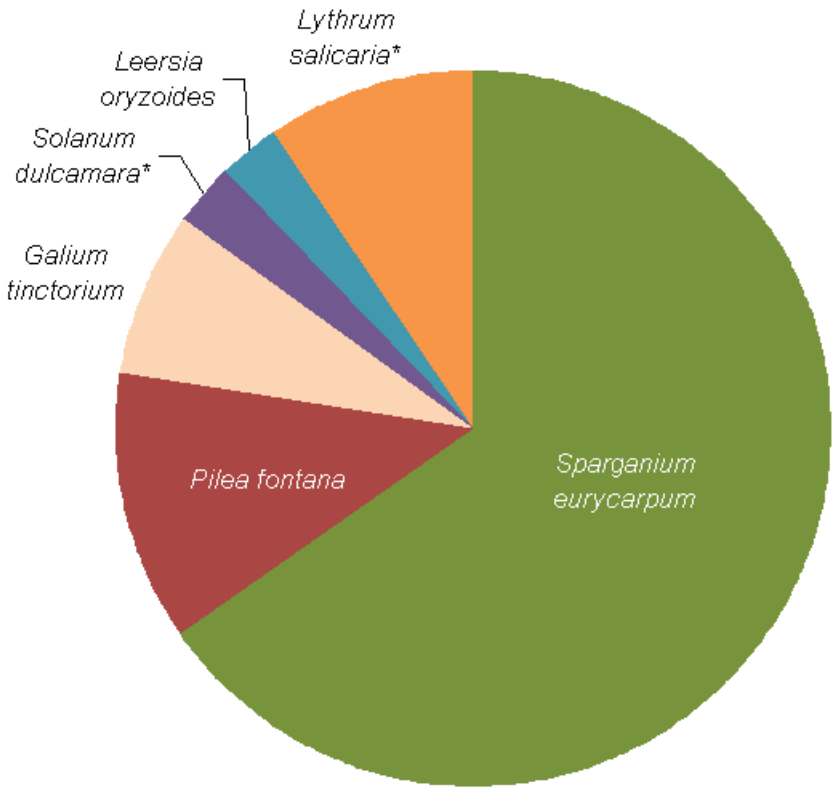
Figure 3. Species Composition of:

a) Mercer's Glen



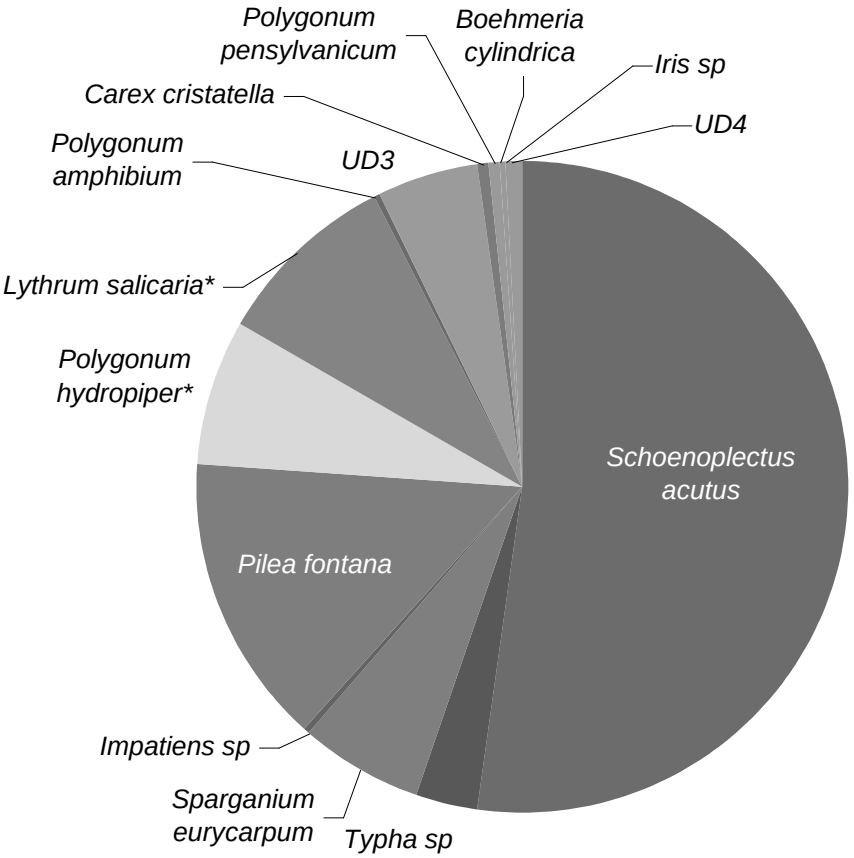
* indicates exotic species

b) Pond 4 (Control)



* indicates exotic species

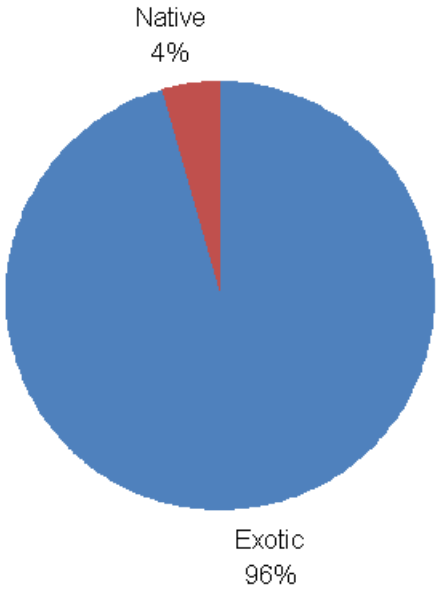
c) Long Pond. UD3 and UD4 are unidentified species



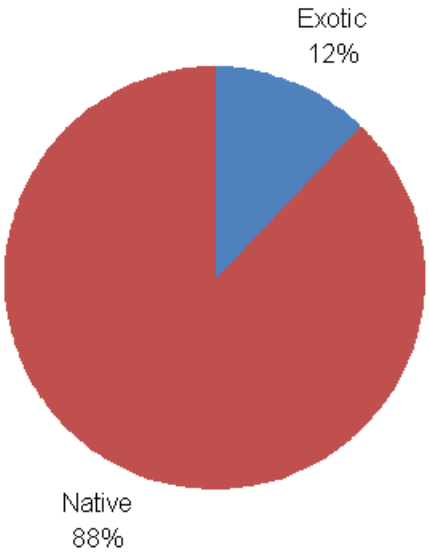
* indicates exotic species

Figure 4. Proportion of natives and exotics for:

a) Mercer's Glen



b) Pond 4 (Control)



c) Long Pond

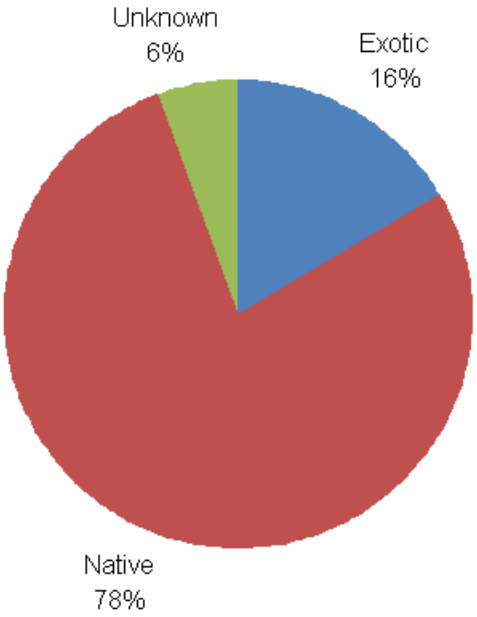


Figure 5. Current and historic stem density of purple loosestrife (*L. salicaria*) for Mercer's Glen, Long Pond and Pond 4

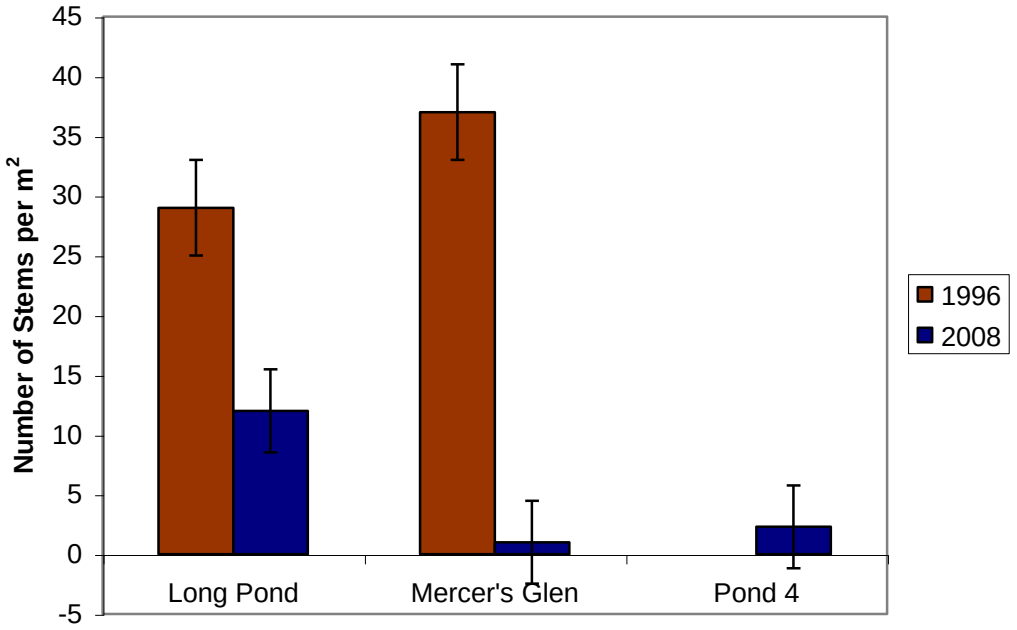
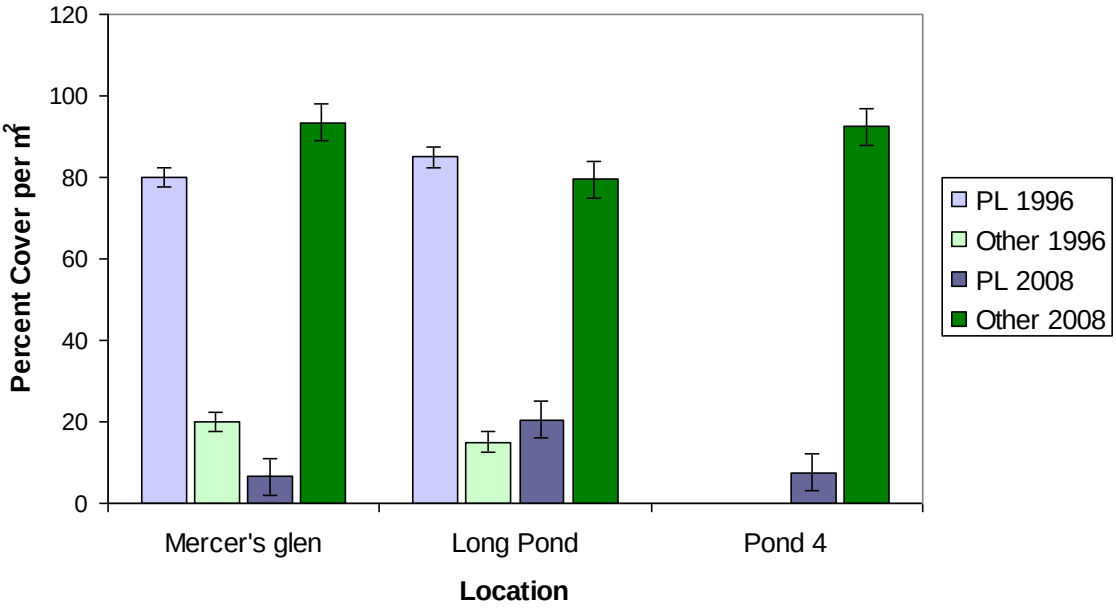


Figure 6. Current and historic percent cover of purple loosestrife (*L. salicaria*) for Mercer's Glen, Long Pond and Pond 4. The error bars represent standard error. PL refers to purple



loosestrife

Figure 7. Winged Loosestrife (*L. alatum*) predation.

Figure 8. *Galerucella* beetle feeding on a Winged Loosestrife plant

Table 1. Stem Density, Shannon-Weaver Diversity Index and Percent Predation for Mercer's Glen, Long Pond and Pond 4.

	Mercer's Glen	**Long Pond**	**Pond 4**
Stem Density per m^2	6	15	6
Percent Leaf Consumption per m^2	45	10	15
Shannon-Weaver Diversity Index	0.413	1.604	1.149

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