IMPACTS OF *GALERUCELLA CALMARIENSIS* AND *G. PUSILLA* ON *LYTHRUM SALICARIA* IN INDIANA

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ABSTRACT.   *Lythrum salicaria,* an invasive wetland hydrophyte native to Eurasia, has spread across Indiana since 1900. Two *Galerucella* spp. have been utilized as biological control agents for *L. salicaria* in Indiana since 1994. This study examines the impact of *Galerucella* spp. over an 8-10 year period at four Indiana wetlands. *Galerucella* abundance varied substantially over time but had low Spearman's ρ at three sites (ρ = 0.21 to 0.40) due to rapid decreases following reduction in *Lythrum*. In contrast, hydrophyte species richness and percent cover were both correlated with time and had higher ρ (0.37 to 0.69) at three sites. The number of *Lythrum* inflorescences and stem density were reduced at all four sites, with inflorescences showing the strongest correlation (ρ = −0.46 to −0.78). Although variation was observed between sites, the introduction of *Galerucella* spp. resulted in significant declines in *L. salicaria* at each of the wetlands. Their impacts at these wetlands strongly suggest that *Galerucella* spp. can play a major role in controlling this invasive plant species.

Keywords: *Galerucella*, *Lythrum salicaria*, purple loosestrife, biological control, invasive species, Indiana wetlands

INTRODUCTION

Lythrum salicaria, a wetland hydrophyte native to Eurasia, is considered an invasive species in North America. Although the first observation of *L. salicaria* in North America occurred in New England in 1814 (Mal et al. 1992) and the earliest record in Indiana is from 1900, its spread was considered minor until after 1940 (Stuckey 1980). Today, *L. salicaria* is found throughout Indiana, though it is most common in the northern counties. Additionally, *L. salicaria* occurs in 47 of the contiguous States (it is absent from Florida) (Blossey et al. 2001).

Lythrum salicaria can form extensive stands which many have characterized as monotypic (Malecki et al. 1993). The slightly square stems can grow in clumps of 30–50 from a single taproot, while a terminal spike of tightly clustered flowers may exceed 1 meter in length (Mal et al. 1992). As a result, a single plant can produce over 2.5 million seeds in a single growing season (Malecki et al. 1993). This prolific seed production, coupled with their persistence in a seed bank, permits high recruitment of seedlings even after removal of adult plants (Welling & Becker 1990). Likewise, adult plants are highly competitive (Gaudet & Keddy 1995; Mal et al 1997; Weihe & Neely 1997; Farnsworth & Ellis 2001), especially in non-flooded conditions (Weiher et al. 1996). This results in reductions in species richness (Schooler & McEvoy 2006) as well as density of associated species such as grasses and sedges (Gabor et al. 1996).

Due to its large showy floral display, *L. salicaria* may reduce seed set of native species such as *L. alatum* through competition for pollinators (Brown et al. 2002). Other studies (Brown & Mitchell 2001; Da Silva & Sargent 2011) demonstrate that pollination of *L. alatum* or *Decodon verticillatus* with mixtures that include *L. salicaria* pollen reduce seed set by as much as 1/3.

Replacement of *Typha* (and other graminoids) by *L. salicaria* may have a notable

impact on wetland function. Its more phosphorus rich leaves drop earlier and decompose more quickly (Bärlocher & Biddiscombe 1996; Emery & Perry 1996; Grout et al. 1997) and likely lead to changes in soil chemistry when compared to communities dominated by *Typha angustifolia* or *Phragmites austalis* (Templer et al. 1996).

Whitt et al. (1999) found that avian species diversity decreased in wetlands where *L. salicaria* was a dominant species. Muskrat (*Agelaius phoeniceus*) use and long-billed marsh wren (*Cistothorus palustris*) nesting were shown to be lower in *L. salicaria* stands than adjacent *Typha* spp. stands (Rawinski 1982; Rawinski & Malecki 1984). Lor (2000) examined the use of *L. salicaria* by many avian species for feeding and nesting. Virginia rail (*Rallus limicola*), sora (*Porazana coaolina*), least bittern (*Ixobrychus exilis*), American bittern (*Botaurus lentiginosus*), and pied-billed grebe (*Podilymbus podicpes*) were found to avoid *L. salicaria* stands. Meanwhile, nearby areas with *Typha* spp. provided habitat for many of these species.

While many studies have identified negative effects of *L. salicaria*, there remains some debate as to whether it is causing damage to native wetland communities. Hager & Vinebrooke (2004) studied six Minnesota wetlands and found that species richness was significantly greater in wetlands that had been invaded by *L. salicaria* compared to *Typha angustifolia* wetlands. A review of the relationship of *L. salicaria* with native flora and fauna by Anderson (1995) determined that, from the literature existing at the time of his review, the affect of *L. salicaria* on wetland ecosystems and native species was unclear. Additionally, Farnsworth & Ellis (2001) found no significant effect between density of *L. salicaria* stems and species richness.

Biological control.—Classical biological control involves the introduction of natural plant enemies to control the introduced plant species (Hight & Drea 1991). In its native European range, *L. salicaria* does not form large monotypic or dominant stands due to control of reproduction and growth provided by native insect herbivores (Blossey et al. 1994) as well as genetic differences in growth patterns (Chun et al. 2010).

Attempts to control *L. salicaria* began in the 1950's, but initial efforts employing flooding, cutting, and burning were largely unsuccessful (Skinner et al. 1994; Blossey et al. 2001). Hand pulling plants was the most successful of early control methods, but required pulling of entire rootstocks, which is highly labor intensive. Chemical control has primarily utilized glyphosate, 2,4-D, or triclopyr; however, because of large and long-persisting seed bank (Welling & Becker 1990), spraying must be repeated (Skinner et al. 1994; Blossey et al. 2001), perhaps every two to three years (Gabor et al. 1996). Additionally, the non-selective nature of chemical control reduced populations of sedges, grasses, cattails, and other native wetland plants (Skinner et al. 1994; Gabor et al. 1996).

Due to the lack of effective control methods and the continued spread of *L. salicaria*, a biological control program was established in North America (Hight & Drea 1991; Malecki et al. 1993; Blossey et al. 2001). Ultimately four insect species were approved for release, *Hylobius transversovittatus* Goeze (a root-mining weevil), *Nanophyes marmoratus* Goeze (a flower feeding weevil), *Galerucella calmariensis* L., and *G. pusilla* Duft (two leaf-beetles) (Blossey et al. 2001). Insect releases began in the United States in 1992 as did the monitoring of their impact on *L. salicaria* and its associated wetland community (Blossey et al. 2001). Results of these releases have been published from across the United States, from two to ten years post-release (Piper 1996; Dech & Nosko 2002; Landis 2003; Grevstad 2006).

The earliest results of biological control of *L. salicaria* occurred in Washington State (Piper 1996) following the release of *Galerucella* spp. in 1992 and 1993. Surveys conducted in 1993 and 1994 found that *Galerucella* spp. had become established at all eight release sites. In another study Dech & Nosko (2002) found that, in Ontario, establishment of *Galerucella* spp. was slow and the populations remained small throughout the three year study period. The *G. pusilla* population crashed in the second year after release, and only minor feeding damage was observed by *Galerucella* spp. over this short time period. In contrast, Landis (2003) found 100% establishment of 24 releases made in Michigan between 1994 and 1999. Within 4–5 years, large populations were observed at all three 1999 release sites. In potted *L. salicaria*, Stamm-Katovich et al. (1999) found that biomass was reduced after a single growing season when *Galerucella* spp. were present. In a natural setting, several years
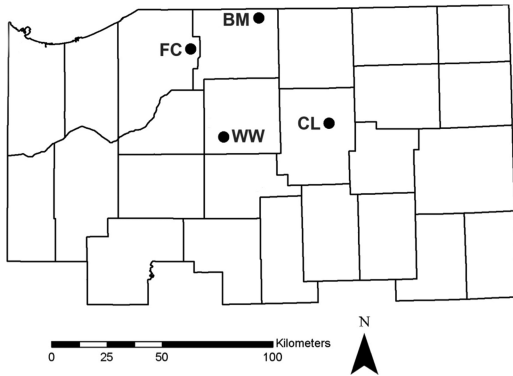
Figure 1.—*Galerucella* spp. transect analysis sites in northern Indiana, USA. BM=Bonneyville Mills, CL=Chapman Lake FC=Fish Creek, WW=Wilson Wetland.

often are required for the impacts of *Galerucella* spp. to be observable (Piper 1996; Dech & Nosko 2002; Landis 2003). Once established, though, Landis (2003) measured suppression of flowering, up to a 98% reduction in stem numbers, and an increase in site species richness. These results, however, did not occur at all sites or in a uniform time. A Minnesota wetland showed a 95% reduction in flowering, and a 50% reduction in stem height four years after release (Blossey & Skinner 2000). In this case, the number of stems per square meter remained constant throughout the sampling period. Grevstad (2006), in New York State, examined the ten-year impacts of *Galerucella* spp. and found that 24 of 36 release sites had *Galerucella* spp. present after ten years. Although only a single site had no persisting *L. salicaria*, as an apparent result of *Galerucella* spp., stem height was reduced by 26% between 1994 and 2004 and reduced flowering was observed at sites with moderate to high plant damage.

In 1994 the Indiana Department of Natural Resources (IDNR), Division of Nature Preserves began releasing *Galerucella* spp. to various wetlands. Additional releases continue as deemed necessary. In order to evaluate the impact of biological control agents in Indiana, the IDNR began monitoring at one release sites in 1996, two additional sites in 1997, and a fourth site in 1999 following the protocol of Blossey (1994). In this study we report the impacts of *Galerucella* spp. on *L. salicaria* and the potential changes in wetland species richness at these Indiana sites.

Table 1.—*Galerucella* abundance categories.

Abundance category	Number of egg masses, larvae, or adults
1	0
2	1–9
3	10–49
4	50–99
5	100–499
6	500–1000
7	<1000

METHODS

Sites and transects.—The releases described in this study occurred in 1996 at three sites and 1999 at the fourth site. The 1996 releases were at Fish Creek, Wilson Wetland, and Bonneyville Mills and the 1999 release site was at Chapman Lake (Fig. 1).

The Fish Creek site (on private property in LaPorte County, Indiana) was a fen grading into a sedge meadow along the west side of the creek. The wetland extended several kilometers above and below the sampling site and, at the start of the study, purple loosestrife was abundant throughout the drainage.

Wilson Wetland (at Culver Academy in Marshall County) was a long, narrow, constructed wetland created by adding sinuosity to a ditched stream channel. The transition from upland to wetland was fairly abrupt and the soils did not have the accumulated organic matter typical of more natural wetlands. Purple loosestrife was also present upstream from the sampling site, and in nearby roadside ditches.

Bonneyville Mill (located along the Little Elkhart River in Bonneyville Mill County Park, Elkhart County) was a ca. 0.2 hectare wetland in a backwater just below the dam for the mill pond. The substrate was a floating mat of plant roots over muck deposits, the least stable substrate of the sites.

Table 2.—Midpoints used for percent cover and percent damage variables.

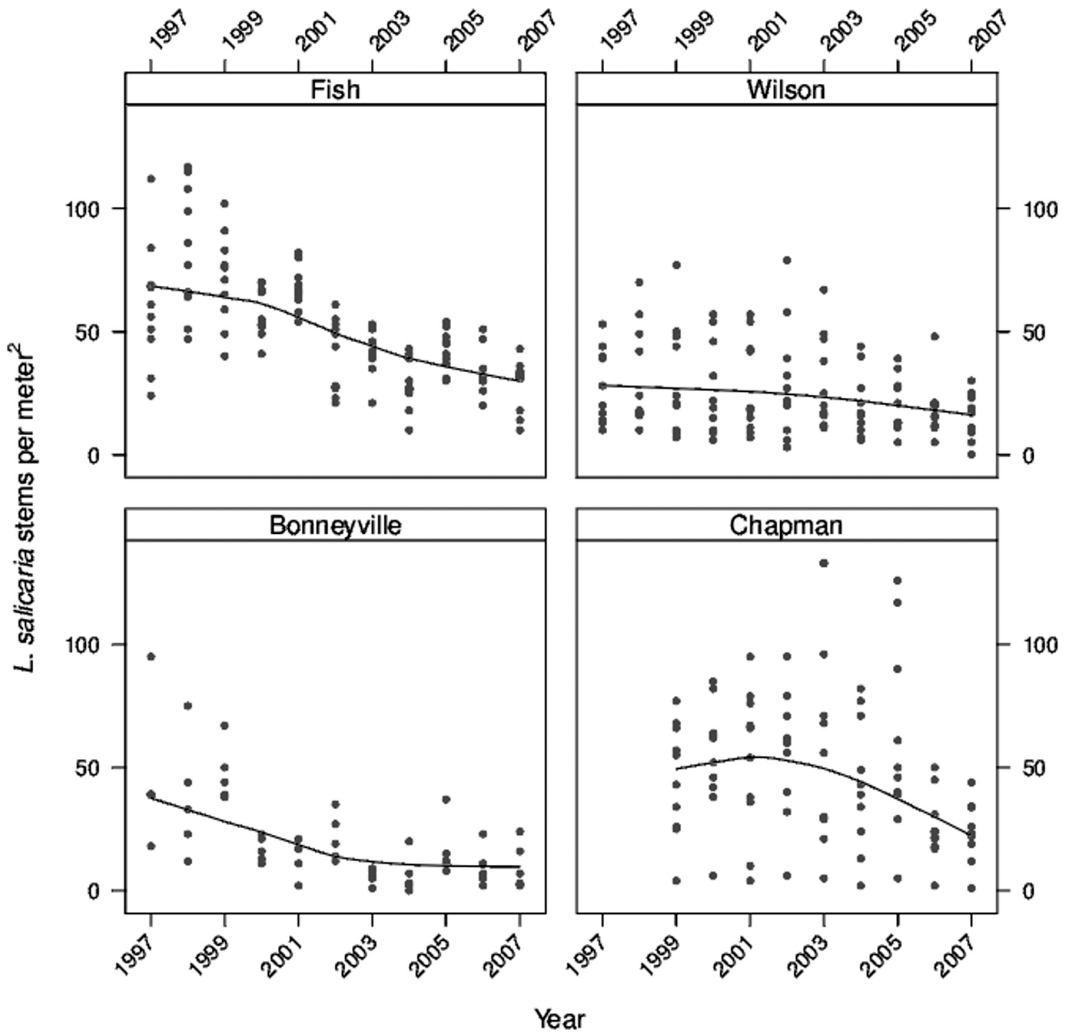| Coded value	Category midpoint	Category range
A	0%	0%
B	3%	1–5%
C	15%	5–25%
D	37.5%	25–50%
E	62.5%	50–75%
F	87.5%	75–100%

Figure 2.—Correlation between the number of *Lythrum salicaria* stems per quadrat over time.

The Chapman Lake site (on private property, Kosciusko County) was a marly flat adjacent to the shore of Big Chapman Lake. The sampling site was a ca. 1.2 hectare wetland dominated by sedges (*Carex* spp.) and abundant cattail (*Typha* spp.). It was the driest of the wetland sites in this study. Other wetlands were scattered around the shore of the lake and many of them had stands of purple loosestrife.

At all sites except Bonneyville Mills, two transects were oriented parallel to one another and one meter square quadrats were placed every five meters for a total of ten quadrats. Spacing between transects varied based on site size, shape, and distribution of *L. salicaria* within the wetland. At Bonneyville Mills the

site was exceptionally small, which restricted the number and positioning of the quadrats. Five quadrats were arranged along two transects. Transects were perpendicular to one another with one quadrat at the intersection and the remaining quadrats place three meters from the intersection in either direction along the transects.

Sampling.—Monitoring was conducted following the protocol outlined by Blossey (1994). This involved sampling each site in the spring and fall, from 1997 through 2007. Spring sampling was conducted between May 18th and June 19th and included insect and vegetative sampling. Each quadrat was surveyed to determine: the estimated abundance of *Galer-*

Table 3.—Spearman rank-sum correlations between year and number of stems, percent cover, and number of inflorescences by *Lythrum salicaria*; species richness; and *Galerucella* abundance. Spearman's followed by an asterisk (*) are significant.

Site	Variable	Spearman's ρ	*p*-value
Bonneyville Mills	Number of stems	−0.64*	<0.0001
	% cover	−0.57*	<0.0001
	Number of inflorescences	−0.46*	0.0006
	Species richness	−0.16	0.25
	Galerucella abundance	0.10	0.48
Chapman Lake	Number of stems	−0.32*	0.002
	% cover	−0.19	0.08
	Number of inflorescences	−0.52*	<0.0001
	Species richness	0.43*	0.0001
	Galerucella abundance	0.21	<0.05
Fish Creek	Number of stems	−0.68*	<0.0001
	% cover	−0.64*	<0.0001
	Number of inflorescences	−0.74*	<0.0001
	Species richness	0.69*	<0.0001
	Galerucella abundance	0.40*	<0.0001
Wilson Wetland	Number of stems	−0.21*	0.03
	% cover	−0.37*	<0.0001
	Number of inflorescences	−0.78	<0.0001
	Species richness	0.39*	<0.0001
	Galerucella abundance	0.21*	0.03

ucella spp. in each life stage (eggs, larvae, adults), percent damage to *L. salicaria*, percent cover of *L. salicaria*, number of *L. salicaria* stems, percent cover of *Typha* spp., number of *Typha* spp. stems. Additionally, in each quadrat the height of the five tallest *L. salicaria* stems and the five tallest *Typha* spp. stems were recorded. Each quadrat also was surveyed to list all present plant species. *Galerucella* abundances for each life stage were recorded as a categorical variable as shown in Table 1. Percent damage and percent cover were assigned to categories as shown in Table 2.

Fall sampling occurred from September 1st to 22nd. At this time *L. salicaria* has finished blooming and *Galerucella* spp. activity had ended. Since insect activity had ceased, no measure of beetle abundance or damage was made. The same measures of *L. salicaria* and *Typha* spp. were made as during the spring sampling. The five tallest *L. salicaria* plants in each quadrat were surveyed for the following: number of inflorescence, length of the terminal inflorescence, number of flower buds in 5 cm of inflorescence. The total number of inflorescence in each quadrat also was recorded. Finally, a list of plant species present in each quadrat was compiled.

Data analysis.—Data obtained from the five tallest plants in each quadrat were averaged

to obtain a single value for each quadrat per year. *Galerucella* spp. abundances presented some challenge. The numbers of each life stage were estimates and were assigned to a category (Table 1). Additionally, the categories had unequal ranges, some of which were quite large; because of this using midpoints as estimates seemed misleading. Therefore, to analyze *Galerucella* spp. abundance the three categorical values were averaged for each quadrat in each year. These calculated abundances are of little use in describing the actual number of beetles (except for an abundance of 1, which means *Galerucella* spp. were not present); rather, they provide an indication of the relative size of the population. Percent cover and percent damage values were defined as the midpoint of the category range (Table 2).

Release timing (Chapman Lake) and replication (Bonneyville Mills) varied between sites, preventing analysis across sites. Therefore, each site was analyzed separately. Statistical analysis and graph plotting was done using R 2.14.2 (R Development Core Team 2011) and the *lattice* package (Sarkar 2008). Fall data were used to assess *L. salicaria* response over time. Data were not normally distributed, and therefore required the use of non-parametric methods. Spearman's rank-sum correlations were calcu-
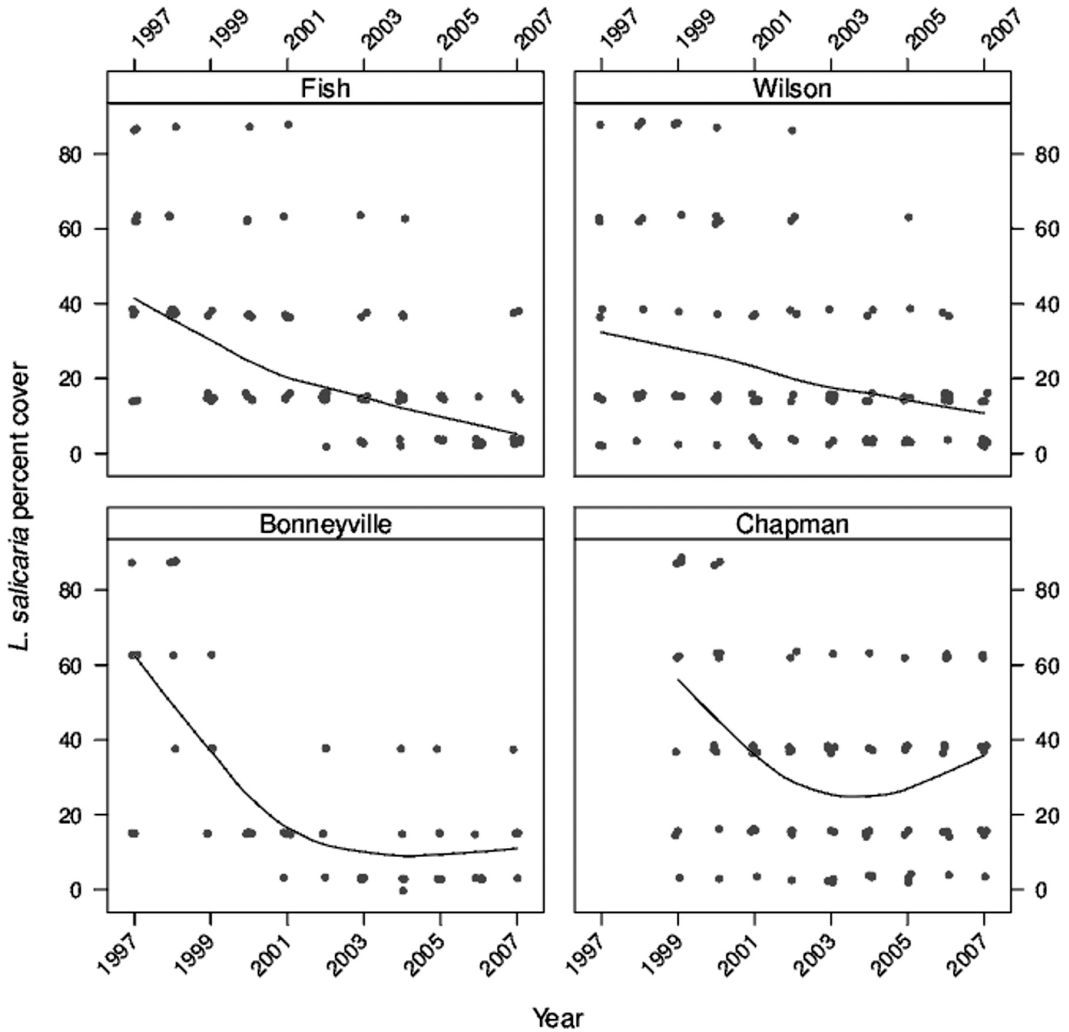
Figure 3.—Change in *Lythrum salicaria* percent cover class over time. Jitter has been added to keep points from overlapping, since both variables are essentially categorical.

lated to examine the relationship between year of observation and the following variables: species richness, number of stems, percent cover of *L. salicaria*, and number of inflorescence. Additionally, to aid in graphical interpretation, best fit lines were drawn through scatterplots of the same data using the *smooth* option of the *xyplot* command which can be found in the *lattice* package. This utilizes loess smoothing which fits the line to the scatterplot using local polynomial regression, providing a graphical view of general trends in the data.

In order to provide comparisons to Landis (2003) and Farnsworth & Ellis (2001), the relationship between *L. salicaria* stem density and species richness was examined. These data were normally distributed at all sites, so a simple linear regression was used. The number of *L. salicaria* stems per quadrat (stem density) was the independent variable and species richness was the dependent variable.

RESULTS

Spearman's ρ showed significant negative correlation at all sites between year and number of stems per square meter (p <0.001) (Fig. 2). Fish Creek (ρ = −0.68) and Bonneyville Mills (ρ = −0.64) had the highest correlations. At Chapman Lake ρ was −0.32 and at Wilson Wetland ρ was −0.21 (Table 3).
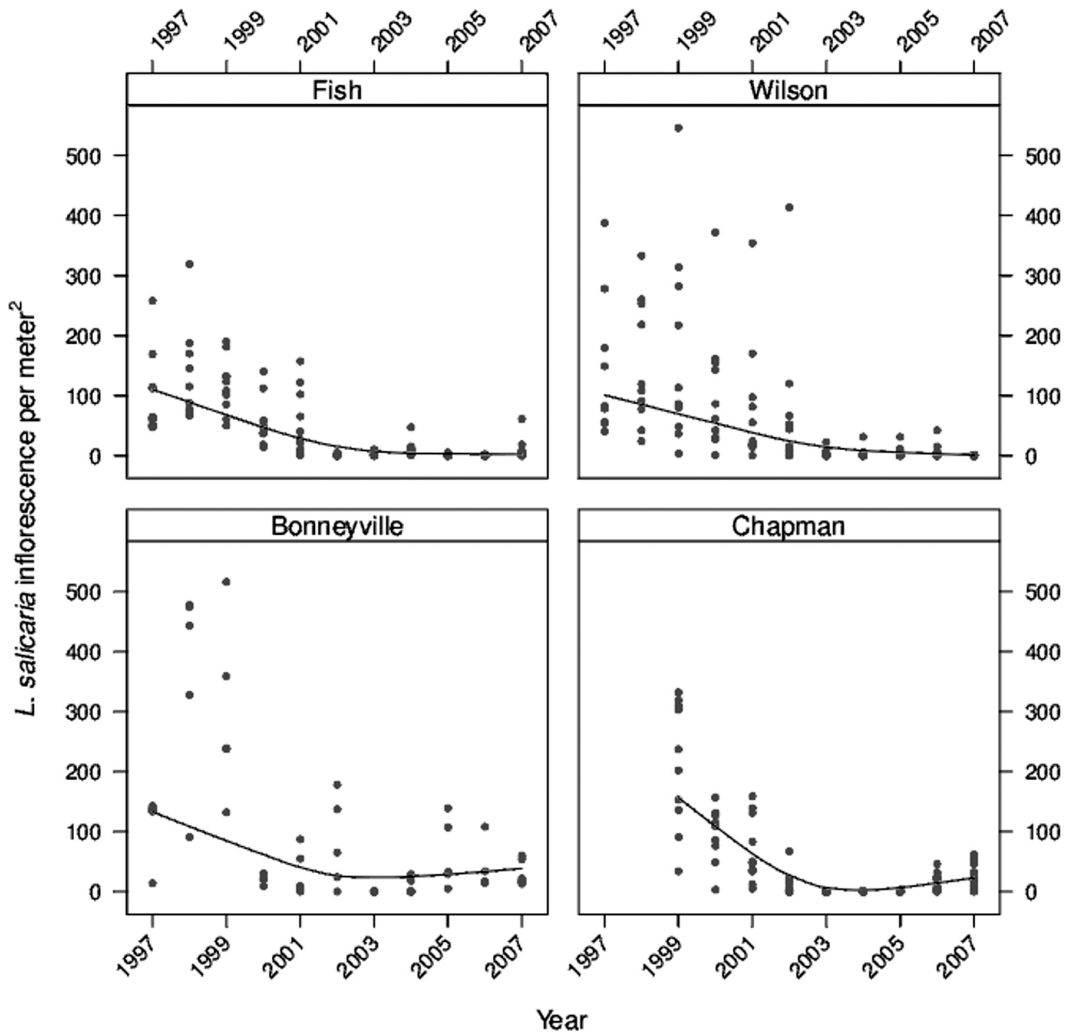
Figure 4.—Correlation between total number of *Lythrum salicaria* inflorescences over time.

Percent cover of *L. salicaria* was significantly correlated to year (p <0.001) at all sites except Chapman Lake (p = 0.08) (Fig. 3). Fish Creek again showed the highest correlation (ρ = −0.64). Spearman's ρ was −0.57 at Bonneyville Mills and −0.37 at Wilson Wetland (Table 3).

Correlations between year and number of inflorescence per quadrat was significant at all sites (p <0.001) (Fig. 4). The highest correlation was at Wilson Wetland, where ρ was −0.78. Spearman's ρ was −0.74 at Fish Creek, −0.52 at Chapman Lake, and −0.46 at Bonneyville Mills (Table 3). In comparison to other metrics, the number of inflorescence showed a consistently high ρ.

Spearman's ρ showed significant positive correlation between species richness and year at Chapman Lake (p <0.001), Fish Creek (p <0.001), and Wilson Wetland (p <0.001), but not at Bonneyville Mills (p = 0.25) (Fig. 5). Fish Creek had a ρ of 0.69, the highest of the sites. Correlations at Chapman Lake and Wilson Wetland were noticeably lower with ρ's of 0.43 and 0.39 respectively (Table 3).

The abundance of *Galerucella* spp. was significantly correlated with time at Fish Creek (p <0.0001), Wilson Wetland (p < 0.03), and Chapman Lake (p < 0.05) and was insignificant at Bonneyville Mills (p = 0.18). At the same time, the Spearman's correlations were consis-
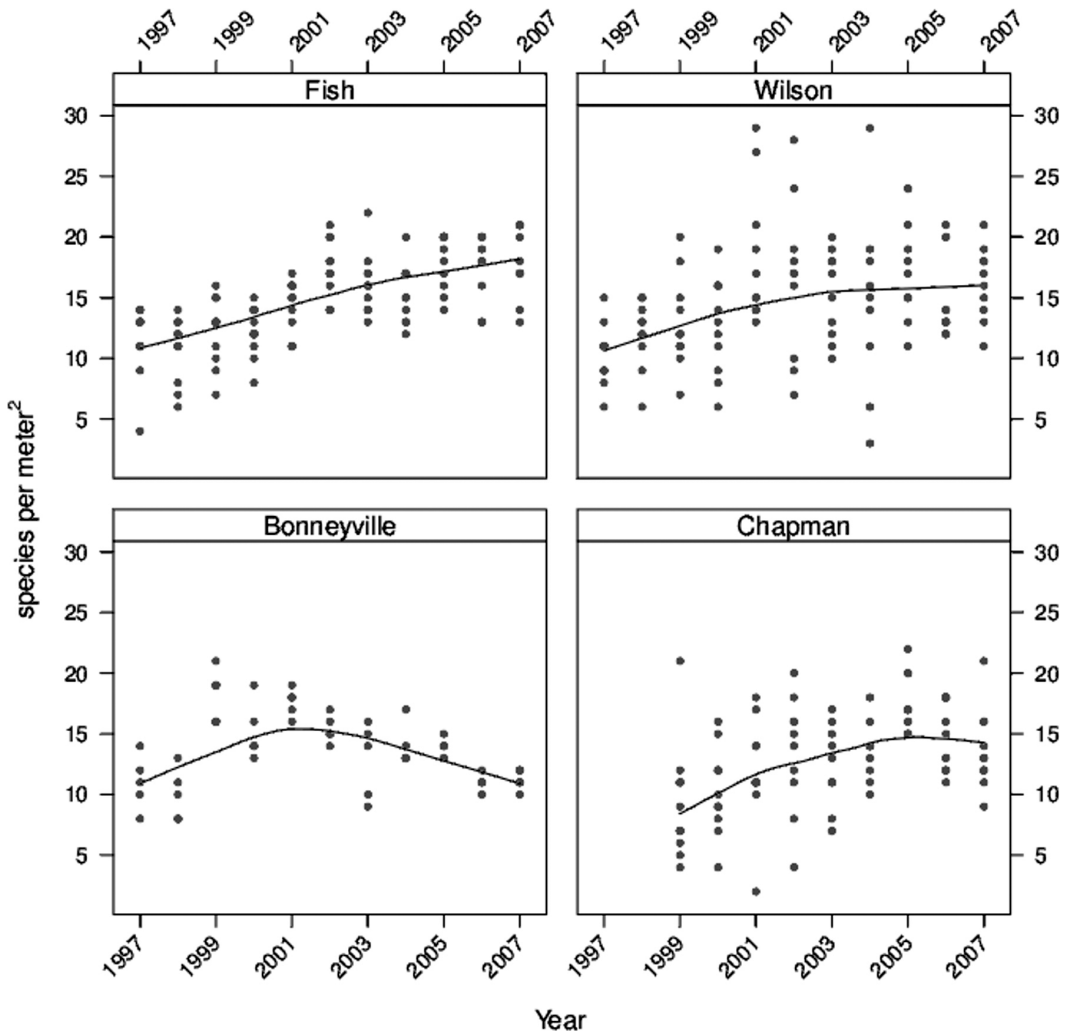
Figure 5.—Change in total autumn species richness over time.

tently low being 0.40 at Fish Creek, 0.21 at Wilson Wetland, and 0.21 at Chapman Lake.

Species richness showed a significant relationship to *L. salicaria* stem density at Chapman Lake ($p = 0.01$; $F(88) = 6.38$, $r = 0.26$), Fish Creek ($p <0.0001$; $F(108) = 43.74$, $r = 0.54$), and Wilson Wetland ($p = 0.02$; $F(102) = 5.39$, $r = 0.22$), but was not significant at Bonneyville Mills ($p = 0.79$; $F(53) = 0.07$, $r = 0.04$) (Fig. 7). When analyzed across all four sites collectively the relationship was highly significant ($p <0.0001$; $F(363) = 26.04$, $r = 0.26$).

DISCUSSION

The response of *L. salicaria* to *Galerucella* spp. varied between sites, with some sites showing a very strong correlation between plant characteristics and time, while others showed only weak correlations.

While all sites showed a negative correlation between number of stems and year, this relationship was very strong at Fish Creek and Bonneyville Mills, but weaker at Chapman Lake, and nearly insignificant at Wilson Wetland. One potential explanation for this is that heavy feeding by *Galerucella* spp. may reduce plant growth early in the season. Following this reduction in height and flowering, plants have been shown to respond by producing new stems (Blossey & Skinner 2000). Regardless, it is clear that in the years following release of *Galerucella* spp., the number of stems decreased at all sites.
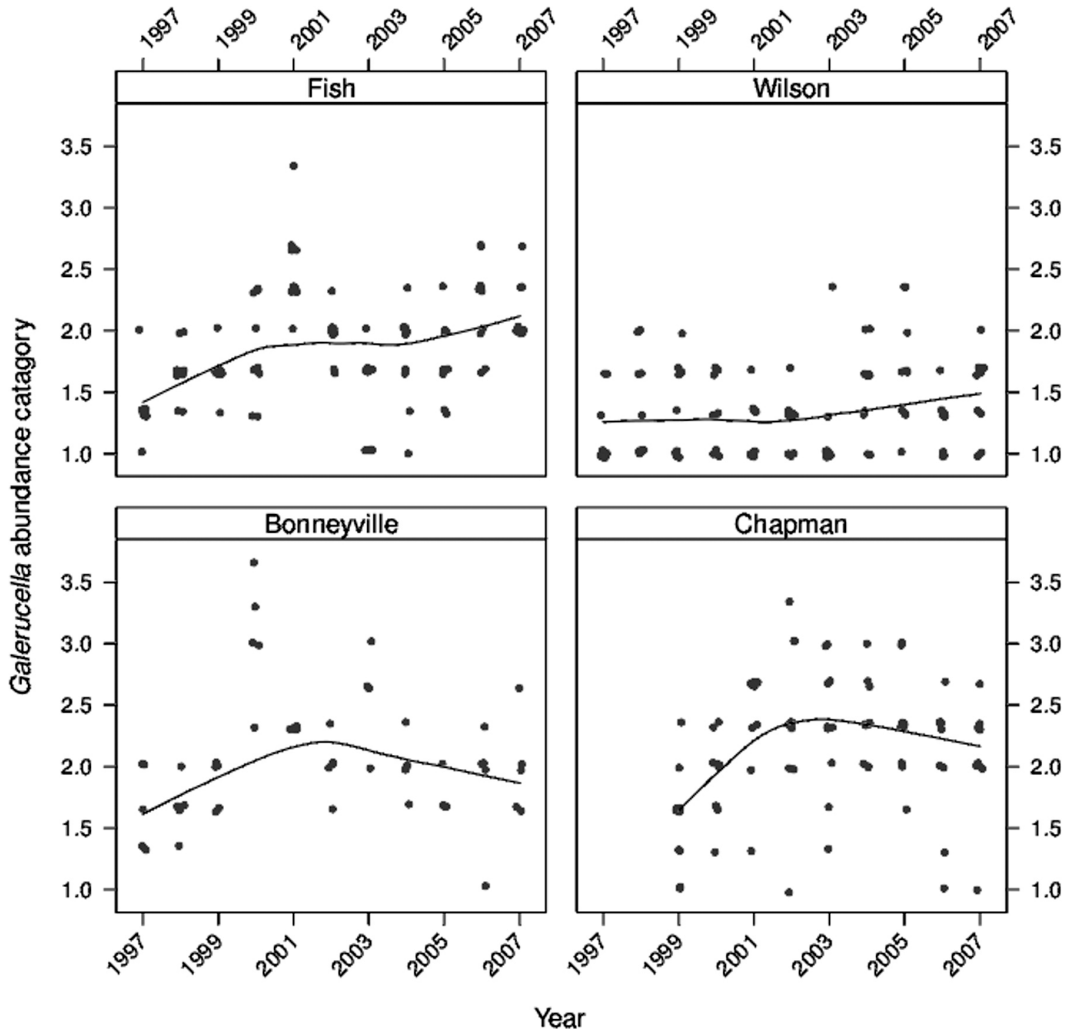
Figure 6.—Change in *Galerucella* abundance over time. Abundance is calculated as the mean categorical value for the three life stages, as described in Methods, and jitter has been added to keep points from overlapping.

Blossey & Skinner (2000) observed similar reduction in stem densities at one site.

Percent cover by *L. salicaria* showed substantial negative correlation with time at three sites (Bonneyville Mills, Wilson Wetland, Fish Creek), but not at Chapman Lake. This could be caused by several factors. Possibly, this illustrates a recovery of *L. salicaria* at the Chapman Lake site. It was the driest of the four sites and potentially *L. salicaria* has greater competitive ability under these non-flooded conditions (Weiher et al. 1996). At the same time, though, other measures of *L. salicaria* health did not show similar trends. Alternatively, these minor changes may be due to the

categorical estimates of cover class or differences in personnel making these observations in subsequent years. Meanwhile, the other three sites demonstrate a significant decrease in cover of *L. salicaria* over time.

All four sites exhibited a correlation between the number of *L. salicaria* inflorescences and year. Consistent reductions in flowering were observed three to five years after biocontrol release, similar to Landis (2003). Flowering was essentially terminated at some point for all sites and in the final year (2007) remained extremely low. The prevention or reduction of flowering could result in major long term impacts on *L. salicaria* populations. Seed accumulation
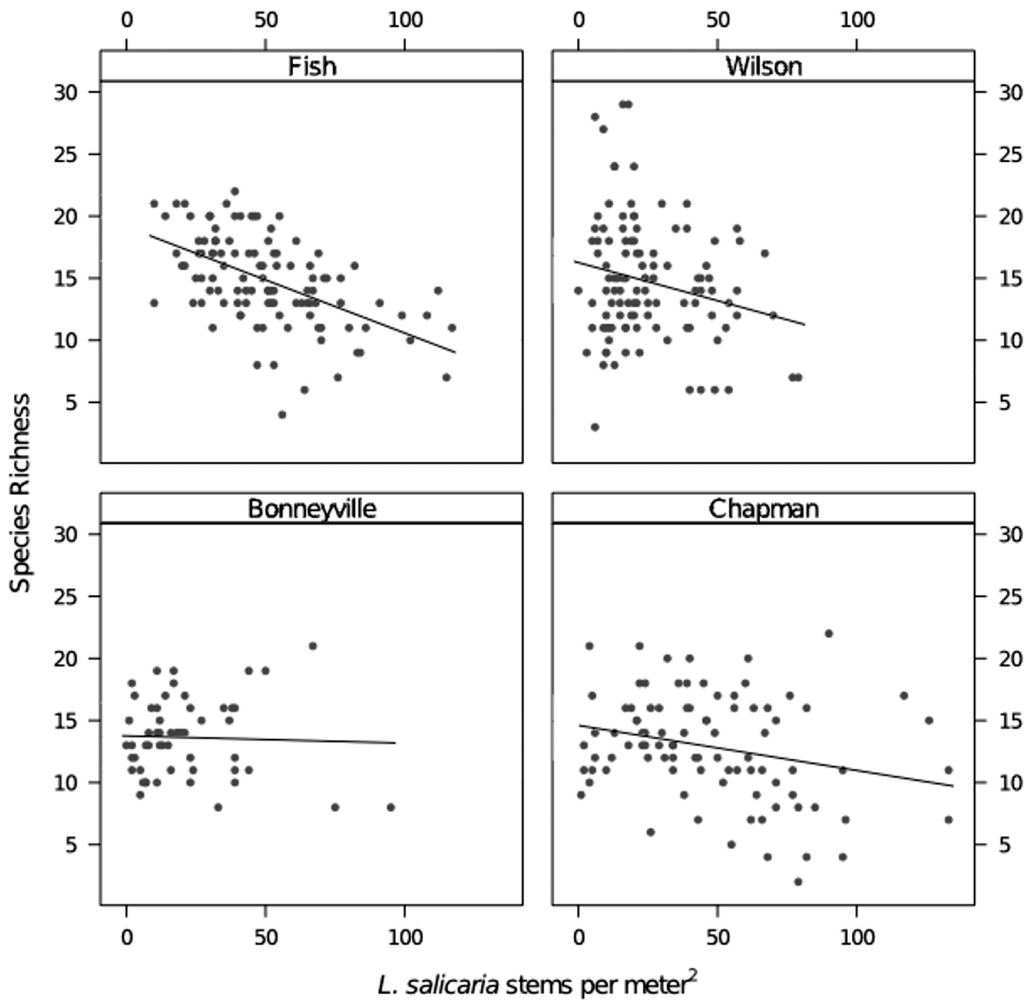
Figure 7.—Linear regression of the relationship between density of *L. salicaria* and plant species richness by site.

in these wetlands was likely high, but if the flowering remains very low, the existing seed bank could become depleted, leading to further reductions in *L. salicaria* in the future.

Species richness was not significantly correlated with year at Bonneyville Mills, but was significant at the other three sites. At Bonneyville Mills, species richness increased steadily for the first 3–5 years, but decreased unexpectedly over the next five years. During the same time period, all measures of *L. salicaria* health continued to decrease.

The correlation identified between *L. salicaria* stem density and species richness at three of the four sites supports the findings of Landis (2003). However, Farnsworth & Ellis (2001)

found no noteworthy relationship one year after release of *Galerucella* spp. This suggests that a longer period of time is needed to observe these impacts. Also, the relationship of stem density and species richness was not significant at Bonneyville Mills. This may have been due to natural species richness within that wetland, particularly since it was such a small area. The smaller number of quadrats within the site may have caused any relationship which did occur to remain unnoticeable.

The correlation of *Galerucella* spp. abundance with time was low, even at sites where it was significant, with the highest correlation being 39.9% at Fish Creek. Examination of the scatter plots and best-fit lines (Fig. 6) better

demonstrates the temporal patterns. A single year with high *Galerucella* spp. abundances occurred at each of the sites except Wilson Wetland. These one-year spikes occurred in 2001 at Fish Creek, 2000 at Bonneyville Mills, and 2002 at Chapman Lake. These spikes in *Galerucella* spp. populations were followed by declined over the next several years. Past studies have shown that large populations of *Galerucella* spp. can decimate *L. salicaria* in an area (Landis 2003). Once this happens, *Galerucella* spp. are forced to disperse to new locations in order to find additional food plants. This is likely occurring at these sites, i.e., several years after release the number of *Galerucella* spp. is sufficiently high to provide substantial control of *L. salicaria* but this control forces the population to disperse to a new area.

CONCLUSIONS

Considering the changes at each of the four sites several conclusions can be drawn. First, not all sites show the same response to *Galerucella* spp. over the 8–10 year time frame. These variations are likely due to subtle differences between sites, which may be biotic, abiotic, or anthropomorphic differences. Despite these differences, the overall trends remain. Species richness generally increased each year after *Galerucella* spp. were released. Similarly, *L. salicaria* stem density, percent cover, and number of inflorescence decreased over time. The general trends suggest that the impact of *Galerucella* spp. is substantial, though the level of control and the impacts of the beetles may be variable between sites.

The one impact which was significant at all sites was a reduction in flowering. This also is the most visible change, as the absence of large purple spike inflorescence is a dramatic change from uncontrolled populations. Early studies of biological control insects suggested that a combination of several insects may best control *L. salicaria* (Malecki et al. 1993). However, one of the approved biological control agents is *Nanophyes marmoratus*, a flower weevil. Considering the impact *Galerucella* spp. had on flower production, significant populations of both insects are likely not sustainable within a wetland.

Finally, our study of biocontrol of *L. salicaria* by *Galerucella* spp. adds a longer time frame than most previous investigations and demonstrates a significant inhibition of vegetative growth and reproductive effort at each of the wetlands in this study. However, *L. salicaria* was more inhibited at some sites than others. In addition to reducing the vigor of *L. salicaria*, species richness generally increased. These negative effects on *L. salicaria*, while important for restoring wetland ecosystem function, did not eliminate this invasive species from these sites.

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