

2012

# The Reduction of Amphibian Species Richness in the Presence of Non-Native Plants

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# The Reduction of Amphibian Species Richness in the Presence of Non-Native Plants

## **Abstract**

Wetlands are critical habitat for many species, making their protection a priority for biodiversity conservation. However, these ecosystems are being degraded at substantial rates. One factor that can be particularly detrimental is the presence of non native plant species. Exotic species have been known to out compete native species, alter trophic interactions between native species and transform habitat structure. To investigate the effect non-native plants have on amphibian species richness, I surveyed for the non native plant species around ten ponds at the University of Michigan's Edwin S. George Reserve. This was done to evaluate their effect on amphibian communities, which have been known to act as good indicator species of habitat quality. These ponds were chosen based on their similar size, hydroperiod and canopy cover. Plant samples were collected every ten paces around each pond's edge. The most abundant plants within a 1 meter radius were collected. Non-native species found were autumn olive (*Elaeagnus umbellata* Thunb.), multiflora rose (*Rosa multiflora* Thunb.), and Japanese barberry (*Berberis thunbergii* DC.). The proportion of non-native plant samples for each pond was calculated (range: 0-100%; mean±SE: 21.6±29.5%), as was the native plant species richness (mean±SE: 0.462±0.233). These data were then compared to the presence of 13 amphibian species. There was a trend toward ponds with a higher proportion of non native plants having fewer amphibian species ( $R^2=0.3257$ ;  $p=0.64087$ ). These results have important implications to understanding the effect non-native plant species have on amphibian communities.

## **Degree Type**

Open Access Senior Honors Thesis

## **Department**

Biology

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THE REDUCTION OF AMPHIBIAN SPECIES RICHNESS IN  
THE PRESENCE OF NON-NATIVE PLANTS

By

Kaitlyn Shott

A Senior Thesis Submitted to the

Eastern Michigan University

Honors College

in Partial Fulfillment of the Requirements for Graduation

with Honors in Biology

Approved at Ypsilanti, Michigan, on this date:

April 12, 2012

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## Abstract

Wetlands are critical habitat for many species, making their protection a priority for biodiversity conservation. However, these ecosystems are being degraded at substantial rates. One factor that can be particularly detrimental is the presence of non-native plant species. Exotic species have been known to out compete native species, alter trophic interactions between native species and transform habitat structure. To investigate the effect non-native plants have on amphibian species richness, I surveyed for the non-native plant species around ten ponds at the University of Michigan's Edwin S. George Reserve. This was done to evaluate their effect on amphibian communities, which have been known to act as good indicator species of habitat quality. These ponds were chosen based on their similar size, hydroperiod and canopy cover. Plant samples were collected every ten paces around each pond's edge. The most abundant plants within a 1 meter radius were collected. Non-native species found were autumn olive (*Elaeagnus umbellata* Thunb.), multiflora rose (*Rosa multiflora* Thunb.), and Japanese barberry (*Berberis thunbergii* DC.). The proportion of non-native plant samples for each pond was calculated (range: 0-100%; mean±SE: 21.6±29.5%), as was the native plant species richness (mean±SE: 0.462±0.233). These data were then compared to the presence of 13 amphibian species. There was a trend toward ponds with a higher proportion of non-native plants having fewer amphibian species ( $R^2=0.3257$ ;  $p=0.64087$ ). These results have important implications to understanding the effect non-native plant species have on amphibian communities.

## **Introduction**

Freshwater habitats are in significantly more danger of anthropogenic degradation than their terrestrial counterparts (Abell 2002). They also contain as much as one third of all vertebrate species, but only cover about 8% of the Earth's surface (Gleick 1996). The value of this biodiversity has many dimensions, including economical productivity, a source of genetic information, supporting ecosystem services, and ameliorating human health (Dudgeon et al. 2006). Because of their high biodiversity and increasing endangerment, these ecosystems are particularly important as targets of conservation biology. The threats to these valuable ecosystems consist of over-exploitation, water pollution, water flow alteration, destruction of habitat, -and lastly invasion by exotic species (Dugeon et al. 2006). Proportionally, wetlands are infested with more alien plant species than any other habitat. The Global Invasive Species Database shows that 8 of the 33 worst alien species grow in wetlands, meaning that 24% of the worst plant invaders grow in systems that only cover up to 6% of the earth (Zedler and Kercher 2004). The highly valuable wetland habitats are in danger and therefore in need of increased conservation efforts.

Invasion of non-native species is considered to be one of the primary threats to the integrity and the function of ecosystems (Blossey et al. 2001), in that they alter resource availability, reduce native diversity and alter habitat structure (Maerz et al. 2005a). Additionally, non-native plant species act directly by altering canopy height, increasing litter, altering litter break down rates, and altering nutrient regimes. They also act indirectly by impacting associations with microorganism, invertebrate and vertebrate

animals (Zedler and Kercher 2004). More specifically, non-native species have proven to be a threat to aquatic species and their habitats (Abell 2002). Wetlands are more vulnerable to invasion because they are landscape “sinks”. This accumulation of materials makes wetlands susceptible to invasion as well as promotes the growth of monotypes (Zedler and Kercher 2004). One example of how detrimental a non-native plant can be is alligator weed (*Alternanthera philoxeroides* Mart.). This plant has taken over Australian wetlands, clogging waterways, increasing sedimentation, and enhancing mosquito breeding (Sainty et al. 1998). Purple loosestrife (*Lythrum salicaria*) has also been shown to impact waterfowl as well as negatively affect various mammals and the bog turtle (Thompson et al. 1987). More recent studies indicate that purple loosestrife plays a role in the declining populations of black terns, rails, grebes, and the least bittern through nest site selection and habitat use (Blossey et al. 2001). Other ecological consequences of purple loosestrife invasion include reduction in native plant species, changes in decomposition rates and timing, changes in porewater chemistry, and increased evaporation rates (Blossey et al. 2001). It is clear that non-native plants have the ability to cause substantial harm to the wetlands that they inhabit. For this reason, increased conservation efforts are needed to more thoroughly understand the effects of non-native plants.

Exotic plant species that have become established in the eastern United States include purple loosestrife, common reed (*Phragmites australis*), multiflora rose (*Rosa multiflora*), narrow leaved cattail (*Typha angustifolia*), and Japanese barberry (*Berberis thunbergii*, D.C.), among many more. Research shows that Japanese barberry has the

potential to change soil conditions, including pH, organic horizon thickness, and increased nitrification rates. It also alters microbial communities in its close proximity (Kortev et al. 2002). Autumn olive (*Elaeagnus umbellata* Thunb.) is another invasive species commonly found in the eastern U.S. that negatively affects ecosystems (Goldstein et al. 2009). This species fixes nitrogen through a symbiotic relationship with an actinomycete *Frankia* (Wang et al. 2005). The increase in nitrogen may alter nutrient cycling and inhibit natural succession, as well as affect surface water quality (Goldstein et al. 2009). The effects of these non-native plants could extend to other taxonomic levels, potentially harming many native species, including amphibians.

Wetland habitat destruction and alteration are the main factors contributing to amphibian population declines, and by 1993, many species of salamanders and frogs were considered organisms of special concern (Alford and Richards 1999). Not only are wetlands in danger, but the rates of extinction overall are greater than any in the last 100,000 years (Eldridge 1998). In fact, the global decline of amphibians beginning in 1989 provides evidence that we are likely in the process of a sixth mass extinction (Blaustein and Kiesecker 2002; Wake & Vredenburg 2008).

Concern for amphibians is due to their ability to indicate environmental stressors (Blaustein and Kiesecker 2002), as well as their important ecological roles. Amphibians' ability to detect stressors has caused them to be referred to as "canaries in a coal mine." This implies that amphibians may be used as early indicators of environmental degradation (Kerby et al. 2010). Factors that make them good indicators include life style habits and rudimentary immune systems (Wake and Vredenburg 2008). As larvae



they are in direct contact with water and many are in contact with the land as adults. Additionally, their moist, permeable skin and unshelled eggs allow them to be directly exposed to both soil and water (Blaustein et al. 1994a). This direct exposure makes them particularly vulnerable to certain chemicals (Kerby et al. 2010). This vulnerability allows them to be used as indicators of declining habitat quality. Another factor that makes amphibian biodiversity important is the impact they have on other organisms. Specifically, amphibians act as important predators, prey, and herbivores in their larval stage (Blaustein et al. 1994a). Therefore, not only are amphibians an important link in the food chain, they are also an invaluable indicator of environmental degradation.

Non-native plant species affect amphibians in various ways. These species can introduce competition, predators, and disease into the amphibians' habitat (Blaustein and Kiesecker 2002), or may directly affect growth or survival. For example, American Toad tadpoles (*Bufo americanus*) showed a dramatic increase in mortality when exposed to the extracts of purple loosestrife (Maerz et al. 2005a). This increase in mortality could be due to oxygen deprivation (Watling et al. 2011). To counter the low levels of oxygen available in the water, tadpoles are observed to make frequent trips to the surface. This in turn makes them more vulnerable to predators, showing that invasive plants cause both direct and indirect detrimental effects (Watling et al. 2009). Another study demonstrated that infestation of Japanese knotweed renders land no longer suitable for foraging by green frogs (Maerz et al. 2005b). Additionally, many exotic plant species are successful based on their unpalatability to insects. This could prove detrimental to herbivorous insect populations as unpalatable plants replace the native palatable species. Organisms that

depend on these insects as a food source, including many species of amphibians, could also be negatively affected (Tallamy 2004).

To better understand the impact of non-native plants on wetland habitats, this study will use amphibians as indicators of wetland quality. Plant samples will be taken around the edges of ten ponds. The non-native plant species will be identified and their abundance determined for each pond. These data will then be compared to larval amphibian community data to determine if non-native plants decrease the number of amphibians present. It is likely that non-native plants will have a negative impact on amphibian richness. Such a reduction would indicate that non-native plants have a detrimental effect on wetland quality and amphibian community structure.

## **Materials and methods**

### **The system**

Plant and amphibian larval populations were studied at ten of the wetlands at the University of Michigan's E. S. George Reserve (ESGR). This fenced in 1500 acre tract located in Pinckney, Michigan (42°28'N, 84°00'W) has had restricted access since 1930 (Werner et al. 2007a). The wetlands at the ESGR (ponds) range in size. They include temporary pools, marshes, swamps and bogs (Fig. 1). The ten ponds chosen for this study are similar in hydroperiod, and canopy cover, but ranged in size from 74 hectares to 1808 hectares (Table 1, Werner et al. 2007a; Werner et al. 2007b). Most of the vegetation on the reserve consists of grasslands and hardwood forests dominated by oak, hickory, and maple (Werner et al. 2007b).

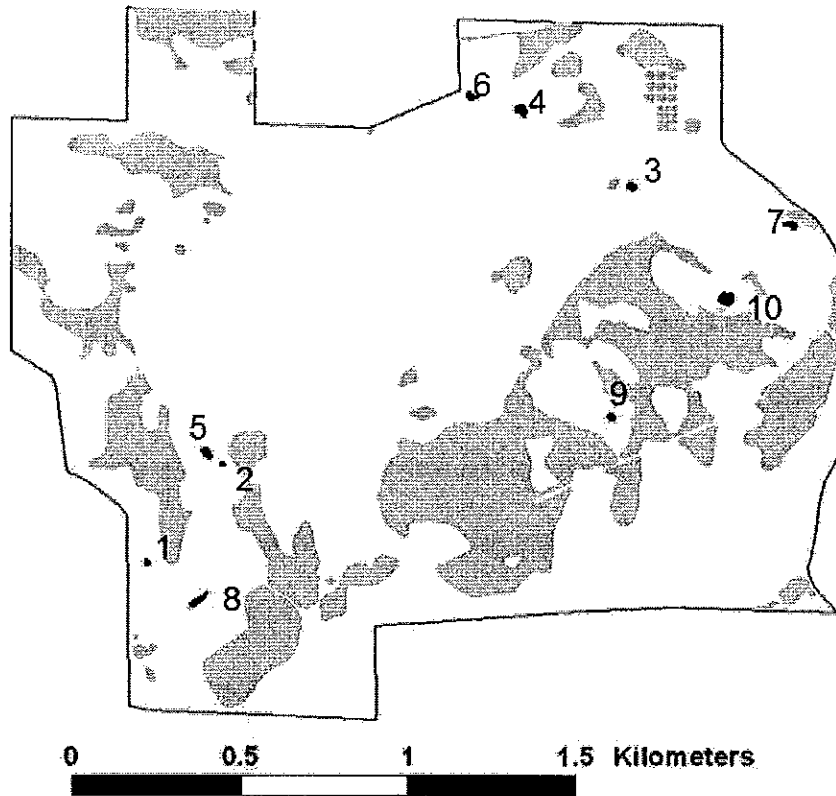


Fig. 1. Map of the E.S. George Reserve. Ponds included in this study are shown in black. All other wetland areas are shown in gray.

Table 1. Pond numbers listed are the same as those shown in Fig. 1. The corresponding pond name is also given. Maximum pond areas are given in hectares.

Pond Number	Pond Name	Area
1	Willow Pond	74
2	West Woods Little	117
3	Uzzell's no. 1	304
4	North Fence Pond	449
5	West Woods Big	537
6	North Fence Swamp	550
7	Dreadful Hollow	581
8	Southwest Woods Pond	893
9	Big Island Pond	897
10	Ilex Pond	1808

A total of seventeen amphibian species actively breed at the ESGR (Werner et al. 2007b), although not all of these were encountered over the course of this study (Table 2). It should be noted that *Ambystoma laterale* was not distinguished from unisexual *Ambystoma* (e.g., triploid *laterale-laterale-jeffersonianum*), as this complex cannot be distinguished morphologically and requires genetic analysis (Uzzell 1964).

Table 2. Amphibian species present at the E. S. George Reserve. Asterisks indicate species included in the present study.

Family	Species	Common Name
Bufo	<i>Bufo americanus</i>	American toad
Hyla	* <i>Hyla versicolor</i>	gray tree frog
	<i>Acris crepitans</i>	cricket frog
	* <i>Pseudacris crucifer</i>	spring peeper
	* <i>Pseudacris triseriata</i>	chorus frog
Rana	<i>Rana catesbeiana</i>	bullfrog
	* <i>Rana clamitans</i>	green frog
	<i>Rana palustris</i>	pickerel frog
	<i>Rana pipiens</i>	leopard frog
	* <i>Rana sylvatica</i>	wood frog
Ambystoma	* <i>Ambystoma laterale</i>	blue-spotted salamander
	* <i>Ambystoma tigrinum</i>	tiger salamander
	* <i>Ambystoma maculatum</i>	spotted salamander
	Unisexual <i>Ambystoma</i>	
Salamandridae	* <i>Notophthalmus viridescens</i>	eastern newt
Plethodontidae	<i>Hemidactylum scutatum</i>	Four-toed salamander
	<i>Plethodon cinereus</i>	red-backed salamander

### The amphibian survey

Amphibian community data were collected by Dr. Earl Werner (University of Michigan) and collaborators in the spring and summer of 2010 as part of an ongoing, long-term project (Werner et al. 2007a; Werner et al. 2007b). Sampling was conducted in the third weeks of both May and July of 2010. Samples were collected using three methods, including “pipe sampling”, dipnetting and seining. The first method was used to

sample columns of sediment and water. Nets were then used to remove all animals from the collection. After the pipe sampling, all ponds were dipnetted. Seining was used in the deeper ponds only. The sampling effort was varied based on the size of the pond. Amphibians collected were then identified to species. More details on amphibian sampling are available in Werner et al. 2007a and Werner et al. 2007b.

### **Plant sample collection**

Plant samples were collected in September of 2011. A circular transect was followed around the edge of each of the ten ponds. Samples were collected every ten paces. The most abundant species within a 1 meter radius was chosen, to ensure samples accurately represented plant communities. Smaller herbaceous specimens were dug up in order for the roots to be included. Larger woody plants were collected by cutting a ~0.5 meter sample from one of the branches. This ensured that both leaves and stem were included in the sample. All samples were sealed in labeled plastic bags and refrigerated. Samples were later removed from their bags, relabeled, and pressed in order to ensure preservation. All samples were identified to genus level; when possible, species level was also determined. Specimen descriptions and names used for identification and keying were found in Voss, Michigan Flora Parts 1, 2 and 3 (Voss 1972; Voss 1985; Voss 1996). The native or non-native status was also determined for each specimen.

## Statistical Analysis

Native plant species richness is the number of native plant species found at each pond. However, since number of samples varied across ponds, this value was used to calculate a proportion of native plant cover. This proportion was calculated by dividing the native plant species richness by the total number of samples for each pond. For example, at Dreadful Pond there were 11 samples taken and 9 of these samples were native plant species. Within the 9 native samples, 7 different native species were identified. The native plant proportion was calculated by dividing 7 by 11 to give a value of 0.636. The percentage of non-native plants at each pond was also found. This value was calculated by dividing the number of non-native plant samples by the total plant samples taken for each pond. At Dreadful Pond, the percentage of invasive plants was found to be 18.2% by dividing 2 invasive plant samples by a total of 11 plant samples.

Linear regression comparisons were done involving pond area, native plant proportions, and non-native plant percentage. Each of these characteristics was compared to amphibian species richness. Residuals between area and amphibian species richness were calculated to account for variability in pond area. These residuals were used in the linear regressions involving native plant proportions and non-native plant percentages. A model including these variables was created to predict the expected amphibian richness at a pond. An ANOVA analysis was done to determine how well all of these characteristics modeled the natural system. Predictive Analytics SoftWare (PASW) was used to calculate the  $R^2$ , B and p-value for all linear regressions.

## Results –

The invasive plant species found at the ESGR wetlands were autumn olive (*Elaeagnus umbellata* Thunb.), multiflora rose (*Rosa multiflora* Thunb.), and Japanese barberry (*Berberis thunbergii* DC.). Autumn olive was found at 40% of the ponds, while both multiflora rose and Japanese barberry were found at 20% of the ponds. The percentage of non-native plant samples for each pond was calculated (range: 0-100%; mean±SE: 21.6±29.5%), as were the native plant proportions (mean±SE: 0.462±0.233) (Table 3).

Table 3. The percentage of non-native plants present at each pond and native plant proportions.

Pond	Non-native plant percentages	Native plant proportions
Willow Pond	100.00%	0.000
Uzzell's #1	30.00%	0.600
Ilex	22.22%	0.444
North Fence Pond	22.22%	0.667
Dreadful Pond	18.18%	0.636
South West Woods	14.29%	0.500
North Fence Swamp	9.09%	0.636
Big Island Pond	0.00%	0.286
West Woods Big	0.00%	0.182
West Woods Little	0.00%	0.667

Data from amphibian sampling was obtained for the ten ponds (Table 4). The number of amphibian species present ranged from 0-7 species. Nine amphibian species were found to be breeding in the ten focal ponds of this study. The most abundant species were the wood frog (*R. sylvatica*) and the blue spotted salamander (*A. laterale*), found at 6 and 8 of the ponds respectively.

Table 4. The amphibian species present at each of the ten ponds on the E.S. George Reserve. A 1 indicates presence and a 0 indicates absence.

Amphibian Species	Big Island Pond	Dreadful Hollow	Ilex Pond	North Fence Pond	North Fence Swamp	Southwest Woods Pond	Uzzell's no. 1	West Woods Big	West Woods Little	Willow Pond
<i>H. versicolor</i>	0	0	1	0	0	0	0	0	0	0
<i>P. crucifer</i>	1	1	1	0	0	0	0	1	0	0
<i>P. triseriata</i>	1	1	1	0	1	0	0	1	0	0
<i>R. clamitans</i>	0	0	1	0	0	0	0	0	0	0
<i>R. sylvatica</i>	1	1	1	0	1	1	0	1	0	0
<i>A. laterale</i>	1	1	1	0	1	1	1	1	1	0
<i>A. tigrinum</i>	0	0	0	0	0	1	0	0	0	0
<i>A. maculatum</i>	0	0	0	0	0	0	0	1	0	0
<i>N. viridescens</i>	0	0	1	0	0	0	0	0	0	0
Total Amphibian Species Present	4	4	7	0	3	3	1	5	1	0
Residuals	0.15	1.35	-0.33	-2.14	0.47	-0.84	-0.59	2.52	0.12	-0.71

Since pond area showed the strongest relationship with amphibian species richness ( $R^2 = 0.6911$ ), the residuals of this relationship were used to observe the effects of non-native plant percentage (Fig. 2) and native plant proportions (Fig. 3) on amphibian species richness. The linear regression between percentage of non-native plant species and the amphibian residuals showed a negative relationship, indicating that amphibian species richness decreases as invasive plant percentage increases ( $R^2 = 0.140$ ;  $p = 0.287$ ). A slightly negative trend was seen between native plant proportions and amphibian species richness ( $R^2 = 0.051$ ;  $p = 0.529$ ).



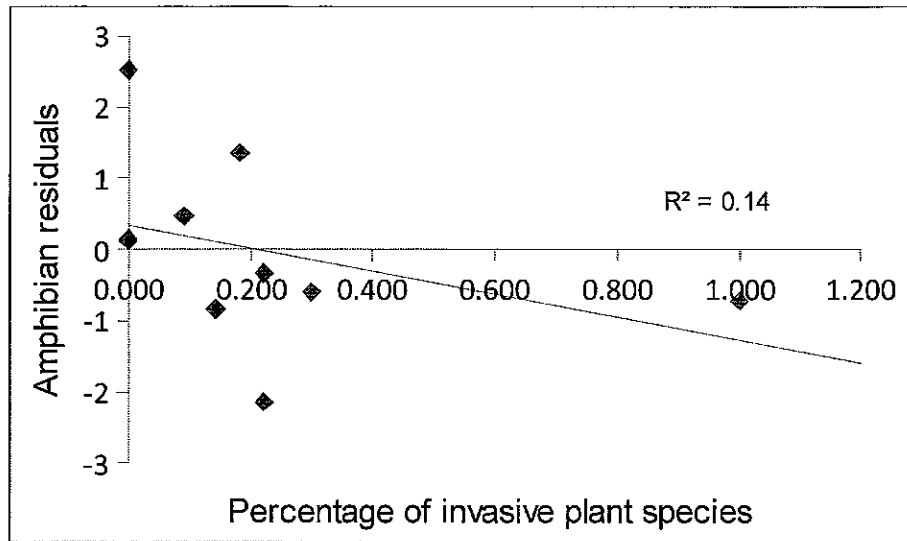


Figure 2. Amphibian species richness correlated with percentage of invasive plant species present at each pond. Residuals of amphibian data were taken to eliminate the effect of pond area.

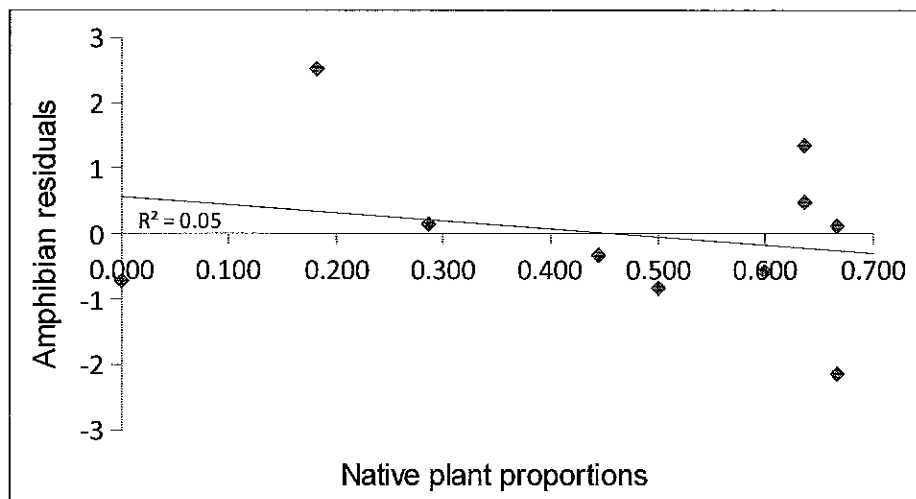


Figure 3. Amphibian species richness correlated with native plant species proportions. Residuals of amphibian data were taken to eliminate the effects of pond area.

An ANOVA was run using amphibian species richness as the dependent variable, and pond area, percentage of non-native plants, and native plant proportions as predictor variables. None of these variables were correlated with one another. The overall model was found to be significant ( $p = 0.011$ ), however this was mostly driven by pond area which showed a positive coefficient ( $R^2 = 0.691$ ;  $p = 0.008$ ). Negative coefficients were

shown by percentage of non-native plants ( $p = 0.093$ ) and native plant proportions ( $p = 0.139$ ).

A linear regression model was created from these data plotting the number of amphibian species observed against the number of amphibian species predicted. The expected number was based on the pond area, percent invasive plants, and native plant proportions regression analyses. These were then compared to observed amphibian species richness data (Fig. 4).

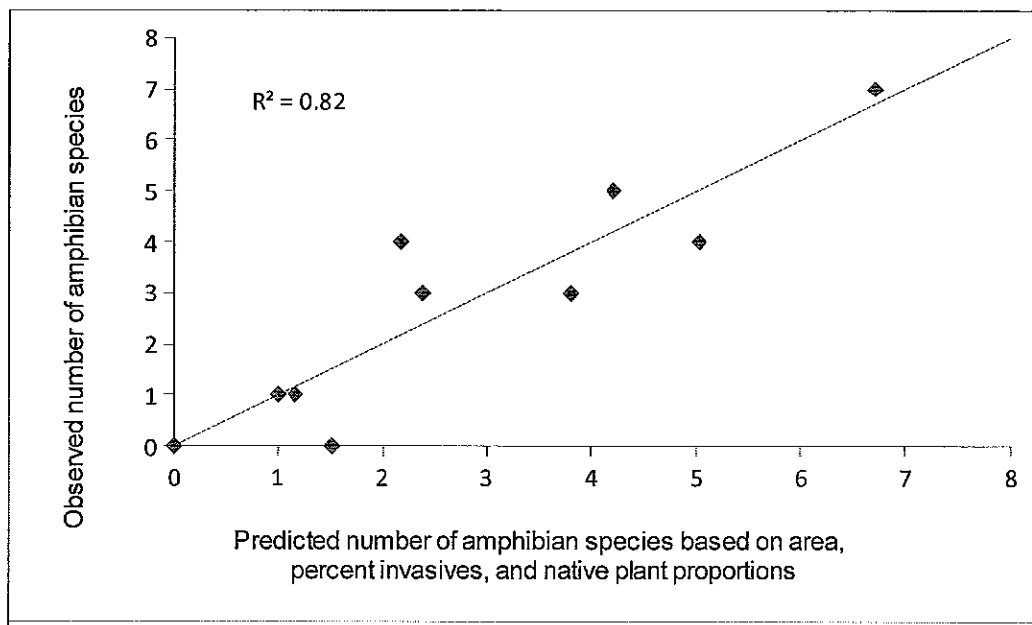


Figure 4. Expected number of amphibian species based on pond area, percentage of non-native plant species and native plant proportions, versus observed number of amphibian species ( $R^2 = 0.824$ ;  $p = 0.011$ ).

## Discussion

These data show a trend toward amphibian species richness decreasing as invasive plant percentage increases, indicating that invasive plants may have detrimental effects on amphibian populations. Amphibian species richness significantly decreased as pond size decreased. Additionally, a trend was seen that indicates that amphibian richness decrease as the proportion of native plant cover decreases. The model including all of these habitat variables effectively predicted the amphibian species richness. This demonstrates the complexity of wetland ecosystems and the impact non-native plant species can have on them. Non-native plants alter the habitats they colonize and amphibian species are sensitive to this alteration. It is likely that non-native species effect these habitats by changing the habitat structure, altering the herbivore and predator interactions, and by modifying the reproductive success of the amphibians (Martin and Murray 2011).

Previous studies have found a similar trend in amphibian decline with increased non-native plant species. Woodland salamander populations have been shown to decline in areas of non-native plant invasions because of habitat alteration. Non-native plants caused an increase in earthworm populations, which causes a reduction of leaf litter. This decrease in leaf litter was associated with a decline in amphibian populations (Maerz et al. 2009). It is possible that the presence of our non-native species could have a similar effect on leaf litter decomposition rates. Another study showed amphibian species richness and evenness decreasing in areas invaded by *Lonicera maackii*. The authors suggested that microclimate alteration by non-native plants could be the cause of

decreased amphibian populations (Watling et al. 2011). It is likely that autumn olive, multiflora rose, and Japanese barberry also alter the microclimates they colonize, which may cause the reduced amphibian species richness seen at specific ponds at the E.S. George Reserve.

Other studies have also shown that a decrease in pond size and a decrease in plant diversity negative effects amphibian richness. Strong positive relationships have been shown between breeding pond area and the population size in the California tiger salamander (Wang et al. 2011). This strong correlation between pond area and amphibian population size is probably due to the rate at which small ponds dry out. Fast evaporation of the pond water reduces both reproduction and survival of offspring, since the larvae have less time to complete metamorphosis. This is likely the reason why fewer amphibian species were found in ponds with smaller areas. Additionally, smaller ponds are less targeted by immigrating amphibians adding to decreased species richness. Reduced native plant diversity due to non-native plant invasion is also detrimental to amphibian populations. Amphibians rely on diverse vegetation as well as structural diversity to increases the amount of niches available to them. These niches are crucial to their own species richness and survival (Martin and Murray 2011).

To better understand the relationship between non-native plant abundance and amphibian communities, more sampling must be done at ponds- located in different regions. All of our samples were taken at the same location, the E.S. George Reserve. Sampling should be done at in other areas in order to look at the effects of other non-native plant species not found at the reserve. Plant samples should also be taken at

various distances away from the pond edge to more clearly represent the vegetative composition of the surrounding area. Additionally, an increased sample size could determine if the trends observed between amphibian populations and non-native plant abundance are significant.

Future research to determine which of the three non-native plant species is most detrimental to the amphibian species and the mechanisms behind this interaction should be investigated. It is possible that either autumn olive, muliflora rose, or Japanese barberry effects amphibian populations more than the other two species. More sampling should be done to look at this relationship. How these non-native plants are affecting the communities should also be xamined. Are they simply reducing native diversity by forming monocultures, or are they more directly affecting the amphibian species? It would also be interesting to determine if certain amphibian species are more vulnerable to the effects of the invasive plants than are others.

Amphibian species richness is negatively affected by non-native plant species presence. This is an indicator that non-native plants have the potential to be harmful to the ecosystem as a whole by altering the habitat structure, ecological interactions, and the reproductive success of the native species. With the large ecological and biodiversity value of wetland habitats, this issue is of serious concern. Therefore, control efforts and the management of exotic plant species should be of high priority in order to protect wetland biodiversity.

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