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Using an Occupancy Modeling Framework to Test the Effects of Habitat Variables on Pond Occupancy of Mabee's Salamander (A mabeei) and Marbled Salamander (A opacum)

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Using an Occupancy Modeling Framework to Test the Effects of Habitat Variables on Pond Occupancy of Mabee’s Salamander \( (A. \text{ mabeei}) \) and Marbled Salamander \( (A. \text{ opacum}) \)

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Scientists are documenting worldwide losses in biodiversity in all classes of plants and animals. Losses in amphibian groups seem to be more severe than in other groups. Habitat loss or alteration is the most thoroughly documented reason for amphibian declines and is often considered the most important. This is particularly true in the southeast United States where forest lands have been converted for agriculture, commercial and residential use. Mabee’s salamander (*A. mabeei*) and the marbled salamander (*A. opacum*) are two species threatened by the loss of suitable habitat in the southeastern United States. Both species live and breed in the Grafton Ponds Natural Area Preserve in the City of Newport News, VA. The temporary ponds in which they breed are destroyed due to ditching or draining and conversion of forest to cropland. Studies are needed to understand the patterns of amphibian distribution and abundance within the Grafton Ponds Natural Area preserve.

In this study both Mabee’s salamander larvae and marbled salamander larvae were studied using site occupancy, an alternative to studying population abundance. The resulting models suggest that ponds with a higher pH have a greater probability of occupancy. Landscape level variables also affected the occupancy of salamanders at breeding ponds, though these models were not as strongly supported. More research needs to be done to determine if there is causation, and to what extent, between decreasing pH levels and decreasing populations of amphibians in the Grafton pond complex.
TABLE OF CONTENTS

Dedication page ii
Acknowledgments iii

Chapter 1: A summary of the problem. 1
Chapter 2: A strong negative effect of acidity on pond occupancy by two species of Ambystoma salamander (A. mabeei and A. opacum). 20

Figure 1: Mabee’s salamander 44
Figure 2: Mabee’s salamander range 44
Figure 3: Marbled salamander 45
Figure 4: Marbled salamander range 45
Figure 5: Map of Grafton Ponds 46

Table 1: Candidate model set 47
Table 2: Highest-ranked occupancy models 48

Works Cited 49
This Thesis is dedicated to my grandmother

Lola M. Christy

May 16, 1913 – May 24, 2009
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Chapter 1:

A summary of the problem.
1. Introduction

Scientists are documenting worldwide losses in biodiversity in all classes of plants and animals (Blaustein and Bancroft 2007; Stuart et al. 2004). When the rates of decline are studied more closely, however, losses in amphibian groups seem to be more severe than other groups (Blaustein and Bancroft 2007; Stuart et al. 2004). According to AmphibiaWeb.org (June 12, 2009), thirty-two percent of amphibians (1856 different species) are considered threatened worldwide. Within the last twenty years, 168 species are thought to have gone extinct and more than 2400 species have declining populations. The areas greatest hit by these losses include Central America, the Caribbean, Australia, South America and western North America.

The growing concern for amphibian declines is not just about protecting frogs and salamanders. Amphibians often act as environmental indicators (Alford and Richards 1999; Blaustein and Bancroft 2007). Amphibians spend their lives in both aquatic and terrestrial habitat. Because of their sensitivity to changes in temperature, precipitation and other factors like increased UVB radiation, amphibians often suggest that factors that negatively affect them may influence entire ecosystems, both aquatic and terrestrial (Alford and Richards 1999; Blaustein and Bancroft 2007; Hopkins 2007).
The general causes for amphibian declines fall into two categories. The first category includes issues that are commonly studied, understood and shared with other types of species. Habitat loss or alteration, competition and predation from introduced species and over-exploitation are three commonly studied reasons for amphibian decline. The second category includes issues that are more elusive, complex and harder to understand. This category includes climate change, UVB radiation, disease, deformities, and synergy among all reasons for decline (AmphibiaWeb 2009; Collins and Storfer 2003)

The most thoroughly documented and most important cause of amphibian declines is habitat loss (Alford and Richards 1999). Examples of impacts on amphibians include destruction of their habitat by clear-cutting or draining wetlands. These events fragment populations and remove breeding sites (Morris and Maret 2007; Rothermel and Semlitsch 2006; Todd et al. 2009; Van Buskirk 2005). For pond breeding amphibians, while the pond area itself may be protected, urban development may change the upland habitat necessary for foraging and overwintering, making it unsuitable for amphibians (Semlitsch 1998; Semlitsch and Bodie 2003).

Another commonly studied cause of amphibian decline is direct predation or competition from introduced species. One commonly cited example is the decline of the mountain yellow-legged frog (Rana muscosa) over 80% of its range due to stocking trout in ponds and lakes throughout the
Sierra Nevada range (Bradford et al. 1993; Knapp and Matthews 2000). Trout predation on the *Rana muscosa* tadpole stage has virtually eliminated the frog.

A second recent example is that of the worldwide introduction and expansion of the bullfrog (*Rana catesbeiana*). Specifically, scientists are interested in its westward U.S. expansion and its effects on native ranid species (Kiesecker and Blaustein 1998). Its large size, high mobility, and huge reproductive capacity has made the American bullfrog a successful invader and a threat to native frog populations. The American bullfrog out-competes and even eats native frogs (AmphibiaWeb 2009; Kiesecker and Blaustein 1998).

Over-exploitation is another commonly understood cause for amphibian declines. During the gold rush of the American West, frogs were harvested for their legs for food (Jensen and Camp 2003). Amphibians have always been collected as pets by children. Professional collectors now sell colorful and distinctive species through shops and dealers (Jensen and Camp 2003). Finally, thousands of frogs are dissected in high school science classes each year. Unfortunately the frogs used are often wild caught leopard frogs (*Rana pipiens*) (Jensen and Camp 2003).

The more complex reasons for amphibian decline include climate change, UVB radiation, disease, deformities and synergy between the
multiple causes of decline (AmphibiaWeb 2009). There have been several accounts of amphibians declining in relatively pristine areas. In these cases, climate change is often considered to be responsible for the declines either by causing the amphibians to change their breeding patterns or because climate change has made conditions favorable for new disease (Collins and Storfer 2003; Lips et al. 2008; Woodhams et al. 2008). Chytrid fungus (Batrachochytrium dendrobatidis) is one particularly devastating fungal infection that decimates populations (Pounds et al. 2006; Rothermel et al. 2008). Because amphibian eggs lack shells and because larvae and adults have thin skin, increases in ambient UVB radiation over the past decades make amphibians vulnerable to damage from increased UVB (Collins and Storfer 2003).

Researchers are also finding factors that may have had little or no effect on populations alone, but when combined with other stressors have huge negative impacts. Combinations of habitat loss and introduced species or climate change and disease have far worse effects than just one of those factors (Alford and Richards 1999; Collins and Storfer 2003; Woodhams et al. 2008).

In the southeastern United States, the most prominent reason for decline is thought to be habitat loss and degradation (Sharitz 2003; Tuberville et al. 2005). This region was once covered with old growth forest that was
removed in the early 1800s for timber or to create cropland (Semlitsch 2003; Sharitz 2003; Wyman 2003). At the same time the forests were removed, wetlands were often drained or ditched to create fields for agriculture. While some of that forest has been allowed to return, much of this land is still managed for timber harvest (Alford and Richards 1999). Alford and Richards (1999) noted that clear-cutting in the Southern Appalachian forest reduced salamander populations by nine percent. There is some evidence that if the forest is allowed to return, the populations will rebound (Ash and Pollock 1999; Semlitsch 2003). The surrounding commercial and residential development and areas dominated by human activity like shopping centers, highways and housing developments, however, make for poor salamander habitat (Semlitsch 2003; Sharitz 2003; Wyman 2003).

2. Study species

2.1 *Ambystoma mabeei*

Mabee's salamander, *Ambystoma mabeei*, is a small mole salamander. As an adult, it has a dark brownish back with light grey to white flecks all over, becoming heavier on the sides (Fig. 1). Adults are typically 8-12 cm total length with the tail being 40% of the total length (Hardy and Anderson 1970; Petranka 1998). The larvae have pond type morphology with bushy gills and dorsal fins that extend up onto the back. They are typically brown and yellow on top and flesh colored below (Hardy and Anderson 1970; Petranka 1998).
Hatchlings have a single yellow stripe on either side of the body while older larvae have two stripes along the side of body usually blotchy and broken with black mottling (Hardy and Anderson 1970; Petranka 1998).

Mabee's salamander is found on the Atlantic coastal plain from South Carolina to extreme southeastern Virginia (Fig 2). The salamander was first identified in Virginia in 1979 in Southampton County and Suffolk (Mitchell and Hedges 1980). There is very little published information about Mabee’s salamander and their habitat requirements. In the few existing studies, *A. mabeei* have been found near river bottoms, tupelo-cypress bottoms in pine woods and cypress and gum swamps (Hardy and Anderson 1970). These data suggest that Mabee’s salamander is limited to “low, wet, bottom lands” (Hardy 1969).

Mabee’s salamander breeds in fish-free, ephemeral ponds including semi-permanent farm ponds, fox holes filled with water, vernal ponds, Carolina Bays and cypress-tupelo ponds in pine woods (Hardy and Anderson 1970). Hardy (1969) also found that *A. mabeei* used acidic ponds (pH ca. 4.5) near extensive stands of pine woods and occasionally used ponds found in open grassy fields. Adults breed in late winter or early spring. In North Carolina they are usually found from February to late March depending on the weather (Hardy 1969). Courtship behavior has not been described for this species, but the entire mating sequence likely takes place in the water (Anderson and Williamson 1977). Eggs are laid individually or in loose strings...
of two to six eggs and are scattered throughout the breeding site (Petranka 1998).

When the larvae hatch they immediately begin feeding on zooplankton and other invertebrates. The *A. mabeei* larvae themselves are regular prey for *A. tigrinum* and are likely prey for beetles and odonate larvae (Hardy 1969; Petranka 1998). In the Grafton Ponds Natural Area Preserve in Newport News, VA, *A. mabeei* larvae are likely preyed upon by the larger *A. opacum* larvae (McCoy and Savitzky 2004).

The larvae grow quickly and the larval period lasts only a few months. The size at transformation varies from 50-55 mm total length (Hardy 1969). Once transformed, the movement of juveniles and adults is poorly documented. Adults are thought to stay relatively close to the breeding ponds and in North Carolina can be found throughout most of the year under surface cover (Hardy 1969). Even less is known about the movement of juveniles. Only one study has documented finding 91 juveniles 800 meters from the nearest known body of water (Hardy 1969).

Mabee’s salamander lives in ponds with a diverse mix of amphibians including frogs and other salamanders but interactions within these communities have not been thoroughly studied. Considered uncommon in the Carolinas, Mabee’s salamander in Virginia has been documented in about 9 sites in 4 counties and 3 cities (Clark 1998). It is considered extremely rare
to very rare in Virginia and is listed as threatened under the Virginia Endangered Species Act. Mabee’s salamander does not have any federal protective status (Clark 1998). It has a national status of N4 (apparently secure; uncommon but not rare; some cause for long-term concern due to declines or other factors) and a global status of G4 (apparently secure; uncommon but not rare; some cause for long-term concern due to declines or other factors). It is considered a species of least concern on the International Union for Conservation of Nature (IUCN) Red list (IUCN 2009).

Mabee’s salamander is threatened in Virginia due to habitat loss through urbanization and agriculture. The temporary ponds in which it breeds are destroyed by ditching or draining and conversion of forest to cropland (Mitchell et al. 2002; Petranka 1998). In Grafton Ponds, the available habitat is fragmented into several parcels by two roads, a railroad and several utility rights-of-way (Roble 1998).

Due to these threats and the status of Mabee’s salamander, many more studies are needed to answer the multitude of questions about Mabee’s salamander conservation. Studies are needed documenting the dispersal movements and distances of adults and juveniles. Studies on habitat utilization patterns could help wildlife managers better protect appropriate areas. Studies on metapopulation dynamics would help managers better understand the colonization and extinction rates within the Grafton Ponds area. Studies on reproductive success are needed to compare abundance in
undisturbed (forest) ponds and disturbed (clear cuts, rights of way) ponds. Studies are needed to investigate age at first reproduction and whether or not Mabee’s salamanders breed annually. It would also be beneficial to study the community ecology and interactions with other salamanders, particularly *Ambystoma opacum*.

2.2 *Ambystoma opacum*

The marbled salamander, *A. opacum*, is a stout black mole salamander with white cross bands from head to tail (Fig 3). Adults generally measure from 77 to 127 mm total length. Hatchlings are dark and drab. Larvae have pond type morphology with bushy gills and a dorsal fin that extends up on to the back almost to the front limbs (Petranka 1998). The larvae are drab brown or black with a series of light spots that form just below the level of limb intersection. Marbled salamanders breed in the fall and larvae collected in the spring are usually much larger than other *Ambystoma* species that share the same breeding habitat (Petranka 1998).

Marbled salamanders are found on the East Coast from southern New England to northern Florida (Fig 4). They can be found as far West as tall grass prairie lands from Indiana to Texas (Petranka 1998). They are generally found in deciduous forest including floodplain forest as well as upland forest that contains suitable breeding sites.
*Ambystoma opacum* is one of only two ambystomatid species that oviposits on dry land, usually in dried ponds beds or along the margins of reduced ponds. Adults move to the breeding habitat on rainy nights in late summer or early fall. The female lays her eggs in a shallow nest and broods by curling herself around the eggs. Most nests are placed at an intermediate depth (Petranka and Petranka 1981). The embryos develop to hatching stage in about 9-15 days but do not hatch until water covers the eggs for 1 to 2 days. Because hatching is environmentally induced, size at hatching varies widely (Petranka 1998; Petranka and Petranka 1981).

Once the eggs hatch, the larvae immediately begin feeding on zooplankton. They eat mostly macrozooplankton but they also eat anything that will fit in their mouths including other amphibian eggs and larvae (Petranka 1998; Petranka and Petranka 1981). Most *A. opacum* larvae suffer tail damage from attacks by other *A. opacum* larvae and the proportion of individuals damaged is positively correlated with larval density (Branch and Altig 1981; Petranka 1998; Stenhouse 1985).

Little is known about the terrestrial ecology of juvenile marbled salamanders. Recently transformed individuals can often be found near the breeding ponds under leaf litter but generally disperse from the ponds during rainy weather soon after transformation (Stenhouse 1987). Juveniles and adults are both fossorial but can sometimes be found on the surface under leaf cover after a summer or fall rain. Marbled salamanders are prey for
woodland predators like owls raccoons, skunks and snakes. When attacked, the adults can secrete a milky substance from the tail to repel the predators.

Historically the marbled salamander was common but secretive throughout its entire range. But, given their dependence on small isolated wetlands for breeding, it is likely that their numbers have been decreasing steadily (Petranka 1998). Little is known about the potential threats to these salamanders.

3. **Study area: Grafton Ponds Natural Area Preserve**

The Grafton Ponds Natural Area Preserve is a 374 hectare property owned by the city of Newport News and located on the Lower Peninsula of Virginia in York County. The area was dedicated as a state natural preserve by the Virginia Department of Conservation and Recreation in January 1995. The property is separated into two large tracts by Ft. Eustis Boulevard, a two-lane highway. The section north of Ft. Eustis is 196 acres and 178 acres are located south of the road. The property is fenced and marked on the north side but only marked with property of Newport News boundary signs to the south.

Currently, the park area is surrounded by forest, residential, commercial and industrial development. Historically, before European settlement, the area was mostly undisturbed forest with only small plots of land cleared by the Chiskiack Native Americans to grow corn and other
plants. The Chiskiack tribes also likely used fire as a forest management tool but it is unclear to what extent (Clark 1998). After the Europeans arrived around 1630-1631, large scale clearing of the land began for agriculture and timber harvest. In the 1700s, the expansion of the colonies and settlements required the expansion of routes for travel. Creeks were dredged to improve navigation and Route 17, the then Yorktown-Hampton Road was completed (Clark 1998). In the 1800s, clearing the land for agriculture and timber harvest continued as well as an expansion of the roads and laying of railroads.

Lands including the preserve and the surrounding area were acquired during the early 1900s to protect the local water supply system. At that time, many agricultural fields were left fallow and converted back to forest. The first timber harvest after the re-growth was in 1942 (Clark 1998). The City of Newport News watershed property has been an actively managed forest since then.

The Grafton Ponds Natural Area Preserve is jointly managed by the City of Newport News and the Natural Heritage Division of the Virginia Department of Conservation and Recreation. The area lies in the Atlantic Coastal plain in a low-lying area called Grafton Plain. The Grafton Plain contains a series of ponds that range in age up to 100,000 years old (Clark 1998). Carbon dioxide in rain and byproducts in the soil produce acidic surface water which percolates through the ground dissolving the shell
material in the layers. As the calcium is leached away it leaves behind a more clay-rich sediment layer which is less porous. The ponds form from the dissolution of shell-rich layers in the underlying sediments and the subsequent subsidence and compaction of the over-lying soil. This slows the percolation of the water and leaves standing water in a depression resulting in a pond. The depressions hold water in the winter and the spring before the increasing rate of evapotranspiration in the summer dries them out. The soil around the ponds and the water in the ponds are usually acidic (Clark 1998).

There are more than 200 depression ponds across the Grafton Plain with about 70 ponds within the preserve boundaries. The ponds range in size from 0.9 to 9.1 meters in diameter with a maximum depth at high water from 0.15 to 1.7 meters deep (Rawinski 1997). The ponds support eleven rare species of plants, animals and insects. Some of the rare plant species are Harper's fimbristylis (*Fimbristilis perpusilla*), featherfoil (*Hottonia inflata*), Cuthbert's turtlehead (*Chelone cuthbertii*) and pondspice (*Litsea aestivalis*). Significant invertebrate fauna include the state rare damselfly, duckweed firetail (*Telebasis byersi*), and state rare dragonfly species, the comet darner (*Anax longipes*). The ponds are also home Mabee's salamander (*Ambystoma mabeei*), considered rare in Virginia, and the state watchlist species, the spotted turtle (*Clemmys guttata*). There are several other species that potentially use the area including the eastern tiger salamander (*Ambystoma tigrinum*) and the Canebrake rattlesnake (*Crotalus horridus*).
The tiger salamander has been found 3 miles from the
preserve in 1973 and 1993 but not in the preserve. Tiger salamanders are
considered globally common but is extremely rare in Virginia. The canebrake
rattler is considered extremely rare in Virginia, although globally common.
The most serious threats to the preservation of the area have been identified
as altered surface water regime (ditching, draining, increased runoff), habitat
degradation and fire deficiency.

Grafton ponds Natural Area Preserve is a great resource for the
Newport News area but many studies are still needed to help land managers
understand and monitor the species that exist there. Other than the
zoological survey, the plant survey and the management plan, all completed
more than 10 years ago when the preserve was dedicated, only one study
has dealt with species in the Grafton Ponds area. Further zoological surveys
are needed given the variable nature of the ponds. Different years and
different amounts of rainfall will allow certain species to be present one year
and perhaps not the next (Roble 1998). More inclusive and larger studies
that encompass more ponds will provided even more information about the
population dynamics in the park.

While the ponds are naturally somewhat acidic, extensive, long-term
water chemistry monitoring is needed to keep track of the changes taking
place in the park given the park’s location in the middle of a large urban area.
Tests on the amphibian species present and the effects of acidic water are
needed as well as tests on the acid tolerance of both eggs and larvae or tadpoles. There is also a need to monitor amphibian abundance or occupancy to document possible declines in the area. A study on road mortality needs to be conducted if a previously discussed four lane expansion of Ft. Eustis Boulevard ever proceeds. Though not in the preserve, the forested Newport News waterworks property to the south of the park is actively managed for timber. Logging operations near ponds should be monitored to determine the possible negative effects on the species present.

4. Occupancy monitoring

Studies are needed to attempt to understand the patterns of amphibian distribution and abundance within the Grafton Ponds Natural Area preserve. Estimating abundance, however, can be expensive, time intensive and in the case of rare species, may be impossible to achieve (MacKenzie et al. 2006). Using site occupancy is an alternative to studying population abundance (Bailey and Adams 2005; MacKenzie et al. 2006). Occupancy studies tend to require less effort per site than surveys that estimate abundance. In cases of rare species, occupancy probability will still be possible to estimate though an estimate of abundance may be impossible to achieve (Bailey and Adams 2005; MacKenzie et al. 2006). In the long term, monitoring occupancy can reveal changes in the status of the study species over large areas and is a particularly appropriate study method for species that exhibit wide population
fluctuations over short time periods (i.e. season to season) (Bailey and Adams 2005).

The problem in any wildlife survey is that species are rarely detected with complete accuracy. Non-detection can be a result of two situations: 1) the animal is truly absent or 2) the animal was present, but not detected. Unless the probability of detecting the species is 100%, the measure of occupancy is confounded with the detectability of the species (Bailey and Adams 2005; MacKenzie et al. 2006; Mazerolle et al. 2007). It is inappropriate to analyze detection/non-detection data as if they were truly presence/absence data. Without accounting for the variation in detectability the results often yield false conclusions (Bailey and Adams 2005).

Occupancy models were developed to solve the problems created by imperfect detectability (Bailey et al. 2007; MacKenzie et al. 2006; MacKenzie and Royle 2005). Occupancy modeling allows the user to distinguish between the probability of occurrence ($\psi$) and the probability of detection ($p$) (MacKenzie et al. 2006; Mazerolle et al. 2007; Mazerolle et al. 2005). These models allow researchers to account for site variables that would affect occupancy (habitat size or habitat type, etc.) as well as survey variables that would affect detectability (air temperature, weather, etc.) (Mazerolle et al. 2005). The parameter estimates for occupancy or detectability are obtained by relating the parameters to the data (observed detection histories) using the logit-link function (Bailey and Adams 2005; MacKenzie et al. 2006).
Models are then selected using Akaike's Information Criteria which selects the most parsimonious model, balancing model fit and parameter precision (Bailey and Adams 2005; MacKenzie et al. 2006). The model with the lowest AIC value is considered the “best” within (or conditional on) the model set. All models with a ΔAIC < 2.0 should be considered when making inferences and reporting parameter estimates (Bailey and Adams 2005).

While occupancy modeling is an excellent solution to the problem of detectability, it is still not perfect. Occupancy modeling does not measure abundance. For many situations, this result is fine. In some situations, such as those of rare or endangered species, after using occupancy modeling to identify likely areas, additional studies that identify abundance may be required. When using occupancy modeling there is also a trade-off between the detailed information researchers may learn about a single pond or small area versus the large scale information researchers can gain about the range of the species.

5. Conclusion

Scientists are struggling to make sense of the worldwide declines in amphibians. Occupancy modeling is one tool helping them gain more information about species at risk. By starting monitoring programs using occupancy modeling, changes in colonization and extinction rates can be tracked. In Grafton ponds, occupancy modeling is an excellent technique
available to monitor these two salamander species, as well as other amphibian species. It may help to infer potential threats to these species in Grafton Ponds and can be a spring board for other more specific research about the species in the future.
Chapter 2:

A strong negative effect of acidity on pond occupancy by two species of *Ambystoma* salamander (*A. mabeei* and *A. opacum*).
1. Introduction

In all classes of plants and animal, biodiversity is declining worldwide (Blaustein and Bancroft 2007; Stuart et al. 2004). A closer look at the rates of decline shows that losses in amphibian groups seem to be more severe than in other groups (Blaustein and Bancroft 2007; Stuart et al. 2004). This is particularly troubling given that amphibians are useful models for studying environmental problems and are often used as environmental indicators (Hopkins 2007; Tuberville et al. 2005). Factors that negatively affect amphibians, because of their sensitivity to changes in temperature, precipitation and other factors like increased UV, are often the same factors that may influence entire ecosystems (Alford and Richards 1999; Blaustein and Bancroft 2007; Hopkins 2007).

One of the best documented and largest threats to amphibian decline is habitat loss and degradation (Alford and Richards 1999; Blaustein and Bancroft 2007; Cushman 2006). Other prominent causes for amphibian declines vary from region to region but usually include climate change, disease, overexploitation, UVB radiation, and introduced species.

In the southeastern United States, habitat loss or alteration is thought to be the most prominent reason for amphibian decline (Sharitz 2003; Tuberville et al. 2005). This area was once covered with old growth forest
that was removed in the early 1800s for timber or to create cropland (Sharitz 2003; Wyman 2003). While some of that forest has been allowed to return, much of that growth is still managed for timber harvest (Alford and Richards 1999). Alford and Richards (1999) noted that clear-cutting in the Southern Appalachian forest reduced salamander populations by nine percent. At the same time the forests were removed, wetlands were often drained or ditched to create fields for agriculture.

Remaining wetlands now face the problem of acidification. Due to burning of fossil fuels, atmospheric deposition of sulfuric and nitric acids often occur in the form of acid rain. Only within the last 15 years have researchers begun to look at the impact of habitat acidification on amphibians. Studies have shown that species richness and amphibian density for terrestrial species decrease when soil pH is <3.8 (Wyman and Jancola 1992). In aquatic habitats, laboratory studies show that acidic water causes an increase in egg and larval mortality as well as sub-lethal effects (slowed growth rates, increased time until transformation) for those individuals that do survive (Horne and Dunson 1994; Pierce 1985, 1993). When pH decreases, acid sensitive species, like the *Ambystoma jeffersonianum* salamander, are excluded from amphibian assemblages while acid tolerant species, like *Rana sylvatica*, may increase because of the absence of the predator (Dunson et al. 1992). These examples clearly demonstrate that habitat acidification has
complex effects on development, growth, and survival but we do not yet know how these effects will change population dynamics (Dunson et al. 1992).

This study focuses specifically on two species of *Ambystoma* salamanders that are threatened by habitat loss and degradation due to development in southeast Virginia. *Ambystoma* species require upland foraging and overwintering habitat and breeding habitat consisting of fish-free ponds. In the Virginia coastal plain, loss of upland habitat is due to residential, commercial and industrial development (Clark 1998). Breeding habitat is lost when ponds that are often too small and too temporary to be considered wetlands are ditched or filled in. Even when these small wetlands or pond complexes are protected, due to their hydrology, the small ephemeral ponds have little buffering capacity against pollutants entering the water (Pierce 1985).

1.1 Study species

Mabee’s salamander (*Ambystoma mabeei*) and the marbled salamander (*Ambystoma opacum*) are threatened by the loss of upland habitat due to development and loss of breeding habitat through ditching and draining or filling in small wetlands (Clark 1998; Mitchell et al. 2002; Petranka 1998; Roble 1998). Both are pond-breeding, mole salamanders. *Ambystoma opacum* breeds in the fall and *A. mabeei* breeds in the late winter or early
spring. As adults, both species rely on upland forest habitat for foraging and shelter and ephemeral, fish free ponds for breeding.

Mabee’s salamander (*A. mabeei*) is a smallish, dark brown, mole salamander with grey flecks that increase in number along the sides. The larvae have pond type morphology with bushy gills and are identified by their dark stripes through a yellowish background. Its range includes the Atlantic coastal plain from South Carolina to extreme southeast Virginia. In Virginia, the species is recognized as state threatened due to loss and alteration of habitat (Clark 1998; Mitchell et al. 2002; Roble 1998). Mabee’s salamander is also related to *A. cingulatum*, a species that in 1999 was federally listed as Threatened due to many of the same reasons (habitat loss, urbanization, fire suppression) that are cited for declining *A. mabeei* populations (Pauly et al. 2007).

The Marbled salamander (*A. opacum*) is a large and stout mole salamander with black and white cross bands from head to tail. The larvae, like the Mabee’s, have pond type morphology with bushy gills. They are dark brown to black with a series of yellowish gold spots along the side of the body below where the legs attach (Petranka 1998). Marbled salamanders range from southern New England south to northern Florida and west to the tall grass prairies (Petranka 1998). *Ambystoma opacum* breed in fall or early winter before the temporary ponds fill with water. Because of their early breeding, they are the first to hatch once the pond fills (Petranka 1998). All
salamanders are carnivorous. As the first to hatch, marbled salamander larvae have a distinct size advantage over other amphibian larvae and are active predators (Petranka 1998). *A. opacum* is a potential predator of *A. mabeei* (McCoy and Savitzky 2004).

### 1.2 Study area

Grafton Ponds Natural Area Preserve is a 374 hectare property located on the lower peninsula of Virginia in York County (Figure 5). The property is owed by the City of Newport News and was dedicated as a state natural preserve area by the Virginia Department of Conservation and Recreation (DCR) in 1995. Grafton Ponds is separated into two separate tracts by Fort Eustis Boulevard, a two-lane, paved road connecting George Washington Memorial Parkway (Rt. 17) and Jefferson Avenue (Rt. 143). The preserve is surrounded by forest, residential, commercial and industrial development (Clark 1998).

Grafton Ponds is located in a low lying area of the Atlantic Coastal Plain called Grafton Plains. In this area, depressions are formed when the shell-rich layers in the underlying sediments are dissolved. As they dissolve, the overlying soil subsides and compacts forming a depression (Clark 1998). The resulting soil is a clay-rich sediment which slows percolation and leaves standing water in the depression. There are more than 70 depression ponds within the preserve boundaries and more than 200 ponds in Grafton Plain.
The ponds are highly variable in hydrology, shape and size but generally hold water from winter to early summer and range in size from 0.9 to 9.1 meters in diameter and 0.15 to 1.7 meters deep at maximum high water (Clark 1998). The ponds support a wide range of insects, amphibians, reptiles and plants including several state rare species like duckweed firetail (*Telebasis byersi*), Mabee’s salamander (*Ambystoma mabeei*), and Harper’s fimbristylis (*Fimbrystilis perpusilla*) (Clark 1998; Rawinski 1997; Roble 1998).

1.3 Occupancy estimation and modeling

Data collected during this study were analyzed using occupancy analyses for single season models (Bailey et al. 2007; MacKenzie et al. 2006; MacKenzie and Royle 2005). Occupancy modeling estimates species occupancy probability while adjusting for imperfect detection. As an improvement to the presence/absence studies of the past, occupancy modeling is designed to account for the fact that non-detection of the species does not always mean the species is absent. Non-detection may mean that the species was present but not detected. Studies using occupancy modeling tend to require less effort than surveys that estimate abundance and may be more appropriate for some situations. For example, in cases of rare species like *Ambystoma mabeei*, abundance may be impossible to estimate, though estimation of occupancy is still possible (MacKenzie et al. 2006). By modeling both the probability of occupancy and the detection probability one reduces “the introductions of errors in spatial models derived from the data” (Mazerolle et
al. 2005). Another advantage of estimating occupancy is the ability to monitor much larger geographic areas than were previously possible with estimating abundance (MacKenzie et al. 2006).

Occupancy modeling uses a probability based model with two parameters. The first, \( \psi \), is the probability that a site is occupied by the target species. The second, \( p \), is the probability of detecting the target species, given the site is occupied. Maximum likelihood methods are used to estimate both parameters based on the observed detection histories associated with each site (pond). In addition, using this method allows the researcher to model either occupancy or detection probabilities as functions of measured covariates using a logistic link function (Bailey et al. 2007; MacKenzie et al. 2006).

The goals of this study were to use occupancy estimation based on detection/non-detection data for both species to:

1) determine the probability of pond occupancy for each species;

2) determine the relationship between pond occupancy and vegetation in both upland terrestrial and pond perimeter habitat and,

3) determine the relationship between pond occupancy and water quality variables (pH, conductivity, turbidity).

2. Methods
2.1 Site selection

Ponds used in this study were found by combining information from three sources: (1) the 1998 Mabee’s salamander breeding pond map from the Zoological Inventory of the Grafton Ponds Sinkhole complex, York County, VA; (2) 2007 Virginia Geographic Information Network aerial photos of the Virginia stateplane South; and (3) by ground-truthing the area. For rare and difficult to find species, MacKenzie and Royle (2005) showed that it is often more efficient to survey more sites less intensively. Therefore, all 46 ponds located and filled with water in 2008 were used in the study. Due to increased rainfall in 2009, additional ponds were added to the study for a total of 55 sites (Fig. 5).

2.2 Sampling

Each pond was surveyed using visual encounter surveys approximately every other week during the breeding season for Mabee’s salamander and the larval season for both species. To survey for breeding adults, we turned over leaves, debris and all logs along the perimeter of the pond until we found a specimen or until we circled the entire pond. We also searched the perimeter and shallows of the ponds with D-loop dip nets. To search for larvae, we just searched the perimeter and shallows of the ponds until we found a specimen or until we circled the entire pond. For the 2008 season, surveys began on February 23rd and ran through June 2nd for a total of 6 surveys. In 2009,
surveys began on February 15th and ran through May 15th for a total of 7 surveys. Each survey took around 5 days to complete.

2.3 Water covariates

Three water quality measurements were made at each pond. We measured turbidity by using a secchi tube. We filled the 125 cm tube with pond water and slowly released the water from the bottom of the tube until the black and white pattern could be seen at the bottom of the tube. The height of the water remaining in the tube is the measurement of turbidity. We measured conductivity in the field using a YSI handheld conductivity meter. Over a period of four days, April 7-10, 2009, we collected multiple water samples from each pond in 60mL polyethylene bottles filled to overflowing to eliminate headspace. We stored the samples at 34°C until they were analyzed (about 1 week). After bringing the samples to room temperature, we tested the pH using an Orion pH meter. Before readings were taken, the pH meter was calibrated with two known standards (pH 7 and pH 4) to establish a linear calibration. We averaged the reading of the multiple samples from each pond to report the pond pH.

2.4 GIS layers for land cover variables

Using georeferenced digital orthophotomaps based on 2006 and 2007 aerial photographs obtained from the Virginia Geographic Information Network (VGIN), we built a GIS map in ArcMap 9.2 (ESRI 2007). We categorized the
landscape into 12 different habitats (grass, hardwood, pine, industrial, pond, residential, road (paved), road-dirt (gravel or sand), road-tree (gas line right-of-way), stream, dirt, and traintracks) based on the aerial photos and ground truthing. We defined buffer areas around each pond at 30, 250, 500 and 1000 meters. Analysis was limited to 1000m because most pond breeding amphibians move within less than 1 kilometer of the breeding pond (Semlitsch and Bodie 2003). The three most common land cover types were grass, hardwood, and pine. In the 1000 meter buffer, the percent of grass land cover ranged from 0.0 – 13.7%, hardwood ranged from 29.1 – 44.7% and pine ranged from 28.0 – 50.2%. In GIS, we created a model using XToolsPro 5.3 to define the buffer, clip out the central pond, dissolve the land cover types within the buffer and then calculate the area of each land cover type within each buffer. For pond level models, the ponds were further subdivided into three categories: open water (pond), swamp with trees and shrubs (pond-tree), or marsh with grasses (pond-grass).

2.5 Analysis

Detection histories from surveys were analyzed using the program PRESENCE (Hines 2006). To be sure the surveys followed the assumptions for a closed season, the detection history for Mabee’s larvae in both 2008 and 2009 consisted of the last four surveys. These histories included the first survey where Mabee’s larvae were detected through the last survey when Mabee’s larvae were still being detected. In 2009, for adult Mabee’s
salamanders, again, we chose the surveys to ensure the season was closed. We choose the first survey, when adults were detected, through the fourth survey, which was the last time adults were detected. For marbled salamanders, all 2009 surveys were used in the analysis because we had detections during every survey.

A set of candidate models (Table 1) was used to examine the data and consisted of both microhabitat (pond) variables and landscape variables (Mazerolle et al. 2005). Microhabitat models included variation in pond area, pond vegetation (open, pond-tree or pond-grass) and pond water characteristics (pH, conductivity, and turbidity). A fourth pond scale model combined variation in pond area and vegetation. All models were tested with the following detection variables as well: date, area, and pond vegetation (pond, pond-tree or pond-grass). The landscape models included variation in total pond area or disturbance (road, clear cut, train track, residential, industrial, rights-of way) within 30, 250, 500 and 1000m buffers. Other landscape models included variation in the total amount of habitat (grass, pine or hardwood) within 30, 250, 500 and 1000m buffers. No combined pond and landscape level models were run due to the sparseness of the data which reduced the power to test models with too many parameters.

The best models were selected using Akaike Information Criterion, AIC (Akaike 1973). Using AIC values, “one can directly weigh the evidence in favor of a model, given the set of candidate models using Akaike weights”
(Mazerolle et al. 2007). The top supported models from each group (pond or landscape) were then run together to see which were best supported overall.

3. Results

In 2008, only two adult Mabee’s salamanders were captured during the entire study. Only two marbled salamanders were captured during the entire study. Due to the small number of detections, we did not analyze these data. In the same season, Mabee’s salamander larvae were detected at 10.8% (n=5) of the 46 ponds surveyed. The larvae were detected in two out of four surveys at every pond where they were seen. The limited detections of both adults and larvae were likely due to the persistent drought through 2007-2008. Despite the recurring detections of the larvae, the general sparseness of the data makes the standard errors large or inestimable.

In the 2009 season, there was much greater success in detecting adult Mabee’s salamanders. They were detected at 20.0% (n=10) of the 50 ponds surveyed. Of the ten captures, however, only one was a repeat detection. Again, due to the lack of repeat detections and the sparseness of the data, many of the standard errors are large or inestimable and we are not reporting the results here. The summary of the top ranked models for the 2009 surveys of A. mabeei and A. opacum larvae can be found in Table 2.

3.1 Ambystoma mabeei larvae 2009
In 2009, Mabee’s larvae were detected at least once at 12.72% (N=7) of the 55 ponds surveyed. At ponds where they were detected, they were caught an average of 1.71 times per pond.

### 3.1.1 Pond level models

For 2009 Mabee’s larvae, models including pH and the detection variable of shrubby vegetation ranked the highest in explaining salamander occurrence at breeding ponds. The best model to explain occurrence was \( p(\text{veg}) \psi(pH) \). The beta estimate describing the relationship between pH and occupancy was 4747.1 ± 11.0. The beta estimate describing the relationship between detection and shrubby pond cover was -0.02 ± 0.01. The results suggest that it is harder to detect Mabee’s larvae at ponds with a higher proportion of shrubby vegetation and that ponds with higher pH have a greater probability of occupancy by Mabee’s salamander larvae.

For the top model, individual site estimates of occupancy range from 0.0 ± 0.0 to 1.0 ± 0.0. Ponds with pH > 3.81 have \( \psi \) estimates of 1.0. Those with pH < 3.81 have \( \psi \) estimates of 0.0. Because the beta estimate is so large, the \( \psi \) estimates are pushed to either zero or one. The individual estimates of detection probability ranged from 0.0 ± 0.0 to 0.6 ± 0.2 depending on the proportion of pond that had shrubby vegetation.

To be sure that there was not a relationship between detection and pH, we ran one final model with pH as a covariate of \( p \). For the model
\[ p(pH)\psi(pH), \] the beta estimate for the relationship between occupancy and pH was 41.6 ± 0.5. The beta estimate for the relationship between detection and pH was -1.8 ± 1.8. The results continue to support the model that a pond with a higher pH will have a higher probability of occupancy. It also confirms that there is not a confounding effect of a positive correlation between pH and detection.

### 3.1.2 Landscape level models

Landscape models that included the proportion of grass area around the pond and the proportion of other ponds within 1000 meters ranked the highest in explaining Mabee’s salamander larvae occurrence at breeding ponds. The best supported model was \( p(.)\psi(1000\text{grass}) \). The beta estimate for the relationship between the proportion of area of grass within the 1000 meter buffer surrounding a pond and occupancy at that pond was 36.0 ± 13.1. The second best model was \( p(\text{veg})\psi(1000\text{pond}) \). The beta estimate that explains the relationship between the total amount of pond area within the 1000 meter buffer around a pond and occupancy was -165.5 ± 75.5. The beta estimate describing the relationship between detection and shrubby pond cover was -0.02 ± 0.01. These results suggest that probability of occupancy by Mabee’s larvae is positively correlated with the amount of grass habitat found within 1000 m of the pond and negatively correlated with the amount of pond habitat found inside the 1000m buffer.
For the top model, $p(.)\psi(1000\text{grass})$, the individual site estimates of occupancy range from $0.5 \pm 0.2$ to $0.8 \pm 0.2$ for ponds located near or along grassy fields and from $0.04 \pm 0.03$ to $0.1 \pm 0.06$ at ponds surrounded by forest. The estimate of detection probability for the top ranked model was constant for all ponds and was $0.4 \pm 0.1$.

When the pond and landscape models were run together, the pond level models were the best supported. The best overall model to explain occurrence of Mabee's larvae at breeding ponds in 2009 was $p(\text{veg})\psi(\text{pH})$.

### 3.2 Ambystoma opacum larvae 2009

During 2008, *A. opacum* larvae were detected at only two ponds. We did not analyze that data. In 2009, however, marbled salamander larvae were relatively common and were detected at 29.1% (N=16) of the 55 ponds surveyed. At the ponds where they were seen, they were detected an average of 2.19 detections per pond.

#### 3.2.1 Pond level models

For 2009 *A. opacum* larvae, the highest ranking model was $p(\text{veg})\psi(\text{pH+turb+μS})$. The beta estimates that characterize the relationship between occupancy and pH, turbidity and conductivity, respectively, were $51.1 \pm 0.4$, $0.04 \pm 0.02$ and $0.2 \pm 0.03$. The beta estimate describing the relationship between detection and shrubby pond cover was $0.01 \pm 0.0$. The
second best model was \( p(\text{grass})\psi(p\text{H}+\text{turb}+\mu S) \). The beta estimates that characterize the relationship between occupancy and pH, turbidity and conductivity, respectively, were 51.8 ± 0.4, 0.04 ± 0.01 and 0.2 ± 0.04. The beta estimate describing the relationship between detection and grassy pond cover was -0.02 ± 0.01. These results continue to suggest that pH plays a critical role in occupancy at the breeding pond.

For the top model, individual site estimates of occupancy ranged from 0.8 ± 0.1 to 1.0 ± 0.0 at ponds with higher pH and from 0.0 ± 0.0 to 0.4 ± 0.1 at ponds with lower pH. The individual site estimates of detection probability for the top ranked model ranged from 0.2 ± 0.1 at ponds with a higher proportion of shrubby vegetation to 0.4 ± 0.1 at ponds with more grass cover.

Again, we ran a final model that included pH as a covariate for detection to be sure that there was not a relationship between detection and pH. For the model \( p(\text{pH})\psi(\text{pH}) \), the beta estimate for the relationship between occupancy and pH was 26.9 ± 0.4. The beta estimate for the relationship between detection and pH was -1.1 ± 0.8. For marbled salamander larvae the results continue to support the model that a pond with a higher pH will have a higher probability of occupancy. It also confirms that there is not a confounding effect of a positive correlation between pH and detection.

3.2.2 Landscape level models
Landscape models that included the total area of hardwood forest around the pond and the area of other ponds ranked the highest in explaining marbled salamander larvae occurrence at breeding ponds. The best model was $p(\text{veg})\psi(1000\text{hard}+30\text{pond})$. The beta estimate for the relationship between the total area of hardwood forest within the 1000 meter buffer surrounding a pond and occupancy at that pond was $22.3 \pm 8.9$. The beta estimate for the relationship between the total area of other ponds within 30 meters was $-16.9 \pm 8.7$. These results suggest that probability of occupancy by $A. \text{opacum}$ larvae is positively correlated with the amount of hardwood forest habitat found within 1000 m of the pond and negatively correlated with the amount of pond habitat found inside the 30 meter buffer.

For the top model, $p(\text{veg})\psi(1000\text{hard}+30\text{pond})$, the individual site estimates of occupancy range from $0.4 \pm 0.2$ to $0.8 \pm 0.1$ for ponds located inside hardwood forest stands and isolated from other nearby ponds. The estimates of detection probability for the top ranked model range from $0.2 \pm 0.1$ to $0.5 \pm 0.1$ depending on the amount of shrubby vegetation.

When the pond and landscape models were run together, the pond level models were the best supported. The best overall model to explain occurrence of $A. \text{opacum}$ larvae at breeding ponds is $p(.)\psi(\text{turb}+\mu\text{S}+\text{pH})$.

4. Discussion
In this study, for both species, *A. mabeei* and *A. opacum*, pH seems to be the determining factor that will predict occupancy at a breeding pond. This is not unexpected considering *Ambystoma* species appear to be relatively sensitive to low pH conditions (Pierce 1985). It is, however, particularly interesting how low the pH is. During this study, the pH at Grafton Ponds ranged from 3.36 to 4.41. The lowest pH where *A. opacum* larvae were found was 3.62. The lowest pH pond where *A. mabeei* larvae were found was 3.81. These pH measurements are far lower than the published ranges of pH that cause of 50% embryo mortality for *A. maculatum* (4.5-5.0), *A. tigrinum* (5.6), *A. jeffersonianum* (4.5) and *A. texanum* (4.2-5.0) (Pierce 1985, 1993; Sadinski and Dunson 1992). The larvae we are seeing at Grafton Ponds are likely the less than 50% that survive such acidic waters. It is important to note, that amphibian larvae are more acid tolerant than embryos and there are even some embryos of frog species that can survive at these low pHs (Freda and Dunson 1985; Pierce 1993). For example, for the Pine barrens treefrog, *Hyla andersonii*, has a 50% mortality rate at pH 3.6-3.8 (Pierce 1985). Wood frogs, *Rana sylvatica*, have a 50% embryo mortality rates at pH 3.5-3.9 (Pierce 1985). Both of these frog species are well within the pH range shown at Grafton Ponds.

For both Mabee's larvae and marbled salamander larvae, the models in this paper suggest that ponds with a higher pH have a greater probability of occupancy. Other studies have also suggested positive relationships
between distribution, abundance and pH (Dunson et al. 1992; Pierce 1985, 1993). Studies of amphibian distribution suggest that both abundance and species richness are reduced in acid environments (Pierce 1985). The absence of certain amphibian species, the decrease in number of egg masses and decrease in hatching success are all associated with pond acidity (Horne and Dunson 1994; Pierce 1993). For example, the number of spotted salamander eggs masses is positively correlated with pH (Pierce 1985).

While these and other studies demonstrate the positive relationship between pH and amphibian distribution, this is the first study to use occupancy modeling and take non-detections into account. For both *A. mabeei* and *A. opacum*, detection probabilities were relatively low for both pond and landscape models (0.2 to 0.5). With such a low probability of finding the species’, without taking non-detections into account, any inferences made about the pond or landscape characteristics as predictors of salamander occupancy are likely to incorrectly estimate their importance (Mazerolle et al. 2005). The benefit of occupancy analysis is despite detection probability being so low, we were accurately able to estimate the proportion of ponds occupied. We were also able to demonstrate that the relationship between pH and occupancy is not due to pH affecting detection probability.
Further research is needed, particularly for *A. mabeei*, to better understand the relationship between occupancy and pH. In Grafton Ponds, are the adult salamanders selecting ponds with higher pH or does low pH reduce the survival rate of eggs, embryos and larvae? Some studies of terrestrial salamanders report that soil pH plays an important role in the distribution of those salamanders: adults actively avoid low soil pH (Pierce 1993; Wyman and Jancola 1992). Our data did not suggest a relationship between pond pH and adult occupancy. Perhaps adults lay eggs in any pond, but only in ponds with higher pH do the eggs develop into larvae. Further study is still needed to determine breeding site selection for adults and whether or not pH plays a role.

The strength of the positive correlation between pH and occupancy shows that in Grafton Ponds there is a strong selection pressure for acid tolerance in these two species. A second line of research could explore the acid tolerance of larvae of both populations of *Ambystoma* species to determine whether this acid tolerance is due to plasticity or has selection pressure for acid tolerance resulted in a local adaptation to low pH in the Grafton Ponds area. How does the acid tolerance of Mabee’s and marbled salamander embryos and larvae from Grafton ponds compare to the those same species elsewhere in their range? Do genetic differences exist between the populations or does acclimation (i.e. plasticity) account for their ability to survive in acidic ponds (Pierce 1985)?
Landscape level variables also affected the occupancy of salamanders at breeding ponds. These results support the numerous other studies that demonstrate the importance of upland habitat (Baldwin et al. 2006; Crawford and Semlitsch 2007; deMaynadier and Hunter 1999; Herrmann et al. 2005; Homan et al. 2004; Otto et al. 2007; Rubbo and Kiesecker 2005). For marbled salamanders, it is more probable that they occupy ponds with extensive stands of hardwood around them. Mabee’s salamanders are more likely found at ponds with more grass surrounding them. For both species, the surrounding upland habitat was considered important at the widest buffer width, 1000 meters. Both species have a higher probability of occupancy at more isolated ponds. This result is counter to metapopulation theory but at the scale of this study and the number of ponds available perhaps no pond is really “isolated”.

For both species, models describing the effect of landscape variables on occupancy were not nearly as well supported as pond level models. This could stem from the fact that the study area is not big enough or the degree to which we characterized the landscape is not specific enough to show significant differences in habitat from one pond to another. It may also mean that the Grafton Ponds area has enough upland habitat for overwintering and foraging to support the existing populations of salamanders within it thereby making habitat distinctions difficult to model.
It is also important to recognize the impacts that drought and time of year may have on the detection probability of these species. For 2008, the lack of rain either made it much more difficult to detect these species, or they were not traveling to the breeding ponds at all. In 2009, while we detected *A. mabeei* adults at the pond, we did not have repeat detections. It is likely that adults reach the breeding site, breed and then leave. For future occupancy studies, it would be more efficient to concentrate efforts on the larval stage, being sure to wait until the larvae are big enough to detect.

5. **Conservation implications**

The results of this study have some important conservation implications. To begin, this is the first study, that we are aware of, to show that low pH appears to limit the distribution of these two salamanders, *A. mabeei* and *A. opacum*.

Secondly, while there is an increasing emphasis on the importance of upland habitat for amphibian conservation and it is well documented that habitat loss and alteration is the major cause of amphibian decline in the Southeastern United States (Sharitz 2003; Todd et al. 2009), it is important not to lose sight of the importance of the pond environment. This study confirms that while upland habitat up to 1000 meters away is important, in this case, the water quality variables were better predictors of pond occupancy.
Thirdly, it is important to note that low pH appears to be negatively affecting the occupancy of breeding ponds for a state threatened amphibian species. In a zoological survey of Grafton Ponds conducted by the Virginia Department of Conservation and Recreation in 1995-1997, Mabee’s salamander was identified at 17 of the 29 ponds surveyed (58.6%). In this study, Mabee’s salamanders were found at only 12.72% of the ponds surveyed. As part of a 1997 vegetation study of Grafton ponds by the Virginia Department of Conservation and Recreation, water quality data was collected on June 11, 1997. At that time, the pH was measured at 34 ponds. The average pH was 4.64 and the minimum value was 4.19. Twelve years later in this study, the pH, taken on April 7-10 2009, at 55 ponds averaged 3.71. The highest value measured (4.41) was still lower than the average for the 1997 study. While different amphibian sampling methods make direct comparisons between the studies impossible, when you combine the facts that occupancy appears to have declined and pH appears to have declined with the positive relationship between pH and occupancy, it is strongly suggestive that pH is causing the declines. More research needs to be done to determine if there is causation, and to what extent, between decreasing pH levels and decreasing populations of amphibians in the Grafton Plains pond complex.
Figure 1. A. Mabee’s salamander adult. B. Mabee’s salamander larvae

Figure 2. Mabee’s salamander range.
Figure 3. A. Marbled salamander adult. B. Marbled salamander larvae.

Figure 4. Marbled salamander range.
**Figure 5. Map of Grafton Ponds Natural Area Preserve and Newport News Waterworks property showing ponds surveyed and species found.***

**Table 1 – Set of candidate models considered to explain Mabee’s salamander and marbled salamander occurrence at 55 ephemeral ponds in Grafton Ponds Natural Area Preserve in 2009. For each group, pond scale and landscape scale, each p model was tested with each ψ model. All models were run for both species larvae except where noted.**

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<td>grass</td>
</tr>
<tr>
<td>occupancy probability varies with field and hardwood (A. mabeei only)</td>
<td>grass hard</td>
</tr>
<tr>
<td>occupancy probability varies with hardwood and pond (A. opacum only)</td>
<td>hard pond</td>
</tr>
</tbody>
</table>

* These ψ models were run for each of the four landscape scales (i.e. 30, 250, 500 and 1000 m)
Table 2 – Highest-ranked occupancy models of 2009 Mabee’s salamander larvae and marbled salamander larvae. Highest ranking models using PRESENCE. Models with Akaike weight < 0.05 excluded for clarity.

<table>
<thead>
<tr>
<th>Model Structure</th>
<th>No. of parameters</th>
<th>ΔAIC</th>
<th>Akaike weight</th>
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</thead>
<tbody>
<tr>
<td>2009 <em>A. mabeei</em> larvae</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Pond level models</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>veg pH</td>
<td>4</td>
<td>0.00</td>
<td>0.655</td>
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<tr>
<td>veg pH turb µS</td>
<td>6</td>
<td>3.73</td>
<td>0.102</td>
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<tr>
<td>veg 1000grass</td>
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<td>0.071</td>
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<tr>
<td>veg 1000pond</td>
<td>4</td>
<td>0.40</td>
<td>0.058</td>
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<td><strong>Pond and Landscape models combined</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>veg pH</td>
<td>4</td>
<td>0.00</td>
<td>0.993</td>
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<tr>
<td>2009 <em>A. opacum</em> larvae</td>
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<tr>
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<td>0.00</td>
<td>0.336</td>
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<td>0.236</td>
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<td>0.114</td>
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<td>3.60</td>
<td>0.056</td>
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<td>veg 1000hard 30pond</td>
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<td>0.995</td>
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