Species-Specific Effects of Acidity on Pond Occupancy in Ambystoma Salamanders

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ABSTRACT.—We used an occupancy modeling framework to test the relative importance of upland habitat composition (proportion of different types of vegetation and development) and pond characteristics (water chemistry and vegetation) in determining the occurrence of two species of pond-breeding salamanders, the Marbled Salamander (Ambystoma opacum) and Mabee’s Salamander (Ambystoma mabeei), in an urban nature reserve in southeastern Virginia, USA. Of 55 ponds surveyed in 2009, we found A. opacum larvae in 17 (30.9%) ponds and A. mabeei larvae in seven (12.7%) ponds. We found a strong positive relationship between A. opacum larval occupancy probability and pH; salamanders were more likely to occupy less acidic waters. Sampled ponds were highly acidic, with pH values ranging between 3.36 and 4.41, but A. opacum occupied only ponds with pH values >3.66. Ambystoma mabeei was more tolerant of the highly acidic conditions and was more likely to occupy larger ponds with fewer trees and scrubs. Landscape-level models were not well supported. Although pond acidification is generally dismissed as a cause of amphibian declines, our results suggest that pH can be an important, species-specific factor in determining occupancy of pond-breeding amphibians.

Amphibians are experiencing alarming rates of decline globally, with approximately 41% of species for which data are available being of conservation concern (Stuart et al., 2004; Blaustein and Bancroft, 2007). Moreover, amphibians have been considered environmental indicators by some (Tuberville et al., 2005; Hopkins, 2007; but see Kerby et al., 2010), making them useful models for studying anthropogenic impacts on wildlife. The factors that negatively affect amphibians are diverse and often the same factors that affect other wildlife species, including habitat loss, climate change, disease, overexploitation, contaminants, and introduced species (Alford and Richards, 1999; Cushman, 2006; Blaustein and Bancroft, 2007; Hopkins, 2007).

Land use changes such as habitat loss, alteration, and fragmentation are the most significant causes of global amphibian declines (Bradford, 2005; Cushman, 2006). Habitat loss or alteration also is thought to be the primary reason for amphibian declines in the southeastern United States (Sharitz, 2003; Tuberville et al., 2005). Extensive tracts of old-growth forest were removed in the early 1800s for timber extraction and creation of cropland (Sharitz, 2003; Wyman, 2003). Some forest has regenerated, but much is still managed for timber production (Alford and Richards, 1999). In addition, 20–50% of wetlands in the southeastern United States have been lost since 1780 (Dahl, 1990), affecting both aquatic and terrestrial amphibian species.

Amphibians in remaining wetlands now face the additional potential threat of habitat acidification. Derived from the burning of fossil fuels, atmospheric deposition of sulfuric and nitric acids occurs in the form of acid precipitation. Previous work has shown that species richness and density of terrestrial amphibians can decrease when soil pH is <3.8 (Wyman and Jancola, 1992). Laboratory studies of amphibians in aquatic habitats show that acidic water causes an increase in egg and larval mortality as well as sublethal effects (reduced growth rates and increased time until metamorphosis) for those individuals that do survive (Pierce, 1985, 1993; Horne and Dunson, 1994). When pH decreases, acid-sensitive species, such as the salamander Ambystoma jeffersonianum, are excluded from amphibian assemblages, and acid-tolerant species, such as the Wood Frog, Lithobates sylvaticus (Rana sylvatica), may increase because of the absence of this predator (Dunson et al., 1992). These examples from laboratory studies demonstrate that habitat acidification has complex effects on development, growth, and survival, but we do not yet know how these effects influence population dynamics in wild populations (Dunson et al., 1992). For example, Vertucci and Corn (1996) found no evidence that acid deposition was responsible for amphibian declines in the Rocky Mountains. In a review of studies conducted across the eastern and western United States, Rowe and Freda (2000) concluded there was no evidence that acidification causes declines in amphibian populations. Thus, evidence for a negative effect of acidification on wild amphibian populations is currently weak.

Here, we examine the relative effects of upland vs. in-pond factors on occupancy for two species of Ambystoma salamanders, Mabee’s Salamander (Ambystoma mabeei) and Marbled Salamander (Ambystoma opacum), that are threatened by habitat loss and degradation due to development in southeastern Virginia, USA. Both species require ephemeral, fish-free ponds for breeding, but A. opacum breeds in the late winter or early spring. As adults, both species rely on upland forest habitat for foraging and shelter, but on the Virginia Coastal Plain, such habitat is subject to habitat loss due to residential, commercial, and industrial development (Clark, 1998). Breeding habitat is lost when ponds become small and have hydropериods too short to support successful larval metamorphoses. Even when these wetlands or pond complexes are protected, the small, ephemeral ponds have little buffering capacity against pollutants entering via storm water runoff or direct precipitation (Pierce, 1985).

The specific goals of our study were to use occupancy estimation based on detection vs. nondetection data for both species to 1) estimate the probability of pond occupancy for each species and 2) test the effects of upland terrestrial habitat and pond habitat variables, including water quality (e.g., pH, conductivity, and turbidity), on pond occupancy.

MATERIALS AND METHODS

Study Species.—Mabee’s Salamander is found in the Atlantic Coastal Plain from South Carolina to extreme southeastern...
Virginia. It breeds in winter or early spring in a variety of lentic water bodies, including vernal ponds, Carolina bays, cypress-tupelo ponds, and ephemeral, naturally acidic ponds in pine stands (Petranka, 1998). In Virginia, the species is recognized as state-threatened due to loss and alteration of habitat (Clark, 1998; Roble, 1998; Mitchell et al., 2002).

Marbled Salamanders range from southern New England south to northern Florida and west to Oklahoma and Texas. They breed in ponds in eastern deciduous forests and tallgrass prairie (Petranka, 1998). *Ambystoma opacum* breeds in fall or early winter before temporary ponds fill with water, and this salamander first to hatch once the ponds fill.

**Study Area and Site Selection.**—Grafton Ponds Natural Area Preserve is a 374-ha property located on the lower Virginia Peninsula in York County (Fig. 1). The property is owned 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 a two-lane, paved road. The preserve is surrounded by forest, and residential, commercial, and industrial development (Clark, 1998).

Grafton Ponds is located in a low-lying area of the Atlantic Coastal Plain where depressions are formed when the shell-rich layers in the underlying sediments dissolve. As they dissolve, the overlying soil subsides and compacts, forming a depression (Clark, 1998). The resulting soil is a clay-rich sediment that slows percolation and leaves standing water in depressions. There are >70 depression ponds within the preserve boundaries and >200 ponds in the surrounding area. The ponds are highly variable in hydrology, shape, and size, but they generally hold water from winter to early summer and range in size from 0.9 to 9.1 m in diameter and from 0.15 to 1.7 m in depth during high water (Clark, 1998). The ponds support a wide range of insects, amphibians, reptiles, and plants, including several state rare species such duckweed firetail (*Telebasis byersi*), Harper’s fimbry (*Eimyrsalis perpusillia*), and Mabee’s Salamander (*Rawinski*, 1997; Clark, 1998; Roble, 1998).

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, Virginia; 2) the 2007 Virginia Geographic Information Network aerial photos of the Virginia State Plane 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. Because of increased rainfall in 2009, additional ponds held water and were added to the study, for 55 sites in total (Fig. 1).

**Field Sampling.**—Each pond was surveyed by one to two observers (CMF was always present to ensure standardization) using visual encounter surveys approximately every other week during the breeding season for *A. mabeei* and during the larval season for both species. We detected breeding adults by searching leaf litter debris every 4 m and turning over all logs on the forest floor within 4 m of the pond perimeter until we found at least one individual of both species or until we circled the entire pond. For detecting larvae, we visually searched the entire perimeter of ponds and swept the shallows with D-loop nets until we found at least one individual of both species or until we circled the entire pond. Because we searched along the entire perimeter of all ponds, time spent sampling was proportional to pond perimeter and varied from 4 to 132 person-min. For the 2008 season, surveys began on February 23 and ran through June 2 (*n* = 6 surveys). In 2009, surveys began on February 15 and ran through May 15 (*n* = 7 surveys). A single round of surveys took approximately 5 days to complete.

**Water Quality and Habitat Variables.**—Three water quality measurements were measured for each pond over a period of 4 days, April 7–10, 2009: turbidity, conductivity, and pH. In prior studies, amphibian abundance in small ponds has been shown to vary with these measures of water quality (Gascon and Planas, 1986; Knutson et al., 2004). We measured turbidity 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 a measurement of turbidity. We measured conductivity in the field by using a model 30 SCT handheld conductivity meter (YSI Inc.). To estimate pH, we collected multiple water samples from each pond (two to five samples per pond, in proportion to pond size) in 60-ml polyethylene bottles filled to overflowing to eliminate headspace (minimizing exchange of CO2 between aqueous and gas phases). Samples were brought to the laboratory within 4 h of collection where they were stored at 1°C. They were then analyzed within 4 days of collection (ensuring low rates of microbial transformations that might affect pH). After bringing the samples to room temperature, we measured pH using a 11 pH meter (Beckman Coulter) with two-point calibration. We averaged the hydrogen ion concentration of the multiple samples from each pond as our measure of pH. Although pH measurements in low-conductivity water can be problematic (ASTM International, 2003), variation in pH among samples was low (average SE = 0.04). Furthermore, a second round of pH measurements later in the season in July yielded similar results among ponds.

Habitat variables within each pond were calculated by subdividing the ponds into three habitat categories: open water, swamp with trees and shrubs (pond-tree), or marsh with grasses (pond-grass). The percentages of the pond consisting of each habitat category are compositional (they sum to 1), causing inherent correlations and inducing an identifiability constraint unless one of the covariates is removed. A preliminary correlation analysis revealed the highest correlation between pond-tree and pond-grass (*r* = −0.80); therefore, we removed pond-grass from our occupancy analyses. Percentages of a pond that were open water (pond-open) and swamp with trees and shrubs (pond-tree) were used as pond-scale covariates, along with pond area (hectares) and the water quality variables described above. We did retain pond-grass as a covariate on detection probability as we believed it was more difficult to detect salamander larvae in ponds with large amounts of dense, grassy vegetation.

We then built a GIS map in ArcMap 9.2 (ESRI, 2007) by using georeferenced digital orthophotomaps based on 2006 and 2007 aerial photographs obtained from the Virginia Geographic Information Network (https://gismaps.virginia.gov/arcgis/services) to calculate landscape-level habitat variables. 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 train tracks—based on the aerial photos and ground-truthing. We defined buffer areas around each pond at 30, 250, 500, and 1,000 m. The maximum buffer distance was set to 1,000 m because most pond-breeding amphibians stay within 1 km of breeding ponds (Semlitch and Bodie, 2003). The three most common land cover types were grass, hardwood, and...
Fig. 1. Map of Grafton Ponds Natural Area Preserve and Newport News Waterworks property showing ponds surveyed and species and life-history stages found at each pond in 2008 and 2009.
In the 1,000-m buffer, the percentage of grass cover ranged from 0.0% to 13.7%, hardwood from 29.1% to 44.7%, and pine from 28.0% to 50.2%. In ArcMap, we created a model using XToolsPro 5.3 (http://www.xtoolspro.com/) to define the buffer, cut out the central pond, dissolve the land cover types within the buffer, and then calculate the percentage of area of each land cover type within each buffer.

**Occupancy Estimation and Modeling.**—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 nondetection of a species does not always mean the species is absent, i.e., species could be 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 such as *A. mabeei*, abundance may be exceedingly difficult to estimate, but estimation of species occurrence is still possible (MacKenzie et al., 2006).

Occupancy modeling uses a probability-based model with two parameters. The first parameter, $\psi$, is the probability that a site is occupied by the target species. The second parameter, $p$, 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 occupancy and detection probabilities independently as functions of measured covariates by using a logistic link function (MacKenzie et al., 2006).

Detection data were compiled into detection histories for each pond and analyzed using the program MARK (White and Burnham, 1999). Occupancy models assume that the state of the pond (occupied or not occupied) does not change among surveys within a season; thus, we used only the last four surveys in our analysis of *A. mabeei* larvae in both 2008 (April 10–June 4) and 2009 (April 2–May 15). These histories span the first and last survey where *A. mabeei* larvae were detected at any pond. Similarly, we truncated survey occasions for adult *A. mabeei* salamanders in 2009 to ensure the season was closed (surveys 1–4 only). For *A. opacum*, all 2009 surveys were used in the analysis because we detected larvae at one or more ponds during each of the seven survey occasions.

A set of 30 occupancy structures was used to test which pond or landscape variables best explained variation in occupancy among ponds. Ten pond-scale occupancy structures assumed that occupancy probability was constant among ponds (denoted (.) or varied with pond area (area), pond habitat (pond-tree or pond-open), or pond water characteristics (pH, turbidity, and conductivity). In addition, we fit additive models with pond area and habitat covariates combined [e.g., $\psi$ (area+pond-tree)] and all three water characteristics [$\psi$ (pH+turb+mass)]. Twenty landscape-scale occupancy structures included variation in total pond area within each buffer, and percentages of disturbance (road, clear cut, train track, residential, industrial, and right-of-way), and habitat (grass, pine, or hardwood) within 30-, 250-, 500-, and 1,000-m buffers. All occupancy structures were combined with three detection structures where detection was either constant among ponds [$p(.)$] or varied by either pond area or the percentage of grassy vegetation within the pond (pond-grass). We attempted to model detection probability as a function of survey occasion, but these models often had convergence problems. Because of sparse detection histories and to avoid overparameterization, no models combined pond- and landscape-level variables and most models contained only a single occupancy covariate, except for models with the three pond scale–combined structures. Our final candidate set included 90 models (all combinations of occupancy and detection structures). We fit these models to the detection history data for each species by using program MARK (White and Burnham, 1999), with the simulated annealing (Goffe et al., 1994) option to ensure the global maximum of the model likelihood was found. The best models were selected using small-sample corrected Akaike Information Criterion, AICc (Akaive, 1973), and we used AIC weights to evaluate the evidence in favor of a model, given the set of candidate models (Burnham and Anderson, 2002).

There is currently no goodness of fit (GOF) test available in program MARK for occupancy models that accommodates covariates or unequal sampling among sites. The parametric bootstrap GOF test developed by MacKenzie and Bailey (2004) and incorporated in program PRESENCE (Hines, 2010) accommodates these components, but it does not contain the simulating annealing option. Accordingly, we evaluated GOF using our simple constant model, $\psi(p(.))$, that converged to the same values with or without the simulating annealing algorithm. Logically, if this simple model fit the data adequately, then more complex models would fit even better. We used 100,000 bootstrap samples for each test.

### Results

In 2008, we did not detect any adult or larval *A. opacum*, and detection data were too sparse to fit our candidate models for *A. mabeei*. Adult *A. mabeei* were detected only at two of 46 ponds (4.3%; Fig. 1), and *A. mabeei* larvae were detected at five ponds (10.9%), but during too few survey occasions to provide reliable estimates of occupancy.

In 2009, we detected breeding adult *A. opacum* at only one of 55 ponds surveyed (1.8%; Fig. 1) and adult *A. mabeei* at 10 ponds (18.2%), but only one of these 10 ponds had detections during multiple surveys. Again, due to the lack of repeat detections and the sparseness of the data, many of the standard errors were large or inestimable. In contrast, there were more repeat detections of *A. mabeei* and *A. opacum* larvae in 2009, allowing us to fit our candidate set of models. *Ambystoma mabeei* larvae were detected at seven ponds (12.7%; Fig. 1), an average of 1.7 times per pond. *Ambystoma opacum* larvae were relatively common and were detected at 17 ponds (30.9%), an average of 2.2 times per pond. Thus, we focus on the analysis of the 2009 larval data here.

### Ambystoma mabeei Larvae

2009.—We found no evidence of lack-of-fit of the constant model for the 2009 *A. mabeei* larval data ($\chi^2 = 76.32, P = 0.15$). The best-supported models suggested occupancy probabilities were higher at larger ponds with fewer trees and shrubs (Table 1). Using a logit-link function, the top model was logit ($\psi$) = $\beta_0$ + $\beta_1$ (area) + $\beta_2$ (pond-tree), where $\beta_1$ = 1.52, with 95% confidence interval (CI) of (0.16, 2.89) and $\beta_2$ = -6.05 (-10.82, -1.29). Some model uncertainty exists regarding the appropriate detection structure for *A. mabeei*, but the model with constant detection probability among sites was ~2.4 times more likely than any other competing model [$\hat{p}(.)$ = 0.43 (0.23, 0.65)]. Still, detection structures suggesting a negative relationship to pond area, $\hat{p}_{area}$ = -0.16 (-0.59, -0.26), or surprisingly, a slight positive relationship to pond-grass, $\hat{p}_{pond-grass}$ = 0.85 (-1.46, 3.17), have some weight of evidence (Table 1). However, the pond-grass...
model has a β coefficient that includes zero, and it has a similar deviation as the model with constant detection probability despite the addition of a parameter, suggesting that it should be removed from the candidate set of models. All other models had \( w < 0.05 \). Landscape-scale occupancy models for \( A. mabeei \) larvae performed poorly: the best of these models suggested that the proportion of grass around the pond within 500 or 1,000 m was positively related to \( A. mabeei \) larval occurrence at breeding ponds, but these models had very little weight.

*Ambystoma opacum* Larvae 2009.—Similar to *A. mabeei*, we found no evidence of lack-of-fit of the constant model for *A. opacum* larvae (\( \chi^2 = 282.42, P = 0.21 \)). The highest ranking model for *A. opacum* larvae was \( \psi(pH, p(pond-grass)) \) (Table 1). The second and third best models, and all models with \( w > 0.04 \), also indicate that \( pH \) plays a critical role in occupancy by *A. opacum*. The top model yielded a \( \beta \) estimate for the relationship between occupancy and \( pH \), of \( \beta_{pH} = 3.96 \) (0.41, 7.52), suggesting that the odds of *A. opacum* occurrence increases \( \sim 50 \) times for a 1-unit increase in \( pH \), thus equivalent to a 10-fold increase in ion concentration (odds ratio = \( e^{\beta_{pH}} \)). Pond-specific occupancy estimates were >0.60 for ponds with higher \( pH \) (\( pH > 4.0 \)) but drop dramatically to <0.25 at ponds with low \( pH \) (\( pH < 3.6 \); Fig. 2). Estimates of detection probability from the top ranked model ranged from \( \sim 0.45 \) at ponds lacking grass cover to 0.05 at ponds with complete grass cover [\( \beta_{pond-grass} = -2.46 (-4.82, -0.10) \)].

As with *A. mabeei*, the landscape-level models were poor descriptors of the data. The best landscape-level model, \( \psi(250pond, p(pond-grass)) \), only had a model weight of \( w = 0.02 \) (Table 1).

**DISCUSSION**

We found that the most important factors affecting pond occupancy differed between our two focal salamander species. In *A. mabeei*, the proportion of shrubs and trees in ponds (pond-tree) and pond area were the most important factors, whereas in *A. opacum*, \( pH \) was the most important variable. This difference in species responses to pond acidity may be due to differences in species’ traits, particularly in habitat use. *Ambystoma opacum* is primarily an inhabitant of eastern deciduous forest and prairie, whereas *A. mabeei* is generally found in pinewoods in the Atlantic Coastal Plain, often breeding in ephemeral, acidic ponds. This difference in habitat use may make *A. mabeei* more adapted to and tolerant of low \( pH \) ponds than *A. opacum*.

The effect of \( pH \) on occupancy in *A. opacum* is not unexpected considering some *Ambystoma* species seem to be relatively sensitive to low \( pH \) conditions (Pierce, 1985). It was surprising, however, just how low the \( pH \) was in our focal ponds. During this study, the \( pH \) at Grafton Ponds ranged from 3.36 to 4.41. Low \( pH \) in Grafton Ponds could be driven by a combination of factors: acid precipitation (an oil refinery is within 6 km of the site), local geology (the dominant Windsor formation is a marine and estuarine soil deposits from which acid sulfate soils form), and organic acids from decaying vegetation. The lowest \( pH \) where *A. opacum* larvae were found was 3.62. This \( pH \) is lower than the published ranges of \( pH \) that cause 50% embryo mortality for *A. maculatum* (4.5–5.0), *Ambystoma tigrinum* (5.6), *A. jeffersonianum* (4.5), and *Ambystoma texanum* (4.2–5.0) (Pierce, 1985, 1993; Sadinski and Dunson, 1992). Survival of *A. mabeei* and *A. opacum* larvae in the highly acidic conditions of Grafton Ponds is remarkable, but not unprecedented. Amphibian larvae are more acid-tolerant than embryos and some embryos of frog species can survive in highly acidic water (Freda and Dunson, 1993).

### Table 1

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\( a \) = constant, Pond-grass = percentage of the pond vegetated as grasses.

\( D \) = pond \( pH \), turb = pond turbidity; mS = pond conductivity; 30pond and 250pond = proportion of other ponds within a 30- and 250-m buffer, respectively; 1000hard = proportion of hardwood forest within 1,000-m buffer; 500 and 1000grass = proportion of field within a 500- and 1,000-m buffer, respectively.

**Fig. 2.** Estimated relationship between occupancy probability and \( pH \) for 2009 *Ambystoma opacum* larvae. Solid line = point estimate; dotted line = lower and upper 95% confidence intervals.
1985; Pierce, 1993). For example, the Pine Barrens Treefrog, *Hyla andersonii*, has a 50% mortality rate at pH 3.6–3.8 (Pierce, 1985). Wood Frogs, *Lithobates sylvaticus*, have a 50% embryo mortality rate at pH 3.5–3.9 (Pierce, 1985). Importantly, it is possible that the correlation between pH and occupancy in *A. opacum* is not caused by low pH itself, but rather by water chemistry (e.g., presence of heavy metals) or biotic (e.g., changes in predators or prey) variables correlated with pH (Sadinski and Dunson, 1992; Horne and Dunson, 1995).

Other studies also have suggested positive relationships between pH and amphibian distribution, abundance, or species richness (Pierce, 1985, 1993; Dunson et al., 1992). An absence of certain amphibian species, a decrease in the number of egg masses, and a decrease in hatching success have all been associated with pond acidity (Pierce, 1993; Horne and Dunson, 1994). Although these studies demonstrate a positive relationship between pH and amphibian distribution, ours is one of the first studies of the relationship between pond acidity and amphibian distribution that uses occupancy modeling to account for detection probability (also see Mazerolle et al., 2005).

For both *A. mabeei* and *A. opacum* larvae, detection probabilities were relatively low for selected models and varied with vegetative features. With such a low probability of finding these species, any inferences made about the pond or landscape characteristics as predictors of salamander occupancy are likely to estimate their importance incorrectly if nondetections are not taken into account (Mazerolle et al., 2005). Despite low detection probabilities, we were able to estimate the proportion of ponds occupied for both species and demonstrate a strong, positive relationship between pH and *A. opacum* occupancy.

Our data do not allow us to determine whether the positive relationship between pond occupancy by *A. opacum* and pH observed in Grafton Ponds is due to adult salamanders selecting ponds with higher pH or to acidic water reducing the survival rates of embryos, larvae, or both. Soil pH has been shown to play an important role in the distribution of adult terrestrial salamanders that actively avoid acidic conditions (Wyman and Jacolca, 1992; Pierce, 1993). Our adult detection histories were insufficient to test for a relationship between pond pH and adult occupancy. Further study is needed to determine whether pH plays a role in breeding site selection, embryo mortality, and larval mortality in *A. opacum*.

The strength of the positive correlation between pH and larval occupancy suggests that in Grafton Ponds, these species are experiencing strong selection for acid tolerance. Previous studies have shown local adaptation to low pH in U.S. and European amphibians (Pierce and Harvey, 1987; Andrén et al., 1989; Räsänen et al., 2003a,b; Merilä et al., 2004). Thus it would also be interesting to investigate whether populations of *A. mabeei* and *A. opacum* in Grafton Ponds are more acid tolerant than populations from less acidic ponds in other areas and, if so, to determine the degree to which greater acid tolerance of Grafton Ponds populations is due to phenotypic plasticity and local adaptation (Pierce, 1985). Testing the relative importance of plasticity and adaptation in acid tolerance would require determining whether geographic variation in acid tolerance has a genetic basis using common garden experiments.

Our finding that *A. mabeei* occupancy was related negatively to the proportion of trees is consistent with previous research showing that shade from trees can reduce amphibian larval growth and abundance (Skelly et al., 2002; Halverson et al., 2003). Alternatively, it is possible that the proportion of trees does not negatively affect occupancy, but rather the proportion of grass positively affects occupancy. These two variables were highly negatively correlated. Thus, occupied ponds tended to have high proportions of grass and low proportions of trees and shrubs. *Ambystoma mabeei* may prefer ponds with high proportions of grass or emergent vegetation, as seen in some other amphibian species (Welch and MacMahon, 2005; Adams et al., 2011).

For both species, landscape-level occupancy models were not nearly as well supported as pond-level models. This difference 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 also may 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 terrestrial habitat seem relatively unimportant in the models. We strongly recommend that other studies testing the effects of upland landscape variables on occupancy by pond amphibians also include pond-level variables in their analyses to assess their relative importance.

It is also important to recognize the impacts that drought and time of year may have on the detection probability of these species. In 2008, the lack of rain either made it much more difficult to detect these species, or they did not breed in as many ponds. In 2009, although 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 large enough to detect.

The results of this study have important conservation implications. This study is the first study of which we are aware to show a positive relationship between pH and amphibian distribution by using a statistically robust occupancy modeling framework. Although previous studies have discounted the importance of acidity in amphibian conservation (Vertucci and Corn, 1996; Rowe and Freda, 2000), our study suggests that acidity can play an important role in determining the distribution of some amphibian populations, especially if pH values approach the physiological limits of amphibian species. Although 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 declines in the southeastern United States (Sharitz, 2003; Todd et al., 2009), it is important not to lose sight of the importance of the local pond environment. This study confirms that although upland habitat up to 1,000 m away is important, in this case, water chemistry variables, especially pH, were much better predictors of pond occupancy.

The second conservation implication of this study is that a comparison of our occupancy data to historical data for *A. mabeei* suggests that this state threatened species is declining. In a zoological survey of Grafton Ponds conducted by the Virginia DCR in 1995–97 (Roble, 1998), *A. mabeei* was identified at 17 of the 29 ponds surveyed (58.6%). Roble (1998) used similar methods to ours (visual encounter surveys and dip-netting), but only checked ponds “on at least one occasion.” In our study, by contrast, *A. mabeei* was found at only 12.7% of the ponds surveyed despite a more intensive survey effort (sampling ponds several times each year for 2 years). Alternatively, it is possible that *A. mabeei* was found at more sites by Roble in 1995–97 due to more favorable weather conditions for detecting.
A. mabeei (e.g., more rain). More research is needed to determine whether a decline has indeed occurred and, if so, assess the extent and causes of this decline. We recommend continued monitoring of A. mabeei populations in Grafton Ponds.

Finally, a comparison of our water chemistry data to historical data suggests that these ponds are becoming increasingly acidic. As part of a 1997 vegetation study of Grafton Ponds by the Virginia DCR (Rawinski, 1997), water quality data also were collected on June 11, 1997. At that time, pH was measured at 34 ponds. Average pH was 4.64 and the minimum value was 4.19. Twelve years later in our study, the pH, taken 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. Given that A. opacum occupancy is affected negatively by acidity, decreasing pH could affect their populations. Moreover, if pH becomes sufficiently low, it also may negatively affect A. mabeei because there is certainly some pH threshold below which even this relatively acid-tolerant species will not be able to grow, function, and survive.

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LITERATURE CITED


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