

**Assessment of Ambystomatid salamander populations
and their breeding habitats in Delaware Water Gap
National Recreation Area**

By

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TABLE OF CONTENTS

SUMMARY	3
INTRODUCTION	4
Background and Justification	4
Objectives	5
Study Area	5
Species Descriptions	6
METHODS	7
Landscape analysis and site selection	7
Salamander and pond habitat sampling	10
Genetic sampling	12
Data analysis	12
RESULTS	17
Weather patterns	17
Status of salamander populations	18
Critical breeding habitats	24
Influence of impoundments	30
Genetic structure	33
DISCUSSION	34
Status and critical breeding habitats	34
Influence of impoundments	40
Management implications	42
LITERATURE CITED	46

SUMMARY

We conducted a three-year study of ambystomid salamander breeding habits and habitats in the Delaware Water Gap National Recreation Area (DEWA). The objectives of the study were to document the population status and critical breeding habitats of the three species of ambystomid salamanders known to occur in the Park; *Ambystoma maculatum* (spotted salamander), *A. opacum* (marbled salamander), and *A. jeffersonianum* (Jefferson salamander). DEWA managers were interested in ecological information on these species for several reasons. First, at the time we began the study there was little known regarding the status of pond-breeding amphibians and their habitats in the Park. Secondly, because they require undegraded habitats in both terrestrial and aquatic habitats to successfully complete their life cycle, the status of ambystomatid salamanders are widely viewed as indicative of overall ecosystem health. And third, because ambystomatid salamanders and other pond-breeding amphibians have been observed in numerous artificial impoundments with the Park, DEWA managers were concerned that their current plans to dismantle or discontinue maintenance on a large number of artificial impoundments may be detrimental to pond-breeding amphibians and possibly other species that use pond or wetland habitats in the park.

In 2001, 2002, and 2003, we mapped the size and location of 200 wetlands, ponds, and artificial impoundments, and related landscape position and site habitat data to salamander occurrence and abundance patterns. The results summarized in this report provide significant new baseline information on ambystomatid salamanders and wetland habitats in the park that will enhance DEWA long-term inventory and monitoring efforts. In addition, breeding habitat assessments suggest that ambystomatid salamanders may be sensitive to a wide variety of stresses important to the park and to the region. In particular, recent trends in near-park and within park development (e.g., roads), regional increases in the acidity of precipitation, and predicted long-term warming trends for the region would all likely be detrimental to pond-breeding salamander populations because of their effects on breeding site quality and quantity, and on the integrity of migration corridors. In contrast, the results of the study indicate management plans to eliminate small impoundments are not likely to adversely affect salamanders.

INTRODUCTION

Background and Justification

Amphibians are integral components of North American wetland and forest ecosystems. In terms of diversity, amphibians represent approximately 12.5% of the total terrestrial vertebrate species in North America (Banks et al. 1987), and the Appalachian Highlands in eastern North America support a particularly high number of endemic amphibian species (Bury et al. 1995). In addition, amphibians frequently comprise the largest fraction of vertebrate biomass. For example, salamander biomass in a northern hardwood ecosystem in New Hampshire was more than twice that of the bird community and nearly equal to that of small mammals (Burton and Likens 1975), while in southern Appalachian forests salamander biomass may exceed that of all other vertebrates combined (Hairston 1987).

Concern over amphibians has heightened in recent years due to increasing evidence of global and regional population declines, range reductions, and extinctions (Wyman 1990). In the United States, about one-third of the amphibians species are suspected of having declines (Bury et al. 1995). These declines are not limited to highly degraded areas. Significant losses of amphibians have been documented in relatively pristine areas such as National Parks that generally lack obvious habitat loss or alteration (Blaustein and Wake 1990). Suspected causes of these regional declines include climate change (Pounds 2001, Carey et al. 2003), acid deposition (Freda 1986, Dunson et al. 1992), depletion of the ozone layer (Blaustein et al. 1995), and disease and parasites (Muths et al. 2003).

Within the nearly 70,000 acres of the Delaware Water Gap National Recreation Area (DEWA) are hundreds of wetlands, vernal ponds, swamps, marshes, beaver ponds and impoundments that provide potential amphibian habitat. Seven amphibian species have been documented in DEWA that are classified as "species of special concern" by the states of Pennsylvania or New Jersey (Stein 1983). Three of these species are pond-breeding ambystomid salamanders: Jefferson salamander (*Ambystoma jeffersonianum*), spotted salamander (*A. maculatum*), and marbled salamander (*A. opacum*). Ambystomatid salamanders (commonly called "mole salamanders") live most of the year underground in forested areas but emerge in early spring and migrate to woodland ponds and wetlands to breed and deposit eggs (Hulse et al. 2001). Adults return to their terrestrial habitats soon after breeding, and the eggs hatch as aquatic larvae that remain in their natal breeding pond for various amounts of time depending on hydroperiod, thermal regime, predation, competition, and other related factors (Dodd and Cade 1998).

In addition to the regional stresses referred to above, there is also concern that more local stresses may affect amphibian populations. In particular, rapid human population growth and associated residential development in areas adjacent to DEWA may negatively affect many species of pond-breeding amphibians because of pollution, direct destruction of terrestrial and aquatic habitats, and the disruption of dispersal corridors by roads. The counties surrounding DEWA have been among the fastest growing counties in Pennsylvania and New Jersey for more than a decade (documented by state statistics).

Activities within DEWA may also threaten salamander populations. Currently, DEWA actively manages beaver and has plans to manage over 120 man-made impoundments within the park. These actions influence the size, number, and distribution of ponds that may be important breeding habitats for ambystomid salamander populations. In addition, DEWA supports a network of roads and trails that may influence migratory behavior and success by fragmenting dispersal corridors. There are currently plans to expand trails and roads in the park. Finally, DEWA leases more than 3,000 acres for agriculture under special permits, and has recently initiated special riparian conservation and restoration measures. Information regarding the distribution, abundance and habitat requirements of these salamander species within DEWA is needed as soon as possible to inform these and other types of management decisions.

Objectives

The objectives of the project were to document the population status and critical breeding habitats of ambystomid salamanders in DEWA. The information will have immediate and long-term implications for the management and preservation of natural resources in DEWA. In the short-term, the data collected will be used to evaluate the importance of small, man-made impoundments in DEWA as habitat for pond-breeding amphibians. We specifically collected and analyzed data in such a way as to address the question of impoundment use by ambystomatid salamanders. In the longer term, the data will provide DEWA management staff with baseline information on status of wetland habitats and ambystomid salamander populations in the park, and can be used by DEWA management to assist in preservation of park biodiversity, and to predict the consequences of landscape changes resulting from DEWA management actions or increased development outside the park. In addition, updated wetland maps and baseline information regarding wetlands and wetland communities that resulted from the study may be used in future long-term monitoring programs in DEWA (e.g., Vital Signs). Because of their need for both optimal terrestrial and aquatic habitat to complete their life cycle, ambystomid salamanders and their habitats may be particularly useful indicators of overall ecosystem health.

Study area

DEWA encompasses 277.4 km² of forested hills, ravines, and bottom lands that straddle the Delaware River. The topographic setting is varied, with terraced benches and ravines on the west (PA) side of the river, and steeply sloping ridge-valley landforms on the east (NJ) side of the river (Snyder et al. 2002). The landscape also supports a wealth of wetlands including marshes, swamps, beaver ponds, and vernal ponds, as well as man-made impoundments. Excluding impoundments larger than 1 ha, DEWA has at least 317 wetlands or water bodies (1.45 wetlands/1 km²) that offer potential ambystomatid salamander breeding habitat. This is a relatively high density of wetlands (Gibbs 2000), especially considering the relatively steep terrain over much of the landscape.

Species Descriptions

The three species of ambystomatid salamanders found in DEWA are *Ambystoma jeffersonianum* (Jefferson salamander), *Ambystoma maculatum* (spotted salamander), and *Ambystoma opacum* (marbled salamander). Ambystomatid salamanders typically breed in vernal bodies of water where they function as the community's top aquatic predator. *Ambystoma jeffersonianum* and *A. maculatum* migrate to bodies of water in the early spring and lay egg masses. Larvae hatch within 2-6 weeks (depending on water temperature) and feed on a variety of invertebrate and vertebrate prey (including other amphibian larvae). Larvae will metamorphose in 2-4 months and initiate a terrestrial lifestyle. *Ambystoma opacum* however, lay eggs during the late summer or fall in dried vernal ponds or the shores of permanent ponds that have dried considerably. When these areas fill with rainwater larvae hatch, commence an aquatic lifestyle similar to other larvae of ambystomatid species, and over-winter at the bottom of ponds. Thus, in the spring, *A. opacum* larvae are considerably larger than sympatric *A. jeffersonianum* and *A. maculatum* larvae and enjoy a competitive and predatory advantage. Previous amphibian surveys of DEWA (Freda 1983, Stein 1978, Brotherton et al. 2000) have noted that *A. maculatum* is the most common ambystomatid salamander at DEWA, breeding in many bodies of water that retain water through the spring months, while *A. jeffersonianum* and *A. opacum* appear to have a much more limited distribution (historically breeding at only 6 and 10 sites respectively).

Although all three species of *Ambystoma* in DEWA are commonly described as breeding primarily in ephemeral bodies of water (Conant and Collins 1998, Petranksa 1998), we have observed each of them in permanent bodies of water during the course of our study. Furthermore, investigators have suggested that artificial bodies of water may prove to be a useful tool in preserving amphibian biodiversity in some areas (Laan and Verboom 1990, Beebe 1997, Monello and Wright 1999), although artificial bodies of water with permanent hydroperiods also frequently support predatory fish and macroinvertebrate species (Skelly 2001), and adults of some amphibian species have been shown to avoid breeding in bodies of water that contain fish (Semlitsch 1988, Resetarits and Wilbur 1991, Hopey and Petranksa 1994). The composition of amphibian assemblages is strongly influenced by wetland hydroperiod (Kolozyvary and Swihart 1999, Snodgrass et al. 2000) and amphibian species that are most successful at colonizing and occupying permanent bodies of water are typically large anurans (notably *Rana catesbeiana* and *Rana clamitans* in the northeastern US) or species that are highly noxious to predators (e.g. *Rana palustris* and *Notophthalmus spp.*). Large anuran species, in turn, will also prey on adult, juvenile, and larval stages of smaller amphibian species (Werner et al. 1995), and *Notophthalmus spp.* are known to prey on both egg and larval stages (Conant and Collins 1998, Petranksa 1998).

In addition to being historically the least common ambystomatid species in DEWA, *A. jeffersonianum* are a species of special concern within the park because their breeding success can be compromised at breeding sites that are shared with unisexual (all female), triploid, populations of *Ambystoma jeffersonianum*-complex biotypes (although this scenario is common in the northern portion of the species range). Unisexual *Ambystoma*

biotypes possess sets of chromosomes from both *A. jeffersonianum* and *Ambystoma laterale*, and the nomenclature used to identify members of this complex reflect their genetic makeup (i.e. LJJ = an individual with one set of *A. laterale* and two sets of *A. jeffersonianum* chromosomes). Unisexual *Ambystoma* biotypes typically reproduce via gynogamy and are sperm parasites of bisexual, diploid *Ambystoma* spp. (typically *A. jeffersonianum* and *A. laterale* populations). Sperm from bisexual *Ambystoma* are used to initiate mitotic division in non-reduced eggs of unisexual biotypes, and the resulting offspring typically do not contain paternal genetic material. In *A. jeffersonianum* populations with only diploid (JJ) individuals, male:female ratios are typically greater than 3:1 (Petranka 1998), but in diploid-unisexual populations male:female ratios can become less than 1:10 (Nyman et al. 1988). When this occurs, egg mortality can be high due to insufficient insemination of both JJ and LJJ females (Petranka, 1998). Traditionally, unisexual and bisexual individuals were distinguished from each other with techniques that worked best when blood/tissue samples are taken from adults (karyotyping, examination of erythrocyte nuclei, and electrophoresis), although recently, molecular techniques have been developed that allow scientists to use individual eggs and pre-hatchlings for biotype identification (Julian et al., 2003).

METHODS

Landscape analyses and site selection

We used GIS to compile and relate geo-referenced information on terrain, vegetation, and hydrology to the location of all known ponds, wetlands, and impoundments in DEWA. The goal was to determine the landscape setting of all the potential breeding habitats in park, and to use the information to develop a stratified sampling design that was representative of landscape variability. Potential ambystomatid salamander breeding habitat was defined as any wetland that contained water during the breeding season of *A. maculatum* and *A. jeffersonianum* (typically March and April), with the exception of artificial impoundments that were larger than 1 ha in surface area. We excluded large impoundments because they contain large populations of predatory fish, and because sampling effort required to adequately characterize breeding habitats in these large water bodies would be time prohibitive.

Initially, we developed a “potential breeding site” map by combining existing digital maps of DEWA wetlands and impoundments < 1ha, and then overlaid this potential breeding site layer with digital representations of terrain and land cover. Subsequently, we used GIS to characterize each of the 317 potential breeding habitats in terms of pond type, vegetation type, and topographic setting as described below.

We created the “pond type” strata by assessing wetland and impoundment maps prepared by DEWA personnel (High 1999). We grouped ponds into strata by selecting polygons with attributes we deemed suitable as potential amphibian breeding sites. The wetland layer was coded with modified wetland codes from Cowardin, et al. (1979) used by the U.S. Fish and Wildlife’s National Wetlands Inventory. We selected pond polygons into three types: wetlands, natural ponds, and impoundments. Wetlands were defined as those polygons coded as palustrine-emergent (PEM), palustrine-scrub/shrub (PSS), or

palustrine-forested (PFO). Natural ponds were those polygons coded as palustrine-open water-permanently flooded (POWH), palustrine-open water-intermittently flooded (POWJ), and lacustrine-littoral-open water-permanently flooded (L2OWH). Impoundments were those polygons coded as having been diked, impounded, or excavated. In addition, polygons were coded as being impoundments by overlaying wetland polygons with a point layer of impoundments compiled by DEWA personnel (A. Ambler, pers. comm.). Some impounded ponds in this layer were not present on the wetlands layer, so these were added into the selection strata separately. Finally, additional locations were added into the set of wetlands by incorporating vernal pond point locations compiled by the Wildlife Conservation Society in their 2000 survey (Brotherton et. al. 2000). Pond type strata were further collapsed into “natural” ponds consisting of all wetlands and natural ponds, and “impoundments” consisting of permanent, impounded water bodies.

Vegetation type strata for ponds were derived from attributes of wetlands polygons and by overlay of pond polygons on land cover maps. Specifically, we grouped ponds into herbaceous, scrub-shrub, forested, and agricultural types based on wetland vegetation type codes of “E” (emergent), “SS” (scrub-shrub), “FO” (forested) and “MOD” (modified/agricultural) respectively. Additionally we assessed vegetation type by overlaying pond polygons on maps of agricultural and forested areas derived from maps of land cover and agricultural fields provided by DEWA personnel. These four strata were further collapsed into three strata of “forest”, “scrub-shrub”, and “open” classes.

We grouped ponds into topographic categories of ridge, valley, and side-slope based on evaluation of terrain shape from digital elevation models. We used an algorithm that took into account concavity and convexity of slopes at multiple scales to classify ponds into categories of topographic position. Field evaluation revealed weaknesses in this approach, and we therefore collapsed topographic types into either “valley” or “ridge” ponds.

Site selection had both random and non-random components. Because of the apparent rarity of *A. jeffersonianum* breeding populations, and because of the stated concern of DEWA resource managers for this species, we non-randomly selected all known *A. jeffersonianum* breeding sites (initially N=9) for sampling all three years. A strictly random design would have yielded few, if any, *A. jeffersonianum* sites, whereas the non-random selection of *A. jeffersonianum* sites would ensure that we collected important ecological and genetic information for this high priority species. Also, any new *A. jeffersonianum* ponds that were discovered would be sampled in the subsequent year(s).

We used a stratified random scheme to select the majority of sampling sites each year. Specifically, we randomly selected sites from the 12 strata formed from the various combinations of pond types, topographic positions, and surrounding vegetation (Table 1). Over the three-year period, we sampled a total of 200 different sites, 187 of these were chosen at random at least in the first year they were sampled (i.e., *A. jeffersonianum* was found in some randomly selected ponds and consequently were non-randomly sampled in subsequent years), and the remaining 13 sites were chosen because of *a priori* knowledge

of the existence of *A. jeffersonianum*. Overall, the 12 strata of potential breeding habitats were sampled in approximately the same proportion as they occurred in the park.

Ponds selected for sampling were mapped in the field using Trimble© mapping-grade GPS receivers during a 2 week period each Spring coincident with biological sampling. GPS data files were returned to the Leetown Science Center and differentially corrected using base station files from NOAA's "Continuously Operating Receiver Station" website. Mean positional error of resulting GPS boundaries was 2.4 meters in year 1, 1.1 meters in year 2, and 1.0 meters in year 3 of the study. Overall accuracy of GPS boundaries was 1.4 meters over the life of the study. Improvements in positional accuracy can be attributed to upgraded GPS equipment in the second year of the study. GPS boundaries were exported as GIS polygon files and edited to remove spikes and other gross errors. Pond area and perimeter were calculated for each pond polygon using GIS software.

Table 1. Distribution of available habitats (All), total number of sites sampled including non-randomly selected sites (Comp), and the total number of sites selected at random (random). Table shows both the number of ponds and percentages in each of the 12 strata.

Type	Site category		Number of ponds			Percentage of ponds		
	Vegetation	Topography	All	Comp	Random	All	Comp	Random
IMPOUND	FOREST	RIDGE	17	14	13	5%	7%	7%
IMPOUND	FOREST	VALLEY	2	2	2	1%	1%	1%
IMPOUND	OPEN	RIDGE	5	5	5	2%	3%	3%
IMPOUND	OPEN	VALLEY	11	7	7	3%	4%	4%
IMPOUND	SHRUB/SCRUB	RIDGE	1	1	1	0.3%	0.5%	0.5%
IMPOUND	SHRUB/SCRUB	VALLEY	0	0	0	0%	0%	0%
NATURAL	FOREST	RIDGE	175	102	92	55%	51%	49%
NATURAL	FOREST	VALLEY	29	20	18	9%	10%	10%
NATURAL	OPEN	RIDGE	16	8	8	5%	4%	4%
NATURAL	OPEN	VALLEY	34	18	18	11%	9%	10%
NATURAL	SHRUB/SCRUB	RIDGE	14	12	12	4%	6%	6%
NATURAL	SHRUB/SCRUB	VALLEY	13	11	11	4%	6%	6%
TOTALS			317	200	187	100%	100%	100%

In addition to site selection, we used GIS to quantify other important landscape variables for use as explanatory variables in habitat selection models (see Data Analysis below). Specifically, we calculated measures of wetland isolation and road density in areas around all natural wetlands selected for sampling. The wetland isolation measures were calculated as the number of other potential breeding habitats in specified buffer areas around each selected wetland (i.e., the fewer the number of other ponds in buffered areas, the more isolated the breeding pond). Both the total number of water bodies (includes man-made impoundments), and the number of natural water bodies were determined for each pond. In addition, each isolation measure was calculated at two scales (i.e., buffer areas around focal ponds): 500-m and 1000-m buffers. Thus, a total of four wetland isolation measures were calculated for each natural wetland selected for sampling. We calculated road density (meters of road per hectare of buffer area) within 250-m, 500-m, and 1000-m buffer areas around each selected wetland. At each scale, we derived the density of primary roads and secondary roads. The distinction between primary and secondary roads was based on traffic statistics compiled by the Pennsylvania Department

of Transportation. Primary roads were defined as roads with greater than 1,000 cars per day. These assessments included only paved roads, and for roads in which no traffic data was available we used our best judgment as to whether a road was primary or secondary based on our own driving experiences in the park.

Salamander and pond habitat sampling

Each year, we sampled selected ponds for the presence and abundance of *A. jeffersonianum* and *A. maculatum* egg masses, and the presence of *A. opacum* larvae. We sampled each pond every two weeks between late February or early March (depending on weather) and mid-May. No selected pond was sampled on fewer than three occasions during the season. We also recorded the presence or absence of fish during each site visit.

We counted egg masses using visual encounter surveys (VES) using one of two sampling schemes. For ponds or wetlands that were either so large, or the number of egg masses so great, that they could not be completely censused by two investigators within 1.5 hours (i.e., 3 person-hours), we established a transect-based sampling scheme that assessed 20% of the entire pond or wetland. Transects were established perpendicular to the long axis of the pond and investigators counted all egg masses within a specified distance on either side of the transect line. The distance between transects and the width of the search path along the transect line varied depending on the size of the pond. We wanted a minimum of 5 transects on each pond so that count data would come from a variety of locations within the pond. Thus, for very large ponds (> 100 m in length along the long axis), transects were laid 20-m apart and the width of the search path along the transect line was 4-m. For very small ponds, transects were laid 5-m apart and the search path was 1-m in width. Intermediate sized ponds had transects set at 10-m apart with a 2-m search path. The location of the first transect in each pond was randomly selected between 0 (one corner of the pond) and the distance between transects. For all other ponds, we conducted a complete census, counting all egg masses in the entire pond.

Although individual egg masses of *A. maculatum* were easily discerned from one another, counting *A. jeffersonianum* egg masses proved more difficult because of conglomerations of closely spaced egg masses and high amounts of embryonic mortality. In instances where individual *A. jeffersonianum* egg masses could not be discerned, the number of egg masses was estimated using an approximation in which each 6cm length of eggs (the approximate length of a thumb) was assumed to equal one egg mass. Although this approximation was primarily based on field observations on the size of a “typical” *A. jeffersonianum* egg mass, Bishop (1941) observed that the mean egg mass length of *A. jeffersonianum* in a New York population was similar (5cm). In addition to recording the total number of *A. jeffersonianum* egg masses in a pond during a survey, we also determined the fraction of egg masses that were viable. Individual masses were categorized as unviable if $\geq 90\%$ of its embryos were dead (covered in fungus). In each year, we estimated the proportion of viable masses for each breeding site with the following calculation:

$$\frac{[\text{Highest total mass count from a single survey}] - [\text{Highest unviable mass count from a single survey}]}{\text{Highest total mass count from a single survey}}$$

The survey date for each site that yielded the highest total mass count typically yielded the highest unviable mass count (86% of the time). At sites where this did not occur, the maximum number of total masses occurred the survey date prior to the observed maximum number of unviable masses. Justification for using “unviable mass counts” from later dates was based on personal observations that unviable egg masses in DEWA persisted for approximately two weeks longer than viable masses laid at roughly the same time.

We used two methods to assess the presence or absence of *A. opacum* larvae. First, we visually searched for *A. opacum* larvae throughout the pond or along pond transects at the same time we conducted egg mass surveys. Second, we conducted a dipnet survey at all sites during the same two-week period when sites were mapped. In 2001 and 2002, we conducted both VES and dipnet surveys at all ponds. In 2003, we only conducted dipnet surveys at sites where at least two investigators had not already observed larvae using VES. Dipnet survey effort (number of sweeps) was proportional to size of the wetland or impoundment, and we used two different sampling techniques to assure that shallow and deeper waters were sampled adequately. For sites that contained deeper waters towards the interior of the pond (most censused ponds), every 10-m around the perimeter of the pond we took two dipnet samples, one sample 1-m from the shore and another sample 5-m from the shore towards the pond middle. At wetlands and transected ponds, we took dipnet samples every 10m along each transect because these sites typically contained irregular depth profiles. For both techniques, we swept a 1-m path along the pond bottom at sampling location.

Habitat assessments in the field included information on wetland or impoundment size and hydroperiod, water chemistry, and in-pond and riparian vegetation. Each selected site was mapped using with a 12 channel, Trimble™ GPS mapping unit in the early spring when water levels were highest.

We derived a relative estimate of seasonal hydroperiod for all 200 sites sampled during the three year study. The estimate was determined by visiting each site during a single two-week period in late May and early June of 2003 (a fairly typical water year), and assigning each pond a drying score based on the difference between the wetted area on that day and the wetted area of the pond when it was at its maximum. Rankings were as follows: 0 = dry; 1 = <10% of seasonal max.; 2 = 11-50% of max.; 3 = 51-90% of max.; and 4 = >90% of max.

Water chemistry was measured at the same time as mapping and included conductance ($\text{ms}\cdot\text{sec}^{-1}$) and pH. Conductance was measured with a YSI model 33 temperature/salinity/conductivity meter and the data later converted to specific conductivity ($\text{ms}\cdot\text{sec}^{-1}$ at 20° C) for comparison. The pH of each site was measured with Orion model 230A pH meters equipped with Orion model 9107BN pH probes.

In-pond and riparian vegetation were assessed at each site during a two-week period in mid-may. For inpond vegetation, we ranked the abundance of 7 vegetation classes (moss, submerged vegetation, emergent vegetation, shrubs, standing timber, downed logs, and

sticks) based on a visual estimation of the proportion of pond area (i.e., fraction of 1 m² cells) in which each vegetation category occurred. Rankings were as follows: 0 = 0% (absent); 1 = >0-10% (rare); 2 = >10-50% (common); and 3 = >50% (abundant). For riparian vegetation we ranked the abundance of six vegetation classes (moss, herbaceous plants, shrubs, coniferous trees, deciduous trees, and total trees) that occurred within a 10-m buffer strip surrounding each pond. Riparian vegetation was ranked the same way as inpond vegetation.

Genetic Sampling

Genetic analyses were conducted on DNA extracted from embryos of *A. jeffersonianum* collected at all sites in which they were found. We collected fewer than five embryos from each egg mass sampled from a maximum of thirty egg masses per site. Embryos were excised from masses with dissecting scissors without dislodging the remaining mass from vegetation from which it was attached. Excised eggs were stored in 90mL of pond water until they could be preserved in 95% ethanol for genetic analysis. This protocol was chosen because it has been shown to have a negligible effect on the hatching success of the embryos remaining in the rest of the egg mass (Julian 2000).

Data Analyses

Status of breeding populations

We used three pieces of information to draw inferences on the status of the three ambystomatid salamander species. First, where possible, we compared ambystomatid salamander occurrence data collected during our surveys with data collected from previous surveys. There were four previous biological surveys in DEWA that involved ambystomatid salamanders. Prior to this study, the most extensive survey was coordinated by John Behler of the Wildlife Conservation Society (WCS) in the spring of 2000 when 148 vernal pools, wetlands, and impoundments were sampled for amphibians (Brotherton and Behler, unpublished). The WCS survey evaluated the occurrence of all three species at each site although each site was visited only one time by investigators. Prior to the WCS survey, 35 temporary wetlands in DEWA were sampled in the spring of 1985 by Joseph Freda, a graduate student at Pennsylvania State University. The temporary wetland survey evaluated the occurrence of only *A. maculatum* and *A. jeffersonianum* but did not attempt to determine the presence of *A. opacum* (Freda 1985). Similarly, in 1977 and 1978, Stephen Nyman, a graduate student at Rutgers University sampled two wetlands in DEWA for *A. maculatum* and *A. jeffersonianum*. However, this survey involved intensive sampling over repeated visits so abundance estimates were obtained in addition to presence-absence data (Nyman 1979). Finally, Joseph Stein conducted surveys at four wetlands in DEWA between 1976 and 1977 and collected occurrence information for all three species (Stein 1978).

Second, we assessed temporal trends in salamander populations at sites we sampled all three years of our survey (N=28) to assess turnover. Turnover is defined as change in occupancy status at a site between years and turnover rate is the proportion of sites that

show turnover. Breeding habitats that exhibit turnover for a given species suggests that habitat conditions may vary with respect to their suitability for breeding.

Finally, we examined the spatial pattern of breeding pond use to determine if salamander populations were distributed at random throughout the DEWA landscape. Non-random distribution suggest other explanations besides habitat may be important in explaining breeding site selection. For *A. maculatum* and *A. jeffersonianum*, we correlated egg mass abundances with geographic latitude and longitude. Because we had only presence-absence data for *A. opacum*, we did not assess spatial patterns in this way.

Assessment of critical breeding habitats

Characteristics of ambystomatid breeding habitats were assessed by relating information on landscape setting and pond habitat to the salamander occurrence and egg mass abundance patterns. For these assessments, we limited our analyses to natural ponds and wetlands that were never observed to contain fish. Effects of fish and the influence of impoundments were analyzed separately (see “Influence of impoundments” below). We pooled data from all three annual surveys and defined a breeding site for a species as a wetland that contained egg masses or larvae of the species on at least one occasion during the course of the study. We recognize that pooling data from different years might have biased our inferences because of species turnover, coupled with survey conditions that result in species detection probabilities < 1 . However, there was almost no species turnover observed at any of our sites for *A. maculatum* and *A. jeffersonianum* and so there should be little bias. In contrast, we observed significant species turnover in larval surveys for *A. opacum*. We tried to account for the potential bias in modified approaches to analyses, and in a more cautious approach to our conclusions for *A. opacum*.

Most habitat variables used in our analyses were measured directly either from digital maps using GIS (e.g., most landscape variables including measures of pond isolation, road density, terrain, and forest vegetation) or in the field (pH, specific conductivity, and wetland hydroperiod). However, we computed other habitat statistics from field measurements. Specifically, pond area was derived from the mapped boundaries of each site using Pathfinder Pro software. In addition, because there was significant correlation between many inpond and riparian vegetation classes, we used a non-hierarchical clustering procedure to summarize inpond and riparian vegetation field measurements into a smaller set of uncorrelated vegetation types. To perform these analyses, we used the “FASTCLUS” procedure in SAS/STAT software, Version 8.2 of the SAS System for Windows. This procedure assigns observations (sites) to a fixed number of clusters (determined by the investigator) using a *k-means* model in which all independent variables are first converted to z-scores (i.e., mean of 0 and a standard deviation of 1).

From Cluster analyses, we classified all natural ponds and wetlands as either mud/bare bottom ponds, leaf bottom ponds, swamps, or marshes according to field-based rankings of seven vegetation classes (see “Sampling Methods” above). The majority of natural sites were categorized as leaf bottom ponds ($N = 69$), which were characterized by low amounts of emergent vegetation, submerged vegetation, and shrubs, and higher than average amounts of sticks and leaves. Swamps were the second most common natural

pond types (N = 44), and were characterized by higher than average amounts of in-pond shrubs, saplings, and timber. Mud/bare bottom ponds (N = 19) were characterized by less than average amounts of timber, sticks and leaves and had highly variable amounts of emerged and submerged vegetation, while marshes (N = 18) contained consistently high amounts of emerged and submerged vegetation, with average amounts of shrubs, saplings, and timber.

The vegetation surrounding ponds were classified as either deciduous tree, coniferous tree, or herbaceous/successional vegetation dominated according to habitat assessments of 10m riparian zones that ranked the abundance of herbaceous vegetation, shrubs/saplings, trees (both deciduous and coniferous), deciduous trees only, and coniferous trees only. Most sites were classified as having deciduous riparian zones (N = 91) that contained a relatively high abundance of shrubs/saplings and trees, with deciduous trees being more abundant than coniferous trees. Sites classified as coniferous (N = 32) contained a high abundance of trees, with coniferous trees being more abundant than deciduous trees. Sites classified as being herbaceous/successional (N = 26), had a high abundance of herbaceous vegetation, but very few trees. Overall, we summarized all habitat data collected for each study site into a suite of 17 predictor variables representing landscape setting and inpond habitat (Table 2).

Table 2. Suite of habitat variables measured for each pond or wetland and used as predictor variables in Classification and Regression Tree analyses.

Variable	Description
<u>Landscape setting</u>	
Topographic Position	-Class variable: Ridge or Valley
Land cover	-Class variable: Forest, Shrub-scrub, Open
1° road density 250-m	-Density of primary (high traffic) or primary plus secondary (low traffic) paved roads (measured as meters of road per hectare) within prescribed buffer areas (i.e., 250-m, 500-m, and 1-km) surrounding each wetland.
1° road density 500-m	
1° road density 1000-m	
1° and 2° road density 250-m	
1° and 2° road density 500-m	
1° and 2° road density 1000-m	
Pond isolation (250-m)	-The number of other natural wetlands within prescribed buffer areas (250-m, 500-m, 1-km) surrounding each wetland.
Pond isolation 500-m	
Pond isolation (1-km)	
<u>Inpond and riparian habitat</u>	
Wetland size	-Area of wetland measured in hectares during early spring.
Wetland vegetation type	Class variable: Mud-bottomed, swamp, marsh, leaf-bottom.
Riparian vegetation type	Class variable: Deciduous; coniferous, herbaceous/successional
Wetland pH	- log of H ⁺ ion concentration of pond or wetland water.
Specific conductivity	-Conductivity of pond water measured as (ms/cm at 20°C).
Drying Score	-Ordinal ranking of wetland hydroperiod.

We used Classification and Regression Tree (CART) analyses (Breiman et al. 1984) to explore associations between egg mass occurrence and abundance data collected for each of the three species of ambystomid salamanders and the entire suite of 18 predictor variables measured for each pond (Table 2). As with traditional modeling techniques, the objective of CART is to explain variation in a categorical response variable (Classification tree) or continuous response variable (Regression tree) using one or more predictor variables. However, unlike many traditional modeling approaches CART analysis remains robust even when subjected to complexities typical of ecological data such as nonlinear and higher order interactions, unbalanced data, and data sets with missing values (De'ath and Fabricius 2000, Vayssieres et al. 2000). For all CART analyses we used only data from randomly selected natural wetlands.

CART works by using rules based on individual predictor variables to repeatedly split the data into relatively homogeneous groups. A relevant example might be that a classification tree splits the group of potential breeding ponds used by *A. maculatum* using the predictor variable pond pH. A split might be that all ponds that had a pH less than 4.5 were not used by spotted salamanders for breeding, whereas, ponds with pH greater than 4.5 were used 75% of the time. Each split, or branch, results in two subsets of the data called nodes (the entire data set is referred to as the root node). If the node is not subject to further partitioning, that node is called a terminal node, or leaf. For each node, an algorithm is used to determine which of the myriad of possible splits best explains the variability in the dependent variable in terms of the independent variables, and whether or not the node should be terminal or subject to further splits. For a more complete discussion of Tree modeling procedures in Ecology see Urban (2002).

We used S-Plus statistical software (Statistical Sciences 1999) to conduct CART. S-Plus uses the binary recursive partitioning algorithm which maximizes homogeneity within individual nodes to choose the best set of splits to partition the data. Homogeneity is based on deviance (comparable to the sums of squares in least-square regression analysis). For classification trees, we used the misclassification error rate to evaluate the effectiveness of the tree model to explain the data (i.e., presence or absence of salamanders). Misclassification error rate is the number of observations that are incorrectly classified by the model *post-hoc*. To determine statistical significance of the tree model, we compared the misclassification error rate of the observed model to a suite of 100 null model misclassification error rates. Null model error rates were determined by randomly assigning presence-absence data in the observed proportions to the set of pond habitat data, and generating a tree model for the randomized data set. The procedure was repeated 100 times to obtain a distribution of null model misclassification error rates. Thus, the p-value of the observed model is the number of times out of 100 iterations that the null model error rate was less than or equal to the error rate of the observed model. We used essentially the same techniques to evaluate the effectiveness of observed regression trees except that, instead of using misclassification error rate, we used the deviance of residuals. As mentioned earlier, residual deviance is comparable to the sums of squares of least-square regression.

We also conducted a model simplification technique developed for tree analyses called “pruning”. This technique attempts to reduce the number of terminal nodes (model complexity) with only minimal reduction in predictive power (De’ath and Fabricus 2000). In order to prune the trees, we used the feature in S-Plus software that allows the user to plot the number of misclassification errors for classification trees or residual deviance for regression trees as a function of the number of terminal nodes. Typically, misclassification errors and residual deviance declined exponentially with number of terminal nodes, and the goal was to locate the inflection point in the graph where increasing the number of terminal nodes resulted in only minor improvements in misclassification error rates or residual deviance. The number of terminal nodes at that point was determined and the model was re-run, but this time forcing the final model to have the reduced number of terminal nodes.

Influence of impoundments

We used logistic regression to model the occurrence and abundance (ranks) of *A. maculatum* as a function of pond type (natural pond versus impoundment). We also included the presence or absence of fish, and the interaction between fish presence and pond type, as predictor variables in the model to determine if any impoundment effect was due largely to a correlation between impoundments and fish. For this analysis, we used data collected from natural sites and impoundments in 2001 and 2002. In our initial analyses, we also included “year” as a main effect and the associated interactions with fish and pond type in the model. However, we found no significant interaction between year and the other main effects and so we pooled the data from the two years and removed the variable “year” from the model. There was no reason to assess statistically the importance of impoundments to the other two species because *A. jeffersonianum* egg masses were never observed in impoundments and *A. opacum* larvae were observed in only one of the 29 impoundments surveyed.

In our logistic regression models we used maximum likelihood methods to estimate cumulative logits and regression coefficients for each main effect (McCullagh 1980). The regression coefficient of a main effect is the natural log of that effect’s odds ratio estimate. The odds ratio estimate is the ratio of the odds of a predicted response between levels of a main effect. For example, the odds of amphibian presence in a fishless site is the probability of amphibian presence divided by the probability of amphibian absence. Likewise, the odds of amphibian presence is formed for sites with fish, and the odds ratio for the effect of fish presence is the odds for fishless sites divided by the odds for sites with fish. Odds ratios are easily interpreted because they express a direct association between levels within a main effect (i.e. a fishless site has “X” times higher odds of containing egg masses than a site containing fish), where lower bounds of confidence intervals > 1.0 or upper bounds < 1.0 indicate statistical significance (Hosmer and Lemeshow 1989). Odds ratios and confidence intervals (90%) were estimated for each species and both years.

RESULTS

Weather Patterns

Breeding activity of many amphibian species, including ambystomatid salamanders, is strongly influenced by weather conditions prior to, and during breeding periods (Semlitsch 2000). Air temperature and the amount and timing of rainfall are important because they directly affect the availability and hydroperiod of breeding habitat. Consequently, it is important to put breeding activity observed in any one breeding season in the context of normal weather patterns.

We compared precipitation and air temperature observed during our three study years to historical patterns measured at locations in DEWA (Fig. 1). We found that, in general, the first and third years of our study were relatively normal in terms of both precipitation and temperature except for the month of October during both years. October of 2000 (year 1) was unusually dry and October of 2002 (year 3) was unusually wet (Fig. 1). In this part of the country, *A. opacum* migrates to breeding sites in September and October and so unusual conditions in October could influence site selection and breeding success of *A. opacum*. However, in large measure the months leading up to the March-May spring breeding for *A. maculatum* and *A. jeffersonianum*, and the nursery period for all three species, was normal for these two years. In contrast, the 2001-2002 study year was extremely unusual (Fig. 1). The eight month period (July-February) leading up to the spring breeding season was the driest on record, receiving nearly 15 fewer inches of rain than normal (normal = 32 inches) for that period. Similarly, the same eight month period was the second warmest on record accumulating nearly 15.5 more degree-days than normal (normal = 78 degree-days).

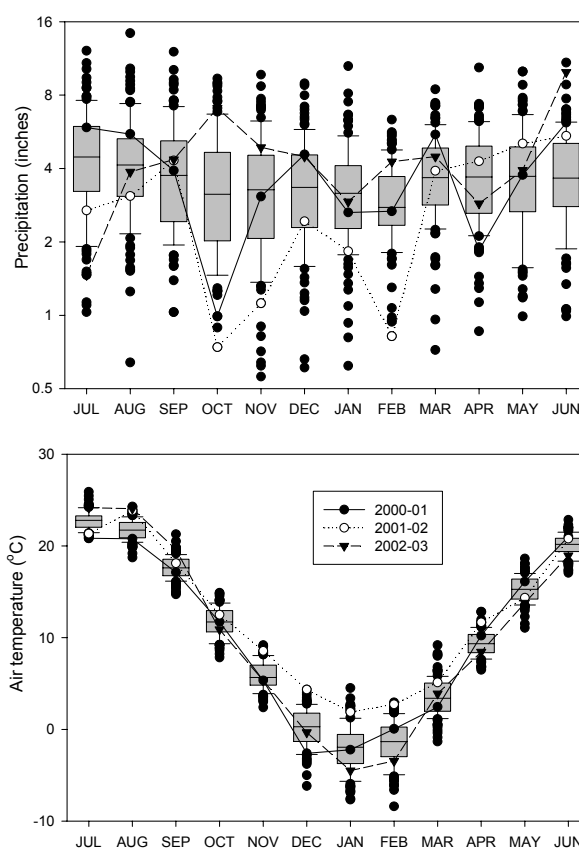


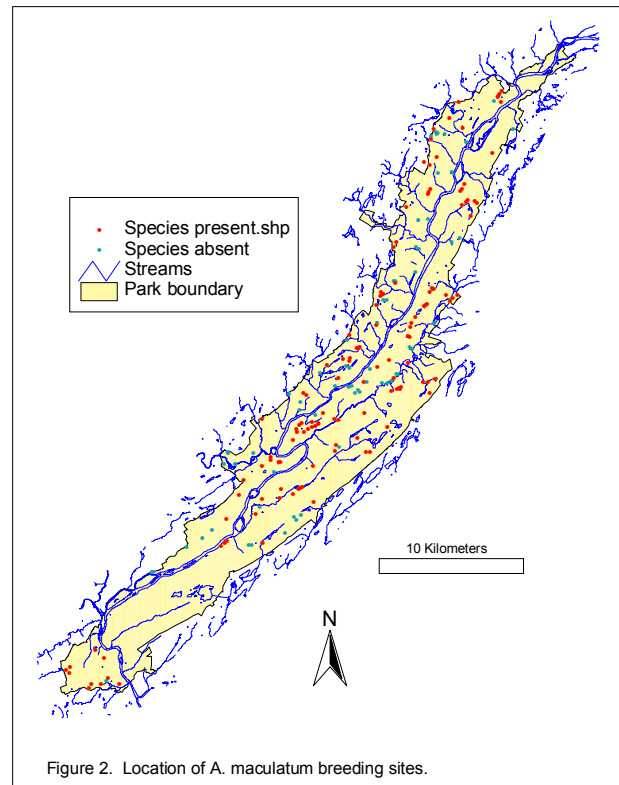
Fig. 1. Precipitation (top) and air temperature (bottom) patterns for north-eastern NJ. Box plots show the range in monthly averages for a 109 year period between 1895 and 2003. For each box, the horizontal line shows the grand mean, the extent of the box represents 50% of the observations, the extent of the whiskers represent 90% of the observations, and individual points show the remaining data. Line graphs show monthly averages for the years we sampled in DEWA.

Status of salamander populations

A. maculatum

Two-thirds of the 200 bodies of water surveyed during our study contained *A. maculatum* egg masses (N=132), with nearly one-quarter of all surveyed bodies of water containing > 100 egg masses in at least one year they were surveyed (N = 46)(Fig. 2). When we consider only natural ponds and wetlands and exclude impoundments, we found that *A. maculatum* used 69.6% (119/171) of wetlands in DEWA.

Nearly 57% of the breeding ponds we identified in our surveys (N=75) were not previously known to harbor breeding populations of *A. maculatum*. Previous surveys collectively identified a total of 81 different *A. maculatum* breeding sites (hereafter referred to as historical breeding sites) out of a combined total of 149 sites surveyed (54.4% occupancy rate for the park). This is a considerably smaller proportion of sites occupied than the 69.6% we observed in our survey. During the course of our study, we re-surveyed 96 of the 149 sites previously surveyed in at least one of the three sampling years, 59 historical breeding sites and 37 sites in which *A. maculatum* breeding was not observed in previous surveys, and we observed *A. maculatum* egg masses in all but 2 (3.4%) of the 59 historical breeding sites, and we observed *A. maculatum* egg masses in 17 of the 37 sites (45.9%) that were found not to contain *A. maculatum* in previous surveys.



Over the three years of our study, the proportion of *A. maculatum* breeding sites in the park increased from 59% of the wetlands in 2001, to 69% in 2002, to nearly 76% in 2003 (Fig. 3A). At the same time, the fraction of sites that supported large populations (>100 masses) decreased from 42.1% in 2001, to 32.6% in 2002, to 27.8% in 2003 (Fig. 3B). For sites sampled all three years (N=28), we compared changes in egg mass abundance among years to changes in pond sizes to determine if the wide variation in rainfall (Fig. 1) and consequently pond sizes, may have influenced temporal patterns. We found that natural sites were typically smallest in 2002 and largest in 2003. Out of 28 sites, 23 were smallest in 2002 and 18 were largest in 2003. Among sites with *A. maculatum*, 91% were smaller in size in 2002 compared to 2001, 82% were smaller in 2002 compared to 2003, and 69% were smaller in 2001 compared to 2003. We observed a strong, positive correlation between change in *A. maculatum* egg mass counts and changes in pond area

between the years of 2001 and 2002. In 2002, 75% of sites were both smaller in size and contained fewer egg masses than in 2001. However, we observed only a weak correlation between the years 2002 and 2003 when half of the sites were larger in size but contained fewer egg masses in 2003 compared to 2002. In addition, we found a negative correlation between egg mass abundance and wetland size between 2001 and 2003 when, in 2003, 62% of sites were larger in size yet contained fewer egg masses than in 2001. Thus, variation in rainfall amounts and resulting pond volumes did not explain temporal variation in *A. maculatum* egg mass abundances. In addition, despite fluctuations in overall egg mass counts between years, egg mass counts at breeding sites rarely changed by an order of magnitude (9.6% of the time). That is, breeding ponds that supported large populations of *A. maculatum* in one year tended to support large populations in subsequent years. Moreover, turnover (i.e., changes in the occupancy status of a pond or wetland) was even less common, occurring at only 5% of the sites.

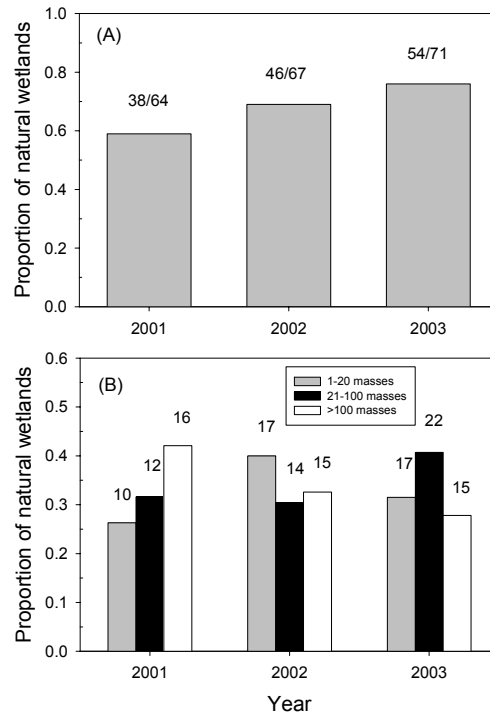


Figure 3. Proportion of randomly selected natural wetlands containing *A. maculatum* egg masses (A), and the proportion of *A. maculatum* breeding sites of different population sizes (B). Numbers above each bar are the actual number of wetlands.

Spatially, *A. maculatum* breeding ponds were distributed throughout the park (Fig. 3), although *A. maculatum* used natural wetlands on the NJ side of the river at a slightly higher rate than on the PA side (78% compared with 66%). We found no spatial relationship

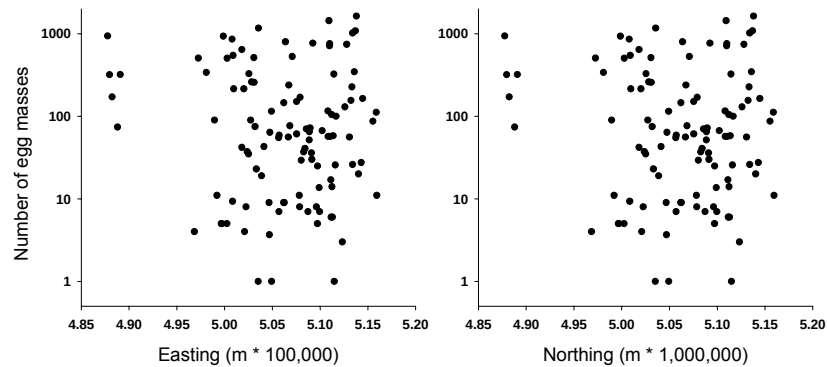


Fig. 4. *A. maculatum* egg mass abundance as a function of wetland location. Spatial location indicated with UTM coordinates, and the relationship with egg mass abundance assessed along east-west (easting) and north-south (northing) directions.

between the extent of pond use (i.e., egg mass abundance) and spatial location within the park. The abundances of *A. maculatum* egg masses within ponds did not correlate with the predominant east-west or north-south directional gradients (Fig. 4).

A. jeffersonianum

Out of 200 ponds, wetlands and impoundments surveyed in this study, *A. jeffersonianum* bred in 20 (10%) (Fig. 5). Limiting our assessment to only natural ponds and wetlands suggests *A. jeffersonianum* used 11.7% (20/171) of the available natural wetlands for breeding. However, these are biased estimates of frequency of occurrence in the park because they include non-randomly selected sites (N=9) (see Methods). Including only randomly selected sites indicated that *A. jeffersonianum* bred in 5.9% (11 of 187) of all sites (including impoundments) or 8% of the natural ponds and wetlands in the park (11 of the 138). Of the 20 wetlands we found to contain *A. jeffersonianum*, five supported large (501-2000 egg masses) or very large (>2000 egg masses) populations.

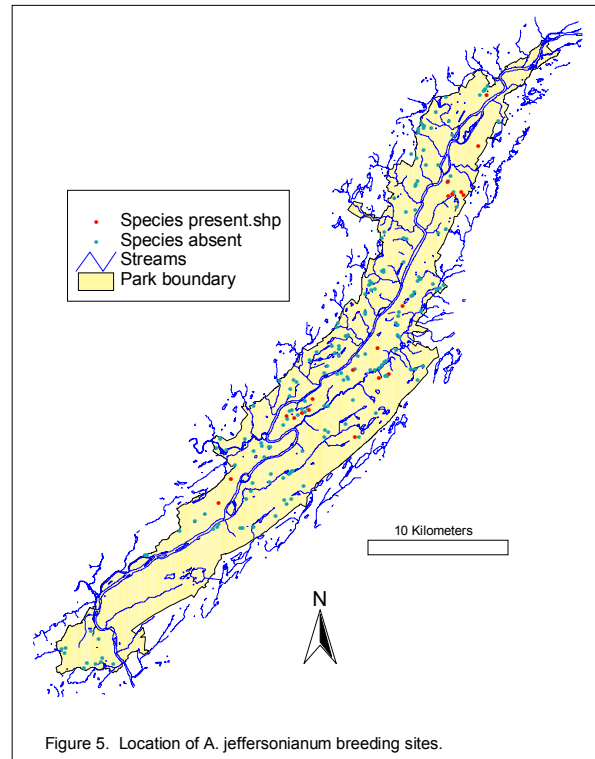


Figure 5. Location of *A. jeffersonianum* breeding sites.

Overall, we identified a total of 14 *A. jeffersonianum* breeding sites that were not documented by previous amphibian surveys in DEWA. Collectively, these prior amphibian surveys identified a combined total of only six different sites that contained *A. jeffersonianum* egg masses, larvae, or adults (hereafter referred to as historical breeding sites) out of a combined total of 96 sites surveyed. The 6.25% frequency of occurrence observed in historical surveys was only slightly less than the 8% rate we observed among randomly selected natural wetlands in our survey. However, we found *A. jeffersonianum* egg masses at nine sites where previous surveys failed to detect them, in addition to all six historical breeding sites.

We compared long-term temporal changes in pond use at two sites that were intensively sampled in 1978 (Nyman 1979) and during the present study (2000-2002). In 1978, Nyman encircled site 2012 (“Youth hostile pond”) with drift fencing and pitfall traps during spring migrations and captured 29 LJJ females, only three JJ females, and no JJ males (determined via erythrocyte examination). Subsequently, all egg masses laid that year were unviable and he concluded that there was a high likelihood the population would go extinct. In contrast, results of our survey indicated that site 2012 consistently harbored very large breeding populations (well over 100 females) despite the fact that a large fraction of the population continued to be LJJ hybrids, and over half of the observed egg masses were not viable (Table 3). In addition, our estimate of nearly 45% egg mass viability suggests that the number of male JJ that bred at this site over the last three years is considerably higher than in 1978.

In contrast, the other population monitored by both Nyman and LSC (site 600, “heater pond”) showed no such dramatic change in egg mass abundance between surveys. At site 600 (Table 3), Nyman counted approximately 160 *A. jeffersonianum* egg masses and estimated that approximately half of the larval population in early June consisted of LJJ individuals. Surveys by LSC at site 600 recorded egg mass counts of 507, 49, and 155 masses in 2001-2003 (respectively), and genetic analyses concluded that approximately 33% of the embryos collected were LJJ individuals.

Site	State	Location	Population Size ¹	Mean %Viable	%Hybrid	Genetic samples ²
688	NJ	BF	Small	13%	NA	10 ³
704	NJ	BF	Small	61%	100%	5
2032	NJ	BML	Small	100%	100%	1
151	NJ	CK	Large	80%	65%	30
232	NJ	CK	Moderate	85%	NA	37 ³
245	NJ	CK	Large	50%	75%	30
2012	NJ	CK	Very Large	45%	88%	45
3012	NJ	CK	Very Large	41%	57%	30
3013	NJ	CK	Large	66%	79%	30
801	NJ	LPR	Small	50%	NA	0 ²
807	NJ	LPR	Small	80%	NA	0 ²
819	NJ	LPR	Small	45%	100%	5
3010	NJ	LPR	Small	87%	NA	0 ²
464	NJ	PV	Small	5%	NA	0 ²
600	NJ	UPR	Moderate	86%	67%	30
758	NJ	UPR	Small	37%	19%	21
3019	NJ	UPR	Small	87%	20%	30
992	PA	HQ	Moderate	50%	0%	30
2035	PA	HQ	Moderate	77%	3%	30
80	PA	M	Small	77%	NA	0 ²

¹ Based on mean population size over all years sampled
² No genetic samples taken due to extremely small population size
³ Genetic samples did not quantify

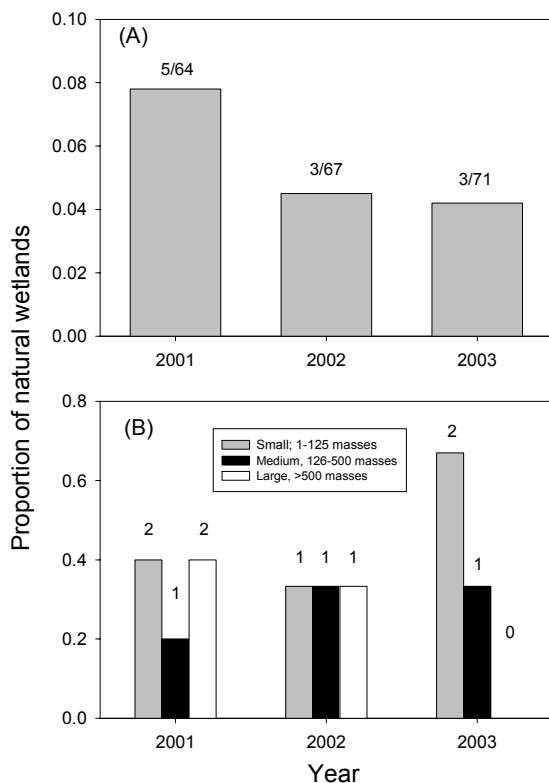


Figure 6. Proportion of randomly selected natural wetlands containing *A. jeffersonianum* egg masses (A), and the proportion of *A. jeffersonianum* breeding sites of different population sizes (B). Numbers above each bar are the actual number of wetlands.

In contrast to *A. maculatum*, the proportion of natural sites used by *A. jeffersonianum* for breeding decreased over the three years of our study from 7.8% in 2001, to 4.5% in 2002, to 4.2% in 2003 (Fig. 6A).

However, like *A. maculatum*, the relative number breeding sites that supported large populations seemed to decline (Fig. 6B). However, the total number of randomly selected breeding sites was small (<5) and so inferences regarding relative population sizes are tenuous.

When we compared egg mass abundances (ranks) among ponds sampled all three years, we found the number of egg masses deposited in a pond was typically lower in 2002 than in 2001 or 2003 (Table 4). In 2002, two-thirds of breeding sites experienced a decrease in egg mass rank from 2001, while the remaining sites showed no change in rank. Sign tests indicated that the number of sites that decreased in egg mass rank in 2002 from

2001 was significantly greater than expected by chance ($N = 12$, $M = -3.5$, $P = 0.0156$). Similarly, the number of sites that increased in egg mass rank in 2003 from 2002 was also significantly greater than expected by chance (Sign test, $N = 16$, $M = 4.5$, $P = 0.0039$). In contrast, when comparing 2003 to 2001, we found that 25% of sites in 2003 had decreased in rank, 16.7% had increased in rank, and 58% remained the same (Sign test not significant). The 2002 study year was significantly drier than 2001 or 2003 (Fig. 1). Thus, temporal variation in egg mass abundances may be explained variation in rainfall and the draught of 2002.

Table 4. Between year comparisons in the proportion of sites that changed in egg mass rank and % viable masses.

	Δ from 2001 to 2002		Δ from 2002 to 2003		Δ from 2001 to 2003	
	Increase	Decrease	Increase	Decrease	Increase	Decrease
Egg mass rank	0%	67%	63%	0%	17%	25%
%Viable Masses	100%	0%	36%	64%	50%	50%

Contrary to the relationship observed between pond size and egg mass abundance (rank), egg mass viability within ponds was highest during the 2002 draught year when pond sizes were smallest. The mean proportion of viable *A. jeffersonianum* egg masses was 34.7% ($N = 10$, $SD \pm 31.7\%$) in 2001, 68.0% ($N = 11$, $SD \pm 27.4\%$) in 2002, and 59.2% ($N = 20$, $SD \pm 34.0\%$) in 2003. All sites experienced an increase in the proportion of viable masses between 2001 and 2002 (Sign test, $N = 7$, $M = 3.5$, $P = 0.0156$) (Table 4). Between 2002 and 2003, the proportion of viable masses decreased in seven ponds and increased in four (Sign test, $N=11$, $M = -1.5$, $P = 0.5488$), and between 2001 and 2003 viability decreased in four ponds and increased in six (Sign test, $N=10$, $M = 1$, $P = 0.7539$) (Table 4).

We observed significant spatial clustering of *A. jeffersonianum* breeding sites in DEWA. Of the 20 *A. jeffersonianum* breeding sites, only three were located in PA. Moreover, although *A. jeffersonianum* breeding ponds were distributed throughout the NJ portion of the park (Fig. 5), breeding ponds that consistently supported large *A. jeffersonianum* populations were all clustered in the Camp Kittatiny area of the Park. In each year of the study, three of the six sites in the Camp Kittatiny area produced $> 2,000$ masses/year, and the remaining three sites produced 500-2,000 masses in at least one year in which they were surveyed. The spatial clustering was evident on both east-west and north-south directions. Excluding PA wetlands, egg mass abundances increased as we move east, and especially as we move north in the park (Fig. 7). Over 75% of the variation in egg mass abundance was explained by latitude (northing).

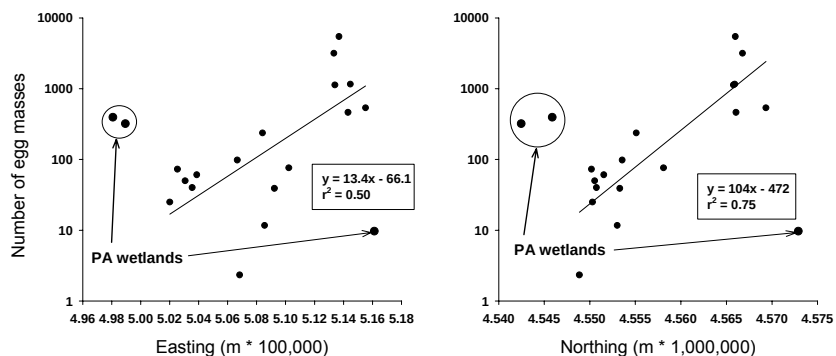


Fig. 7. *A. jeffersonianum* egg mass abundance as a function of wetland location. Spatial location indicated with UTM coordinates, and the relationship with egg mass abundance assessed along east-west (easting) and north-south (northing) directions. PA wetlands not included in regressions.

Ambystoma opacum

Out of 200 ponds, wetlands and impoundments surveyed over the three year period, we identified a total of 40 sites (20% of total surveyed) that contained *A. opacum* larvae (Fig. 8). Of the 171 natural ponds and wetlands, we observed *A. opacum* in 22.8% (N=39).

Prior to our surveys, *A. opacum* had been observed at only 11 sites in DEWA. We re-surveyed 9 of the 11 historical breeding sites in our study and found *A. opacum* in all nine ponds or wetlands. In addition, we found *A. opacum* larvae at 19 sites where prior studies had failed to detect them, and 12 additional sites that had never been surveyed for *A. opacum*.

We found that, despite large differences in rainfall patterns observed between years, *A. opacum* occurrence patterns in the park were remarkably stable over the three year study. Specifically, *A. opacum* larvae were observed in about 20% of the natural ponds and wetlands surveyed in all three years (Fig. 9). However, although the probability of occurrence of *A. opacum* in the park varied little, we observed high temporal variation in the probability of occurrence among years at individual ponds or wetlands (i.e., turnover). Specifically, we found that 10 of 13 *A. opacum* breeding sites (77%) monitored for at least two years changed occupancy status at least once between years. Of the 12 breeding sites sampled in both 2001 and 2002, eight (66.7%) changed occupancy status (7 sites where *A. opacum* was present in 2001 and absent in 2002; 1 site where *A. opacum* was absent in 2001 but present in 2002). Of the 11 sites sampled in both 2002 and 2003, eight (72.7%) changed occupancy status (2 sites where *A. opacum* occurred in 2002 but was absent in 2003; 6 sites where *A. opacum* was absent in 2002 but occurred in 2003).

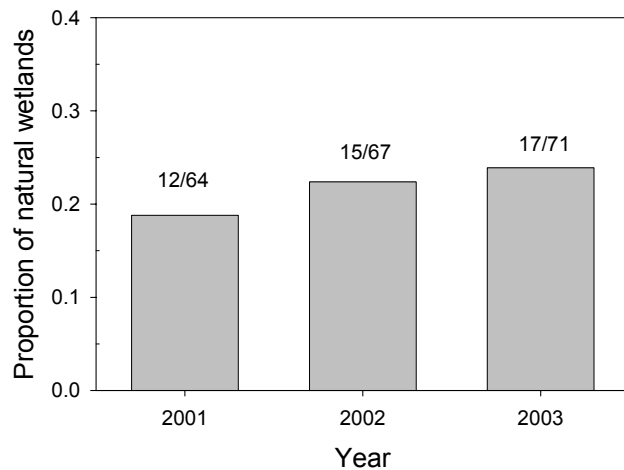
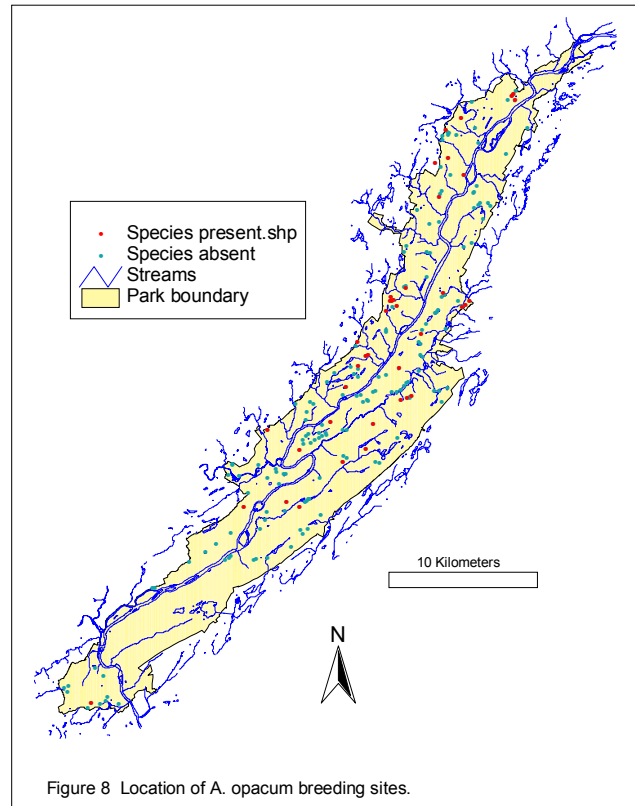


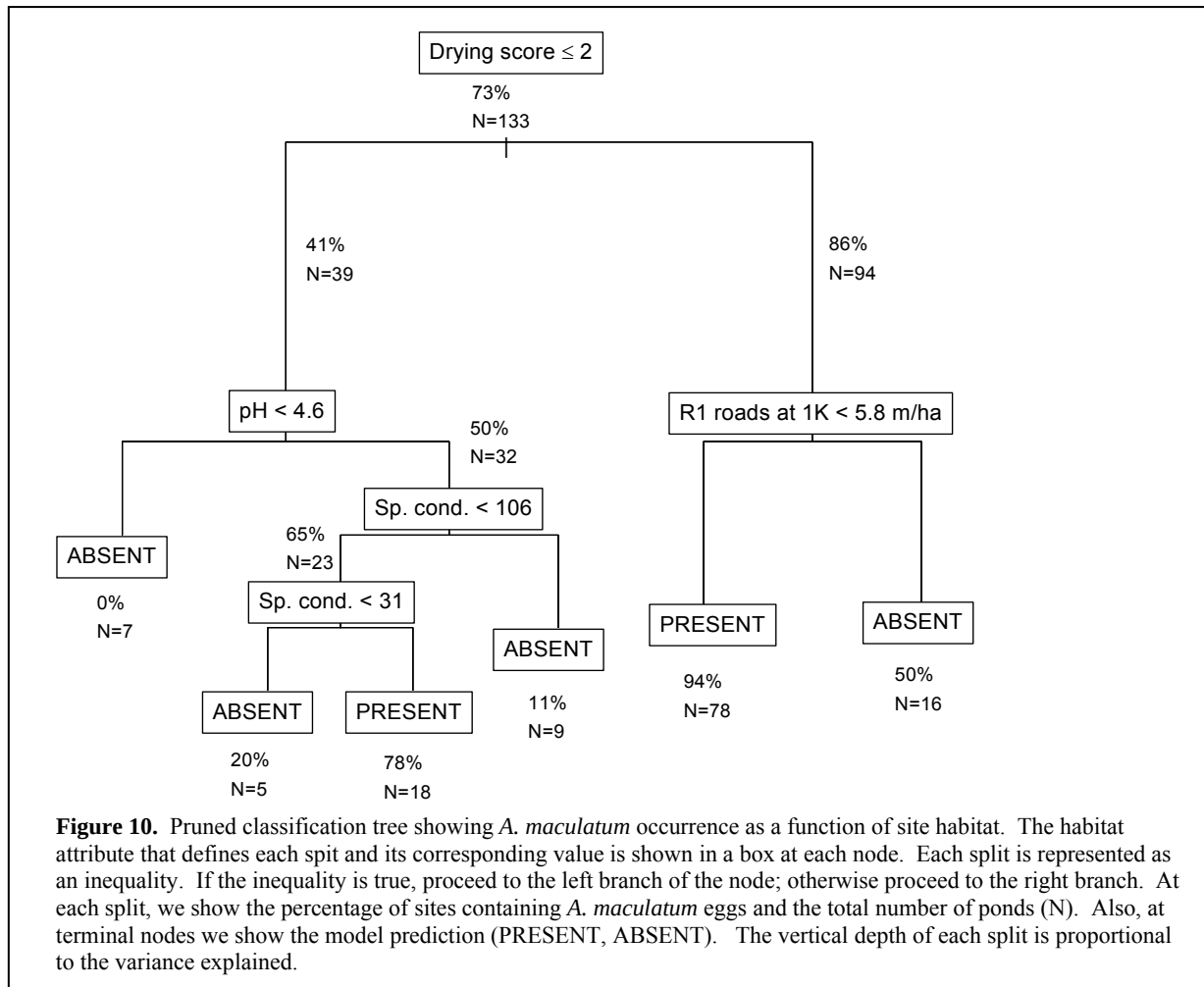
Figure 9. Proportion of randomly selected natural wetlands containing *A. opacum* egg masses of different abundances. Numbers above each bar are the actual number of wetlands and the N number is the total number of wetlands sampled for each year.

Spatially, *A. opacum* breeding sites were more prevalent on the PA side of the river. Nearly half (49.4%) of all PA natural sites surveyed contained *A. opacum* larvae compared to only 17.4% of natural sites surveyed in NJ. In contrast, *A. opacum* sites seemed to be widely distributed across latitude (north-south) on both sides of the river (Fig. 8).

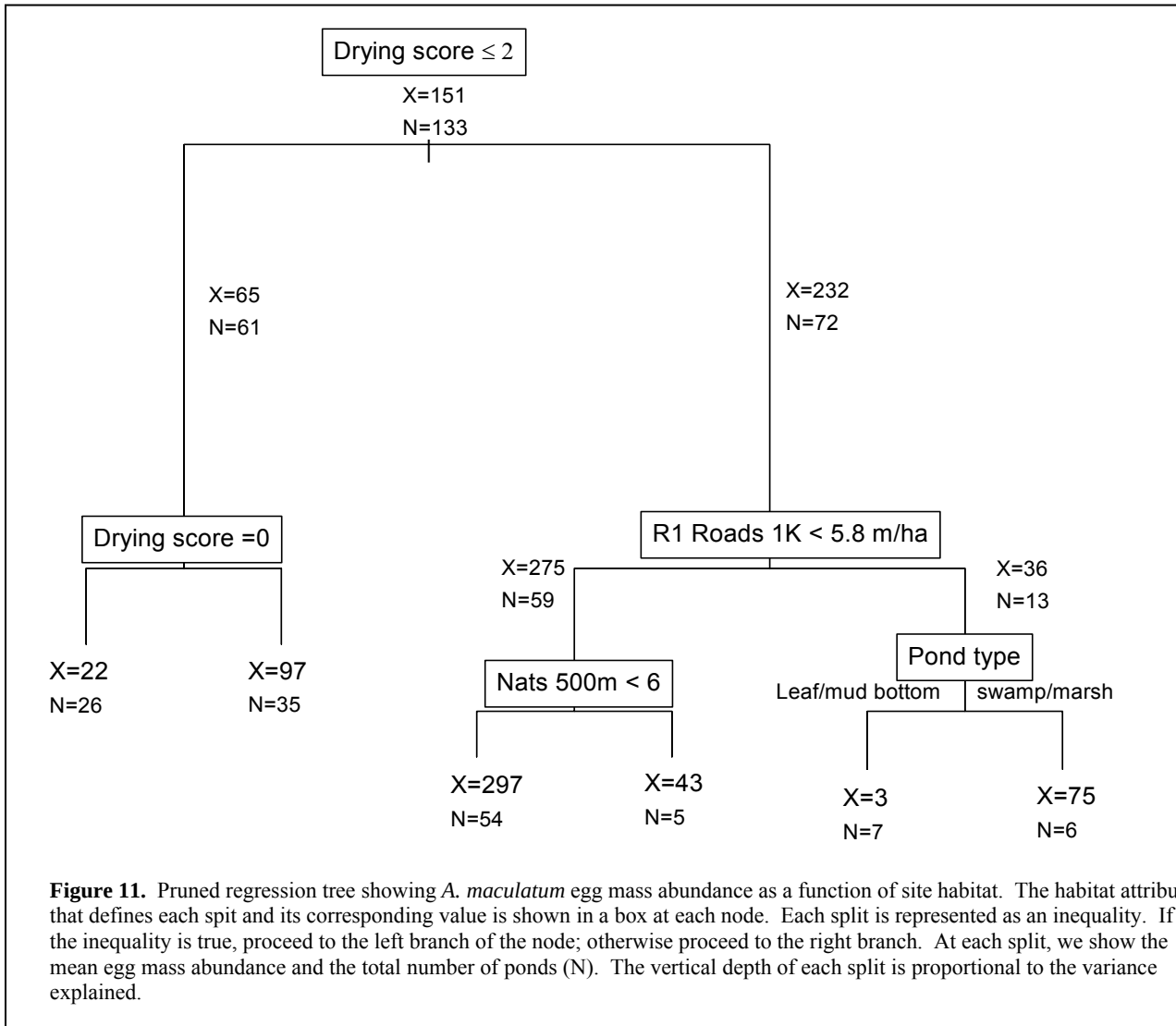
Critical breeding habitats

Ambystoma maculatum

A. maculatum eggs were found in 97 of the 133 (73%) randomly selected natural wetlands surveyed in DEWA over the three years of the study. The classification tree model that best explained egg mass occurrence patterns had six terminal nodes or leaves (Fig. 10). Out of the 18 potential predictor variables in the model, only 4 were selected by the algorithm to be used in the final tree and included drying score, primary road density within 1-km buffers, pH, and specific conductivity (Fig. 10). The four-node model had a misclassification rate of 14.3%. That is, 19 of the 133 wetlands were misclassified *post hoc* by the model. In comparison, none of the 100 null models (standardized for node size) we ran resulted in fewer misclassification errors than the observed model, and therefore the observed model is statistically significant ($p < 0.01$).



A large fraction of the variation in *A. maculatum* occurrence was explained by the first split on drying score. Nearly 86% of the sites with drying scores that were > 2 contained *A. maculatum* egg masses compared to only 41% of sites with drying scores of zero or one (Fig. 10). Presence of primary roads within 1-km buffers around sites boundaries was also an important predictor. Nearly 94% (73/78) of sites with drying scores of 2 or more and with < 5.8 m/ha of primary roads within 1-km contained *A. maculatum*. In contrast, half of the sites that did have primary roads within 1-km were not used by *A. maculatum* despite having high drying scores. Sites least likely to support *A. maculatum* breeding populations had drying scores of zero or one and pH less than 4.6 (Fig. 10). Sites with low drying scores were likely to contain *A. maculatum* egg masses (78%) only if pH was greater than 4.6 and specific conductivity was between 31 and 106.



A. maculatum egg mass abundance ranged between 0 and 1,640 egg masses among sites. The pruned regression tree model that best explained variation in egg mass abundance had six terminal nodes and used four predictor variables to explain 47% of the total variance. Predictor variables included in the regression tree were drying score, primary road density within 1-km, number of other wetlands within 500-m, and pond type (Fig. 11). As with egg mass occurrence

patterns, hydroperiod as measured by drying score, by itself explained a significant amount of the total variation. The mean abundance of egg masses in the 72 sites with drying scores > 2 was 232 compared to a mean of 65 egg masses in the 61 sites with drying scores ≤ 2 . In addition to having drying scores > 2 , sites with the greatest average number of egg masses also had < 5.8 m/ha of primary roads within 1-km and had fewer than six other wetlands within 500-m. In contrast, sites with the fewest average number of masses had drying scores < 1 , or, were leaf- or mud-bottomed ponds that were within 1-km of primary roads (Fig. 11).

A. jeffersonianum

A. jeffersonianum eggs were found in 11 of 130 randomly selected natural wetlands surveyed in DEWA over the three years of the study. The classification tree (Fig. 12) model that best explained egg mass occurrence patterns had six terminal nodes and used five of the 17 potential predictor variables to explain *A. jeffersonianum* occurrence patterns. Predictor variables were primary road density within 1-km buffer, drying score, number of other wetlands within both 500-m and 1-km buffers, and specific conductivity (Fig. 12). The misclassification rate of the observed model was 5.4% (7/130). In comparison, 10 of 100 null models that were generated had misclassification error rates that were lower than those of the observed model. Thus, the model was only marginally significant statistically (i.e., $p = 0.10$).

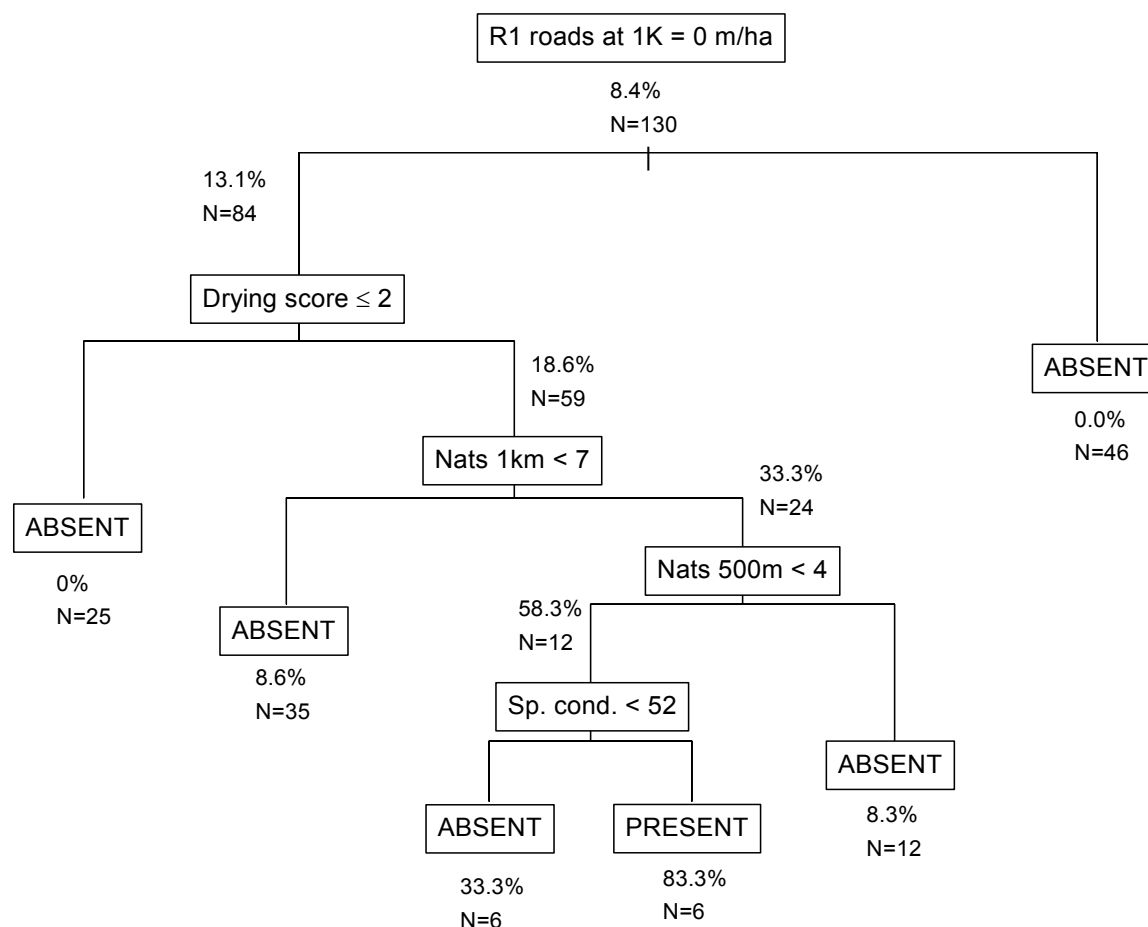
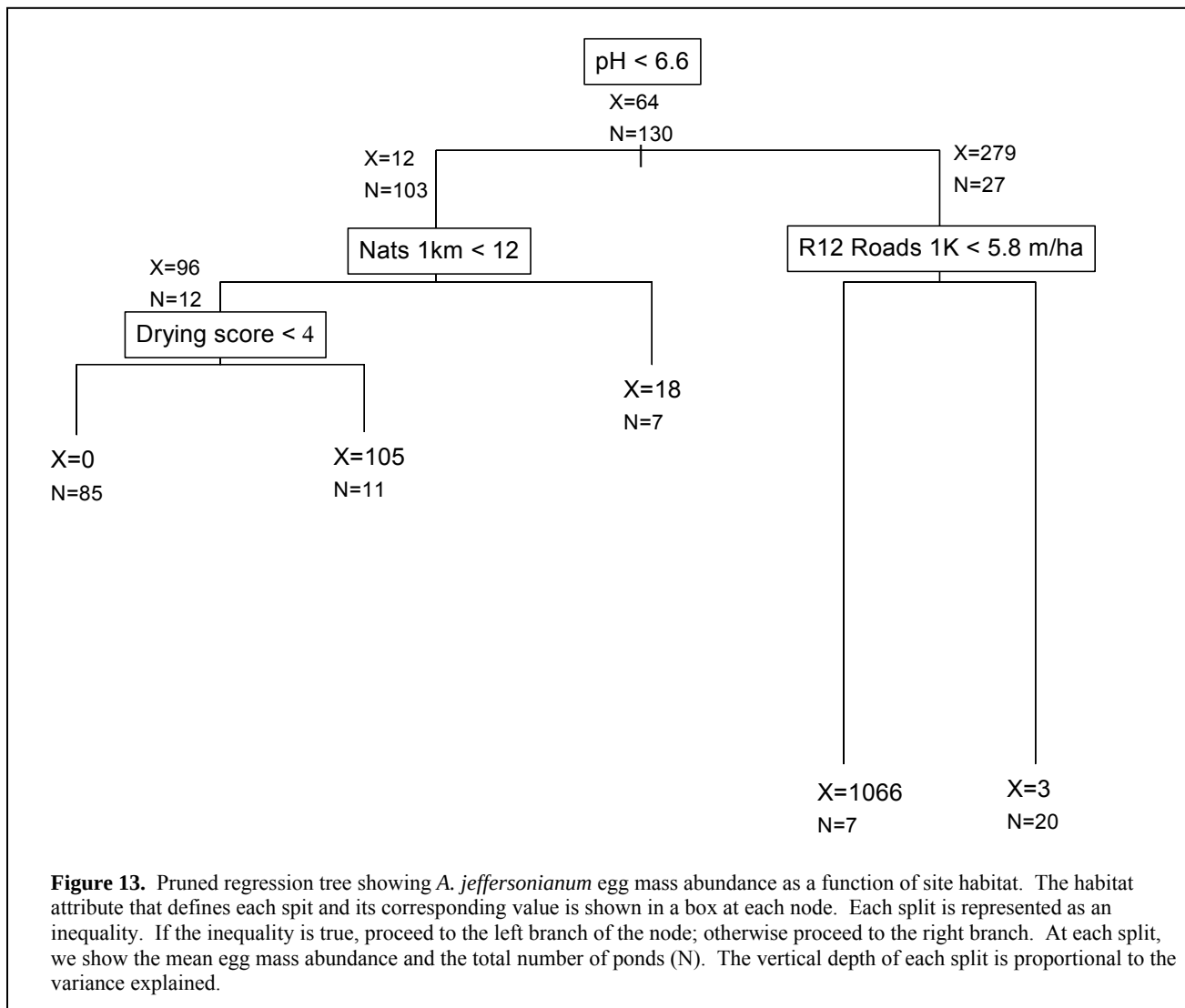


Figure 12. Pruned classification tree showing *A. jeffersonianum* occurrence as a function of site habitat. The habitat attribute that defines each split and its corresponding value is shown in a box at each node. Each split is represented as an inequality. If the inequality is true, proceed to the left branch of the node; otherwise proceed to the right branch. At each split, we show the percentage of sites containing *A. maculatum* eggs and the total number of ponds (N).

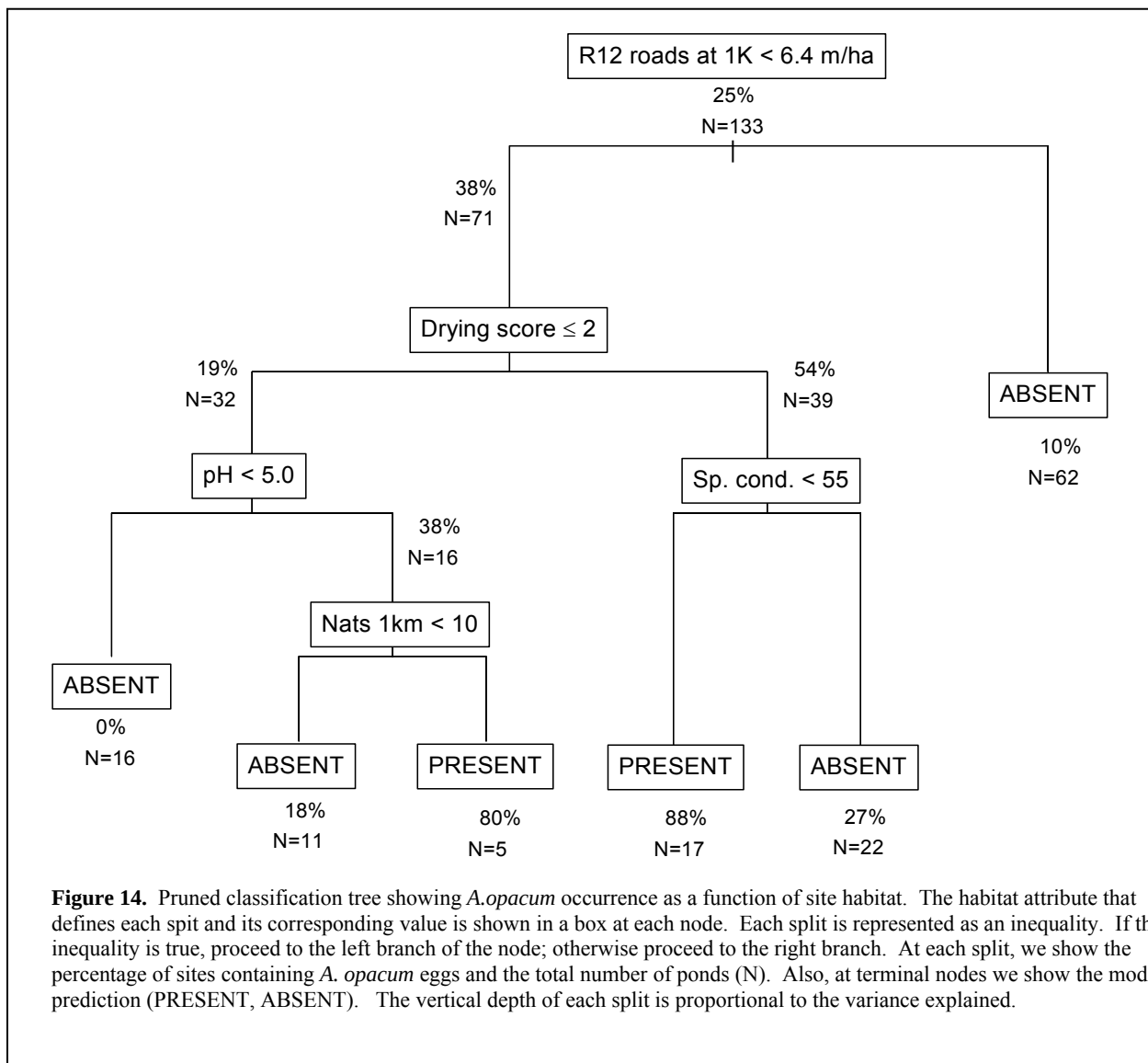
A. jeffersonianum egg mass abundance ranged between 0 and 5,448 at randomly selected natural wetlands. The pruned regression tree model that best explained variation in egg mass abundance had five terminal nodes and used four predictor variables to explain 45% of the total variance. Unlike for *A. maculatum* where the suite of predictors that explained occurrence and abundance of egg masses were essentially the same, there were some important differences between the predictors selected to explain occurrence (Fig. 12) and those used to explain egg mass abundance (Fig. 13). For example, pH was an important predictor of egg mass abundance but not egg mass occurrence. The 27 sites with pH greater than 6.6 averaged 279 egg masses compared to 12 egg masses for sites with pH less than 6.6 (Fig. 13). Also, although sites containing primary roads within a 1-km buffer tended to not be used by *A. jeffersonianum*, the abundance of egg masses tended to be much lower in sites with either primary or secondary roads within 1-km. The seven sites with pH >6 and with no primary or secondary roads within 1-km buffers contained an average of 1,066 egg masses (Fig. 13). The only other sites that supported relatively large populations of *A. jeffersonianum* (mean=105 masses, N=11 sites) had pH < 6.6, fewer than 12 other natural wetlands within 1-km, and drying scores of 4 (Fig. 13).



Ambystoma opacum

A. opacum larvae were observed in 33 of 133 (25%) randomly selected natural ponds and wetlands (Fig. 14). The classification tree model that best explained *A. opacum* occurrence had six terminal nodes and used five of the 17 potential predictor variables. The model had a misclassification error rate of 12.8% (17/133). In comparison, none of the randomly generated null models, standardized for comparable node size, had fewer errors than our observed models (i.e. $p < 0.01$).

A. opacum tended to avoid sites near roads. Specifically, *A. opacum* larvae were observed in only six of the 62 sites that contained either primary or secondary roads within 1-km. Of those sites with low road density, *A. opacum* larvae were not found in any of the 16 sites with shorter hydroperiods (drying score ≤ 2) and lower pH (< 5) (Fig. 14). Sites with a high likelihood of supporting *A. opacum* either had low road density, short hydroperiods (drying score ≤ 2), high pH (> 5), and numerous other wetlands within 1-km (≥ 10); or, had low road density, long hydroperiod (> 2), and low specific conductivity (< 55) (Fig. 14).



However, as stated earlier and in contrast to the other two species, we observed high turnover rates for *A. opacum*. As a result, larvae would be more likely to be observed in sites sampled more frequently (i.e., in multiple years) than sites sampled in only one year. Moreover, because habitat attributes of sites with turnover were essentially unchanged from year-to-year (see Methods), variation in sampling frequency could introduce a bias that might make any inferences regarding occurrence-habitat associations suspect.

To evaluate the potential effects of bias associated with sampling sites with different frequencies, we first examined the relationship between sampling frequency and probability of occurrence of *A. opacum* larvae. As expected, sites sampled more often were more likely to contain *A. opacum* larvae than sites sampled in only one year (Fig. 15). However, the association between sampling frequency and occurrence was relatively weak and observed differences were marginal statistically (Fisher's Exact Contingency Test, $\chi^2 = 2.59$, $df = 1$, $P = 0.12$).

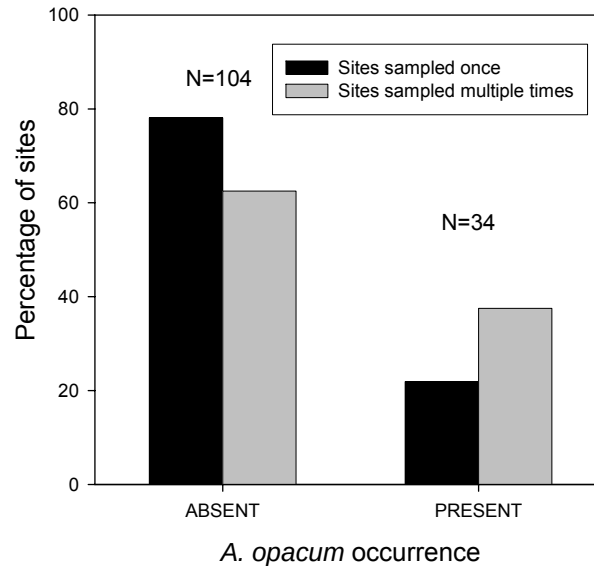


Figure 15. Comparison of *Ambystoma opacum* occurrence patterns between sites sampled in only one year (black bars) and sites sampled in two or three years (gray bars). Numbers above each set of bars is the total number of sites.

Secondly, we examined and compared the sampling frequency distributions among the terminal nodes of our classification tree model (Fig. 14). If the sampling frequency bias was important in our classification model, we would expect *A. opacum* occurrence probabilities to be higher for those nodes containing a higher frequency of sites that were sampled in multiple years, compared with nodes containing more sites sampled in only one year. We found that four of the six terminal nodes had sampling frequency distributions very similar to the overall sampling frequency of all 133 sites (i.e., approx. 82% sampled in only one year; 18% sampled in more than one year) (Table 5). Of these four terminal nodes, three had low occurrence probabilities (0 – 22%) and one (node 4) had the highest *A. opacum* occurrence probability (88%) (Table 5). The two other terminal nodes had a substantially higher frequency of sites sampled more than one year (> 31%). One of the two nodes (#3) had a high probability of containing *A. opacum* (80%) and the other node (#5) had a relatively low probability of containing *A. opacum* (27%) (Table 5). Thus, although there is clearly a bias associated with sampling frequency, the bias appeared to be relatively equally represented among the nodes in the classification tree regardless of occurrence probability.

Table 5. Comparison of sampling frequency and *A. opacum* occurrence probabilities among classification tree terminal nodes (see Fig. 14). Table shows the proportion of sites sampled in only one year, proportion of sites sampled in 2 or 3 years, probability of occurrence of *A. opacum* larvae, and the sample size of each node.

Node	% of sites with larvae)	No. of Sites	% of sites in each node sampled:	
			1 year	2-3 years
Root	25	133	83	17
1	10	16	81	19
2	18	11	82	18
3	80	5	60	40
4	88	17	82	18
5	27	22	68	32
6	10	62	89	11

Influence of impoundments

In addition to seasonally flooded wetlands and temporary ponds, all three ambystomatid species bred in permanent natural bodies of water as well. However, only *A. maculatum* bred in a significant number of artificial impoundments. *A. maculatum* breeding sites (N=132) were distributed among natural sites and impoundments in roughly the same proportions as they occurred in all 200 sites surveyed (Fig. 16). In contrast, *A. opacum* larvae were observed in

only one of the 29 impoundments sampled, and *A. jeffersonianum* egg masses were never observed in impoundments, though they were observed in permanent natural wetlands (Fig. 16).

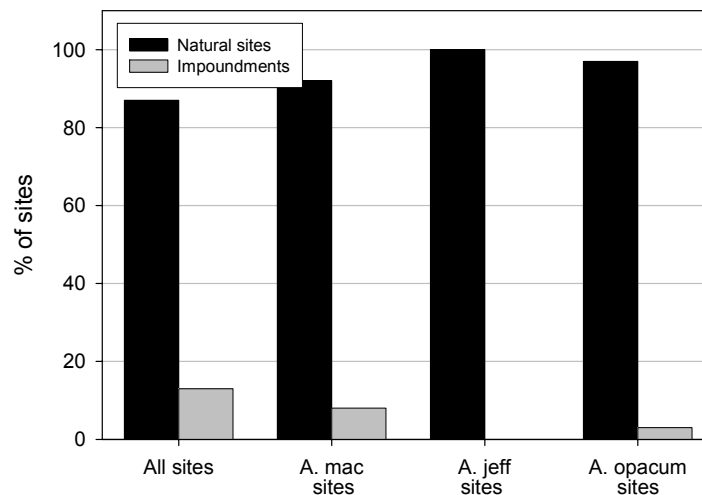


Figure 16. Comparison of the distribution of natural sites and impoundments between all sites sampled, *A. maculatum* sites, *A. jeffersonianum* sites and *A. opacum* sites.

We observed fish in 22% of all sites surveyed (Fig. 17), including 13% of natural wetlands and 79.3% of impoundments. We found that occurrences of all three species were negatively correlated with presence of fish. Only one out the 20 identified breeding sites *A. jeffersonianum* contained fish, and we only observed fish that site (site #80) in only one the three years the site was sampled. Moreover, the site is known to dry up completely in years (as it did in 2002) suggesting a resident fish

population or community is unlikely. Its proximity to a major road leads us to believe that the presence of fish at this *A. jeffersonianum* breeding site in 2001 was likely due to someone purposefully releasing fish into it, perhaps as bait. Fish presence also seems to have a strong effect on *A. opacum* and *A. maculatum* presence. Sites without *A. opacum* contained fish 25% of the time, while only 10% of sites with *A. opacum* contained fish. Furthermore, *A. opacum* were found in less than 9% of sites containing fish, compared to 23% of fishless sites. Sites without *A. maculatum* contained fish 37% of the time, while only 15% of sites with *A. maculatum* contained fish.

A more detailed examination of *A. maculatum* occurrence patterns indicate that they consistently used impoundments less frequently than natural ponds, and sites with fish less often than sites without fish (Fig. 18). In 2001, *A. maculatum* bred in approximately 60% of the available natural sites compared to about 47% of available impoundments. In 2002, the difference between natural sites and impoundments was similar, with *A. maculatum* breeding in 69% of natural sites and 55% of available impoundments (Fig. 18). More pronounced were differences in the fraction of natural ponds and impoundments that supported large breeding populations (i.e., > 100 masses). Large breeding populations were found in 27% of natural bodies and 6% of impoundments in 2001, and in about 20% of natural ponds and 10% of impoundments in 2002.

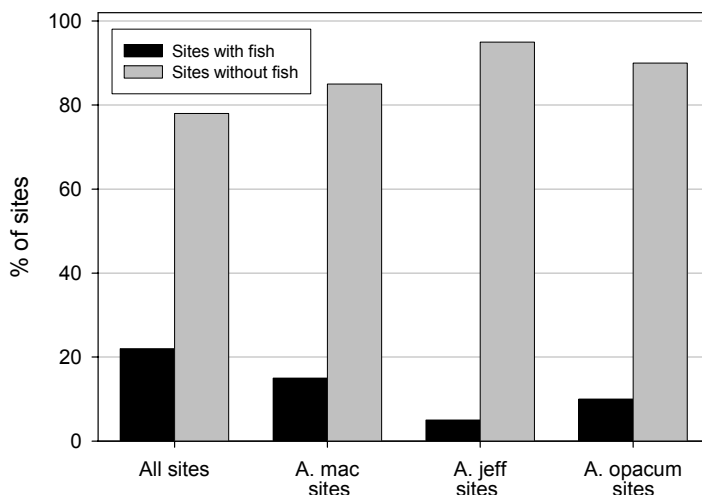


Figure 17. Comparison of the distribution of sites with and without fish between all sites sampled, *A. maculatum* sites, *A. jeffersonianum* sites and *A. opacum* sites.

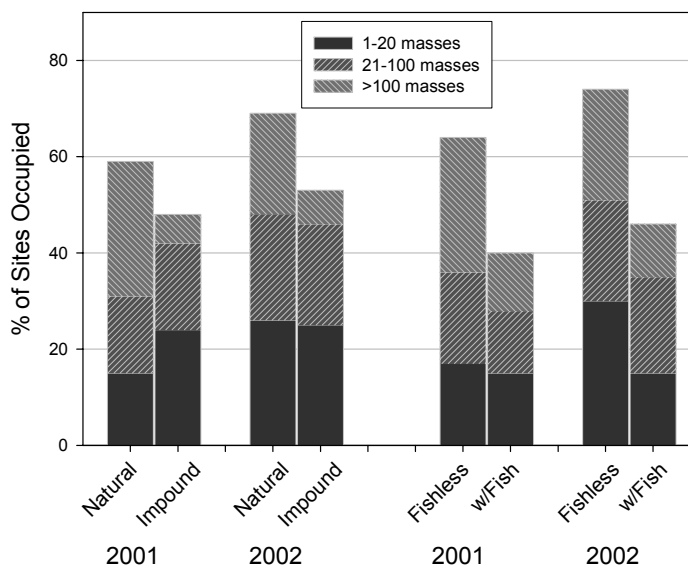


Fig. 18. Percentage of sites with *A. maculatum* as a function of site type (impoundment versus natural wetland) and fish presence.

The presence of fish negatively affected the presence and relative abundance of *A. maculatum* in both years. In 2001, *A. maculatum* bred in over 60% of fishless sites, while breeding in only 40% of sites with fish. In 2002, *A. maculatum* bred in nearly three quarters of fishless sites and in less than half of sites with fish (Fig. 18). As with pond type, differences in the proportion of sites occupied between fish and fishless habitats were more pronounced when comparing occurrence of large populations. Large breeding populations of *A. maculatum* were found in approximately 27% of fishless sites compared to 12% of sites with fish in 2001, and 23% of fishless sites and 12% of sites that contained fish in 2002 (Fig. 18).

Logistic regression results indicated site type and fish presence had similar effects on *A. maculatum* egg mass occurrence in both 2001 and 2002. After accounting for the effects of site type, sites without fish were about 2.5 times more likely to have *A. maculatum* egg masses than sites with fish in both 2001 ($\beta = 0.442$, $P = 0.10$, $df = 1$) and 2002 ($\beta = 0.472$, $P = 0.10$, $df = 1$). In contrast, after accounting for the effects of fish presence, site type had no effect on egg mass abundance in either 2001 ($\beta = -0.120$, $P = 0.69$, $df = 1$) or 2002 ($\beta = -0.003$, $P = 0.99$, $df = 1$) (Fig. 2B). Furthermore, logistic regression models predicted egg mass abundances to be highest in natural fishless sites, followed by fishless impoundments, natural sites with fish, and impoundments with fish in both 2001 and 2002.

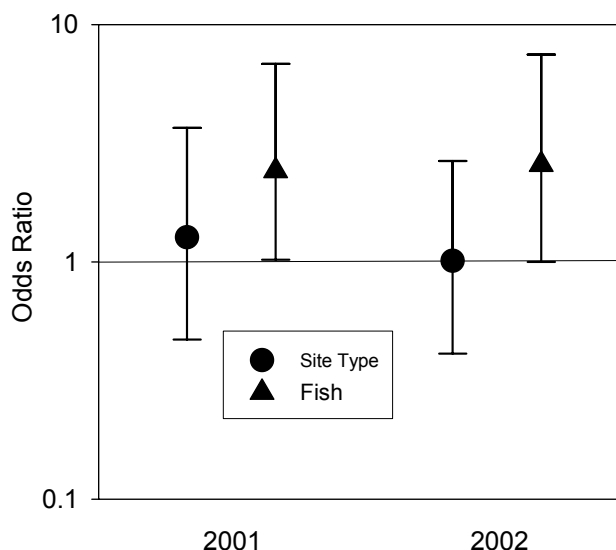


Figure 19. Results of logistics regression to determine the effects of site type and fish on the *A. maculatum* breeding site selection in the years 2001 and 2002.

Because the 2002 study year was extremely dry (Fig. _) there were fewer natural wetlands available for breeding. At the same time, it is unlikely that any of the impoundments in the park dried up completely. Consequently, we were interested in whether the use of impoundments by *A. maculatum* may have increased in 2002 in response to the drought. The below average precipitation in DEWA prior to the spring of 2002 (Fig. _) resulted in 25 out of 29 natural sites having smaller pond areas in 2002 than in 2001 which could have affected *A. maculatum* breeding patterns. In natural sites we observed a strong correlation between changes in *A. maculatum* egg mass counts and changes in pond area (Spearman: $r = 0.829$, $P < .0001$, $N=22$). We compared changes in egg mass counts between years, and found that 71% of natural sites with *A. maculatum* decreased in mass counts (17 of 24 sites) from 2001 to 2002, while only 43% of impoundments with *A. maculatum* decreased (3 of 7 sites). Sign tests indicated that the number of sites with breeding populations that decreased from 2001 to 2002 was significantly greater than expected by chance for natural sites ($M = -5$, $P = 0.06$), but not for impoundments ($M = 0$, $P = 1.00$).

Genetic structure

DNA microsatellite analysis of embryos revealed that 75% of sites in which viable genetic samples were taken contained LJJ individuals (Table 6). LJJ individuals were detected in all 10 New Jersey populations of *A. jeffersonianum* in which genetics samples were taken. The proportion of LJJ individuals in each NJ population ranged from 19 – 100% ($N = 10$, $\bar{x} = 71.3\%$, $S.D. = \pm 30.3\%$). In contrast, only one embryo (out of 60) sampled from a Pennsylvania population was identified as LJJ in which only 1 embryo. Although *A. jeffersonianum*-LJJ complexes with large proportions of LJJ individuals suffer from high egg mortality, we saw no direct correlation between the proportion of hybrids in a genetics sample and the proportion of viable masses within sites.

Table 6. Population size, percent of viable egg masses, and percent hybrids in individual populations of <i>A. jeffersonianum</i>						
Site	State	Location	Population Size ¹	Mean %Viable	%Hybrid	Genetic samples ²
688	NJ	BF	Small	13%	NA	10 ³
704	NJ	BF	Small	61%	100%	5
2032	NJ	BML	Small	100%	100%	1
151	NJ	CK	Large	80%	65%	30
232	NJ	CK	Moderate	85%	NA	37 ³
245	NJ	CK	Large	50%	75%	30
2012	NJ	CK	Very Large	45%	88%	45
3012	NJ	CK	Very Large	41%	57%	30
3013	NJ	CK	Large	66%	79%	30
801	NJ	LPR	Small	50%	NA	0 ²
807	NJ	LPR	Small	80%	NA	0 ²
819	NJ	LPR	Small	45%	100%	5
3010	NJ	LPR	Small	87%	NA	0 ²
464	NJ	PV	Small	5%	NA	0 ²
600	NJ	UPR	Moderate	86%	67%	30
758	NJ	UPR	Small	37%	19%	21
3019	NJ	UPR	Small	87%	20%	30
992	PA	HQ	Moderate	50%	0%	30
2035	PA	HQ	Moderate	77%	3%	30
80	PA	M	Small	77%	NA	0 ²
¹ Based on mean population size over all years sampled						
² No genetic samples taken due to extremely small population size						
³ Genetic samples did not quantify						

DISCUSSION

Status and critical breeding habitats of salamander populations

Ambystoma maculatum

Ambystoma maculatum was the most frequently encountered ambystomatid salamander during our surveys, as well as previous surveys of wetlands in DEWA. Although we found considerable variation in *A. maculatum* egg mass production among years, known populations rarely failed to breed unless sites were dry during the breeding season, which did occur at numerous known breeding locations in the spring of 2002. We detected breeding populations in all historical breeding sites for this species, and we also detected breeding populations where previous surveys failed to detect them. Although we observed *A. maculatum* breeding at a larger fraction of wetlands than previous surveys, and although we found *A. maculatum* at a large fraction of sites where they were not previously observed, we cannot conclude *A. maculatum* has expanded its range in DEWA because sampling protocols in previous surveys were less intense. Specifically, most of the ponds or wetlands in previous surveys were only visited once during the spring breeding compared to a minimum of three site visits each year surveyed by LSC. As a result, previous surveys probably failed to observe breeding when it actually did occur in some instances. Nevertheless, the combined information from our surveys and previous surveys indicate that *A. maculatum* has been, and continues to be, both abundant and widely distributed throughout the park. These patterns combined with the low turnover rates we observed between successive years suggest that the larger population within DEWA is stable and requires no immediate management action directed at maintaining population viability in the park.

In terms of breeding habitats, we found *A. maculatum* to be more of a generalist than the other two species, breeding in all types of habitats including man-made impoundments, although breeding adults tended to avoid impoundments with fish. Nearly all natural wetlands with longer hydroperiods and with a relatively low density of primary roads within 1-km of the site were used for breeding. Wetlands with these attributes are common in DEWA representing about 62% of all available natural wetlands.

We found that even wetlands with shorter hydroperiods were frequently used by *A. maculatum* for breeding, so long as the water chemistry was suitable. Specifically, 78% of wetlands with shorter hydroperiods (i.e., drying score ≤ 2) were used for breeding when pH was above 4.6 and specific conductivity was between 21 and 106. Sites with these habitat attributes were relatively rare in DEWA, representing only slightly more than 14% of available natural wetlands. Shorter hydroperiod sites were used much less often (0 - 20% of the time) when pH was lower or specific conductivity was more extreme. These patterns combined with the observation that natural sites with longer hydroperiods tended to be used for breeding independent of water chemistry suggests that growth, development, or survival of eggs or larvae is compromised by low pH or more extreme specific conductivity levels, but that longer hydroperiods may moderate these stresses in some way.

Ephemeral wetlands make good breeding habitats for amphibians because they typically do not contain fish that prey on their larvae. The tradeoff however is that amphibians that use seasonally flooded wetlands must grow and develop rapidly or they will not mature before pond drying (Semlitsch 1987). Thus, any stress that reduces growth or increases time to metamorphosis would decrease the likelihood that an individual would survive the larval period.

Previous field and laboratory experiments have shown that low pH (<5) causes significant declines in embryonic survival and larval growth rates of ambystomatid salamanders (Sadinski and Dunson 1992, Kutka 1994, Horne and Dunson 1995). Some previous research has also noted that *A. maculatum* egg deposition was inversely associated with specific conductivities (e.g., Rowe and Dunson 1993, Bunnell and Zampella 1999). However, data from comparative field surveys suggest that survival of natural populations exposed to low pH is highly variable (Cook 1983, Portnoy 1990) supporting the argument that other factors such as hydroperiod probably interact with water chemistry to affect reproductive success in ambystomatid salamanders. Simlitsch (1987) observed a similar pattern where the effects of intraspecific competition among *A. talpoideum* larvae depended on hydroperiod. He argued that competition for food had a similar effect as low pH in that it reduced growth and increased time to metamorphosis, but these effects were less consequential in ponds with longer hydroperiods because individuals had sufficient time to develop despite lower growth rates associated with competition.

It does not necessarily follow that factors that affect breeding site selection (as inferred by presence-absence data) are the same factors that affect population abundance (as inferred by total number of egg masses). If, for example, terrestrial habitat variables were more responsible for regulating overall population size but in-pond habitat variables were more important in determining breeding site selection by migrating adults, a different set of habitat predictors might be found to explain variation in occurrence and variation in abundance. However, for *A. maculatum*, we found that the intensity of breeding as inferred by the total number of egg masses laid at a site was strongly related to the same two primary habitat variables as those related to pond use (i.e., presence/absence). Hydroperiod and the density of primary roads within 1-km explained much of the variation in egg mass abundance among breeding sites. Our finding that hydroperiod was the most important predictor of both egg mass occurrence and abundance is not surprising in light of a wealth of past research that documented the importance of hydroperiod to ambystomatid populations (e.g., Pechmann et al. 1989, Rowe and Dunson 1995, Paton and Crouch 2002). Although not as well studied, recent evidence suggests that road density negatively affects Ambystomatid salamanders (Fahrig et al. 1995, Lehtinen et al. 1999, Houlihan and Findlay 2003).

A. jeffersonianum

In contrast to the relatively ubiquitous *A. maculatum*, *A. jeffersonianum* was rarely encountered, occurring at less than 10% of available natural wetlands. Moreover, except for the sites within the Camp Kittatiny area, populations at breeding sites were relatively small. However, based on the data from previous surveys, it appears that *A. jeffersonianum* is no less common, and their breeding populations no smaller now, than in years past. Despite the apparent persistence of *A. jeffersonianum* populations in DEWA, we believe there remains cause for concern about the status and likelihood of persistence of the population in the future. The high incidence of hybridization, the patchy nature and small sizes of most breeding populations, and the almost non-existent gene flow among breeding populations, all suggest that the long-term outlook for *A. jeffersonianum* may be uncertain and that DEWA management should consider additional monitoring of the population. These factors are discussed in detail below.

Egg mass mortality in DEWA populations of *A. jeffersonianum* was considerably higher than has been reported by others for purely diploid populations of *A. jeffersonianum*, and for other species of *Ambystoma* (Rowe and Dunson 1993, Brodman 1995, Cook 1983, Julian 2000). We attribute

this high amount of mortality to the presence of *Ambystoma laterale*-*Ambystoma jeffersonianum* hybrids at *A. jeffersonianum* breeding sites. These hybrids are genetic triploids composed of one set of *A. laterale* chromosomes and two sets of *A. jeffersonianum* chromosomes (hereafter we refer to these individuals as LJJ and their diploid counterpart as JJ), and are morphologically indistinct from purely diploid *A. jeffersonianum* individuals. During breeding, these purely female triploids require sperm from diploid male *A. jeffersonianum* to stimulate meiosis but do not incorporate the associated genetic material and pass it along to their offspring (Bogart and Lecht 1987).

In *A. jeffersonianum* populations with only diploid (JJ) individuals, male:female ratios are typically greater than 3:1 (Petranka 1998), but in populations where diploid and unisexual populations are sympatric, male:female ratios can become less than 1:10 (Nyman et al., 1988). When this occurs, egg mortality is typically very high due to insufficient insemination of both JJ and LJJ females (Petranka 1998). Triploid populations have been previously reported from western New Jersey (Nyman et al. 1988) and genetic data we collected confirmed the presence of a large number of LJJ individuals in many of the *A. jeffersonianum* breeding sites (Julian et al. 2003).

We estimated very high proportions (as much as 100%) of many DEWA breeding populations to be made up of LJJ hybrids. The effect of these triploid hybrids on breeding populations is unclear but obviously has the potential to be dramatic given that all individuals of the triploid biotype are female and therefore compete with a smaller number of diploid females for male sperm. Moreover, the triploid biotype has a numerical advantage over the diploid form because all females lay eggs. These patterns suggest that the diploid form may be vulnerable to “swamping” by the unisexual form, and since the mortality rates of the unisexual biotype will continue to rise as the male:female ratio declines, the overall population would become increasingly vulnerable to extinction through time. However, although we have limited data to make strong conclusions, there is some evidence that the elimination of the diploid form by the unisexual form is not occurring. Specifically, Nyman (1979) measured the proportion of JJ and LJJ individuals in two ponds in 1978 that we also monitored in 2001-2003. A comparison of the two surveys indicates that breeding populations at neither site has declined and that the fraction of hybrids has not increased suggesting that the elimination of *A. jeffersonianum* via unisexual “swamping” has not occurred, perhaps because aspects of each biotype’s reproductive ecology would alternately favor them over time. These data also suggest that population size and LJJ:JJ ratios at a breeding site may fluctuate over time as conditions alternately favor each biotype.

The specific conditions that favor the alternative biotypes and consequently allows for the long-term persistence of both biotypes is not clear but we have two alternative hypotheses that are based on temporal changes in the size and viability of *A. jeffersonianum* breeding populations over the three study years. In the second year of our study (2002) we observed lower numbers of egg masses, yet higher proportions of viable egg masses than during the other two years. Decreases in *A. jeffersonianum* egg mass abundances during 2002 were similar to observations for *Ambystoma maculatum* in DEWA and were correlated with declines in pond areas between 2001 and 2002 that were associated with a severe drought that occurred during the spring and summer. In the following year (2003) *A. jeffersonianum* breeding populations again increased in size and decreased in viability. One hypothesis to explain this pattern is that male:female sex ratios increase in response to drought because females may be less likely to breed than males, or that males are more persistent in terms of attempting to breed than females in the face of poor

environmental conditions. Since individual *A. jeffersonianum* males are capable of breeding every year and females are typically not (Downs 1989), the “cost” of a failed reproductive effort is relatively greater for females. Therefore, if females are less likely than males to risk the potential costs of breeding during drought conditions, we would expect to see higher proportions of viable egg masses during drought years because typically low male:female ratios would be increased and consequently relatively more sperm would be available for fertilizing females of both biotypes.

An alternate hypothesis to explain the persistence of both biotypes within breeding populations relates to the timing of breeding migrations. In addition to the drought that occurred during the spring and summer of 2002, the preceding winter was unusually warm with relatively little snow cover. As a result, ponds thawed earlier and the onset of *A. jeffersonianum* breeding occurred 2-3 weeks earlier in 2002 than in the other two years. Unisexual embryos typically have higher mortality rates than JJ embryos, and adult LJJ are less successful at competing for breeding male JJ than female JJ. However, female LJJ typically compensate by migrating to breeding sites earlier than female JJ, thus affording them a competitive edge in initially sequestering male JJ sperm. Because the breeding season in 2002 was earlier than 2001 and 2003, the lag time between female LJJ and female JJ migrations may have been reduced, thus limiting the competitive advantage of female LJJ in monopolizing available male JJ sperm.

Clearly much is not known regarding the potential effects LJJ hybrids on the long-term viability of the species in the park and more research is needed. It is possible that long term genetic monitoring of both *A. maculatum* and *A. jeffersonianum* breeding populations might shed light on historical changes in population structure, gene flow, and colonization events that may help explain mechanisms that allow for the coexistence of the diploid and triploid forms. Beebe and Rowe (2001) confirmed the practicality of using genetic techniques as a method of predicting population bottlenecks in populations of *Bufo calamita* comparing demographic data to DNA microsatellite data. If genetic analyses were performed on populations of *A. jeffersonianum* and *A. maculatum* sharing the same breeding site, and there was evidence to suggest that a population bottleneck occurred in *A. jeffersonianum* but not *A. maculatum*, then sources of environmental stochasticity that would effect both species equally (like prolonged drought) could be ruled out as the cause of the bottleneck and evidence would point towards more species specific mechanisms as the cause, such as deleterious effects of high ratios of LJJ:JJ.

In terms of breeding habitats, we found that *A. jeffersonianum* would not breed in sites with certain habitat characteristics. For example, *A. jeffersonianum* did not breed in any artificial impoundments or in any natural wetland with primary roads within 1-km of the pond edge. Artificial impoundments represent about 12% of the potential breeding sites, and natural wetlands that contain primary roads within 1-km of pond boundaries represented just over 35% of all natural wetlands. However, unlike for *A. maculatum*, the suite of landscape and habitat variables we measured was not particularly useful in predicting pond use by *A. jeffersonianum*. Although the best classification tree model we derived was statistically significant, a relatively large number (10%) of randomly generated null models (i.e., random combinations of predictor variables) had as low or lower misclassification error rates than our model.

Likewise, the best regression tree model we derived to predict *A. jeffersonianum* egg mass abundance from landscape and site habitat was also not particularly robust, explaining only 45% of the variability in egg mass abundance. In addition, most of that variance was explained by the

amount of both primary and secondary roads within pond edges, which we found to positively correlate with egg mass density, a correlation that is most likely spurious. The most effective predictor of *A. jeffersonianum* egg mass abundance did not relate to habitat at all, but rather to the spatial location of breeding sites on the landscape, independent of habitat. Latitude by itself explained 75% of the total variation in egg mass abundance.

More specifically, although *A. jeffersonianum* breeding sites typically contained small breeding populations (mode = <125 masses for all three years), the sites around the Kittitini Camp area of New Jersey consistently produced high egg mass abundances. In each year of the study, half of the sites in the area produced > 2,000 masses/year, and the remaining sites produced 500-2,000 masses in at least one year in which they were surveyed. Sites outside the Kittitini Camp area in New Jersey included a grouping of small-sized populations along Lower Pompey Ridge (LPR), pairs of mostly small populations near Upper Pompey Ridge (UPR) and Buttermilk Falls (BF), and small, isolated breeding populations near the Peter's Valley (PV) and Blue Mountain Lake (BML). In Pennsylvania, we observed a small, isolated breeding population in the north end of DEWA < 1km south of the town of Milford (M), and a pair of moderate sized populations near National Park Service Headquarters (HQ). However, over the three years of the study, the reproductive output of the 30% of sites located in the Kittitini area (N=6) produced on average 89.2% of all *A. jeffersonianum* egg masses in the park. Sites in this area are in relatively close proximity to secondary roads which explains the positive correlation with roads referred to above.

The reason for this spatial pattern in *A. jeffersonianum* recruitment is unclear. However, landscape and inpond habitat characteristics were clearly not, by themselves, particularly important, or the habitat models would have been more robust. That is, there does not seem to be anything unique about the Kittitini Camp area from a habitat point of view. The observed patterns may be consistent with a metapopulation that is either expanding or contracting its range. Another possibility is that the occurrence of the triploid biotype is a relatively recent event in the park, and may be causing local extinctions for intrinsic reasons described above. Whatever the explanation, the observation that such a large fraction of this species recruitment output is centered on such a small spatial area in the park is cause for concern and begs for more research.

A. opacum

Ambystoma opacum was intermediate relative to the other two species in terms of abundance of breeding sites, occurring at about 20% of the sites surveyed. We found *A. opacum* at all historical breeding sites, as well as at many sites previously surveyed but where they were not previously found. However, as for the other two species, we sampled this species with greater effort than previous surveys including visual encounter surveys (VES) over multiple visits and a relatively rigorous dipnetting of each site. Thus, the higher occurrence probability observed for *A. opacum* in our study compared with previous surveys does not necessarily imply an increasing population.

We believe the relatively low occurrence frequency of *A. opacum* relative to *A. maculatum* is likely due to differences in their life history that make them more vulnerable to stochastic weather events. Specifically, because *A. opacum* lays their eggs in dry pond beds, they require sites that dry down substantially in the summer and early fall during the egg laying period, but refill again and maintain water levels long enough for hatching and larval development. Thus, A

opacum is vulnerable to extraordinarily wet summers because ponds would not dry down sufficiently for egg laying, and to unusually dry winter and spring seasons because sites would have sufficient hydroperiod to support larval and growth and development. In contrast, *A. maculatum* requires only the latter. Thus, a much smaller set of sites would meet hydrologic conditions for successful reproduction for *A. opacum*.

In contrast to the other two species, we found high turnover rates for *A. opacum* at breeding sites. High turnover at individual sites was observed despite the fact that overall occurrence probabilities did not vary significantly between years, ranging between 19% and 24%. There are two possible explanations for the high turnover rates observed at breeding sites. Either detection probabilities were relatively low leading to a high likelihood of not observing larvae at a site when they actually did occur, or between-year variation in some important habitat characteristic resulted in *A. opacum* breeding at a site one year but not in another year, or some combination of the two.

We believe that *A. opacum* larval detection probabilities were relatively high in our study, though likely not as high as for *A. maculatum* and *A. jeffersonianum* egg masses. In all three years, we used visual encounter surveys (VES) and dipnetting to sample *A. opacum* larvae. In 2001 and 2002, all sites were dipnetted for *A. opacum* larvae, even sites in which larvae were detected previously during VES, whereas in 2003, we did not dipnet sites in which we already observed larvae with VES. For the years 2001 and 2002, there were instances where we failed to detect larvae using VES but did find them in dipnet surveys and visa versa. Also, neither technique appeared to work better than the other, although in 2002 we were able to detect larvae in more ponds using dipnetting than VES. In a previous study, Jenkins et al. (2002) found that VES for *A. opacum* larvae during the spring resulted in approximately 80% success rates when sites were visited at least 3 times. All of our sites were visited at least three times. Moreover, since both dipnetting and VES were equally effective in our surveys, then dipnetting should have a similar sampling success rate. Thus, independently employing both techniques as we did should provide a success rate of approximately 95% for combined techniques.

The more likely explanation for the high turnover observed for *A. opacum* at breeding sites is related to the drought that occurred in 2002. During that year, a large fraction of sites that normally fill with water in the late fall and winter, apparently did not fill until very late in the spring; and many of those only partially. At the same time, most instances of *A. opacum* turnover that we observed were associated with sites where we observed larvae in 2001 or 2003, but not during the 2002 drought year. This pattern suggests *A. opacum* eggs deposited in ponds that did not fill until the spring of 2002 may have failed to hatch. It is not clear why eggs would not continue to be viable through the winter and hatch normally in the spring. If that were to occur, larvae may be at a relative disadvantage because they would compete with, rather than prey on, *A. maculatum* and *A. jeffersonianum* larvae at sites where they are sympatric, but we would still expect to see some of them in our samples. We did not visit sites until spring and so we do not know what the hydrologic conditions were throughout the fall-winter seasons. It may be that many of the sites with turnover did fill early in the winter but dried up later in the season before again refilling in the spring. If so, larvae may have hatched and then died after the pond dried up again.

In terms of breeding site habitat, we found that *A. opacum* almost never bred in artificial impoundments or in wetlands that contained fish. They also tended to avoid natural wetlands

with either primary or secondary roads within 1-km of the pond edge. Avoidance of sites with either primary or secondary roads suggests *A. opacum* may be even more vulnerable to road traffic than the other two species that showed a negative correlation with only primary roads. It may be that *A. opacum* is less likely to migrate across any paved substrate (i.e., behavioral characteristic), whereas the observation of low pond use of sites with primary roads nearby for the other two species reflects higher than normal mortality rates from cars (i.e., population characteristic). Our habitat model further suggested that *A. opacum* preferred breeding sites either with longer hydroperiods and low conductivity, or, for sites with shorter hydroperiods, sites with higher pH and other wetlands nearby. This pattern is similar to the one observed for *A. maculatum* where pH was negatively correlated with pond use but only in sites with lower hydroperiods suggesting that hydroperiod may ameliorate the effects of acid stress.

Influence of impoundments

Based on survey data, we find no evidence that artificial impoundments provide critical or preferred breeding habitat for *A. jeffersonianum* or *A. opacum*. Although the propensity for impoundments to contain fish (23 out of 29 contained fish) likely contributed to the exclusion of *A. opacum* and *A. jeffersonianum* from these sites, we believe that static water levels of impoundments further deterred the colonization the *A. opacum* in these habitats because *A. opacum* require nesting sites that are dry when they deposit their eggs, but later fill with water to initiate hatching in their larvae. Furthermore, we do not believe that artificial impoundments in DEWA contribute significantly to the amount of *A. maculatum* breeding habitat across the landscape in light of the abundance of more suitable, fishless, natural bodies of water. Although impoundments were used frequently by *A. maculatum* for breeding, only 2 out of 24 supported large breeding populations. Our results suggest that the tendency for impoundments to contain fish was adequate to explain the lower occurrence and abundance of *A. maculatum* in impoundments than in natural bodies of water. Although we also observed that *A. maculatum* egg mass abundance decreased in natural sites between drought and non-drought years (2001 and 2002, respectively), salamanders did not colonize previously uninhabited impoundments during drought conditions, nor did their abundances in impoundments increase significantly. Therefore, we could not conclude that impoundments offer a “rescue effect” during drought years for populations from nearby natural bodies of water that do not fill. However, due to the limited duration of this project, we could not assess the importance of these habitats to the persistence of *A. maculatum* populations during extended periods of drought. In addition, we can not extrapolate our conclusions regarding the potential influence of man-made ponds to landscapes where natural wetlands and ponds are less common.

However, in making the final decision to deactivate individual impoundments, DEWA resource managers may want to consider the degree in which the impoundment is isolated from other bodies of water, especially if the impoundment in question contains *A. maculatum*. Summarized in the table below are measures of isolation for impoundments occupied by *A. maculatum*. Relatively isolated impoundments (those > 500m from other sites or those with fewer than three bodies of water within 1km) may provide the only available breeding habitat for *A. maculatum* in a given area of the park and may prove to be important “stepping stones” in maintaining gene-flow between areas with higher densities of breeding habitats that flank them.

Table7. Measures of isolation for impoundments containing *A. A. maculatum*.

Site#	#Masses	Sites within 1k	Sites within 500m	Dist. to nearest site (m)
1250*	1-20	0	0	9,194
905	21-100	2	0	697
1267	21-100	1	0	555
710	1-20	3	1	343
785	> 100	1	1	222
236	1-20	3	1	220
244	21-100	3	2	220
72	1-20	7	4	217
971	1-20	6	6	145
968	21-100	6	5	145
517	1-20	7	1	126
119	21-100	1	1	68
1295	> 100	4	1	17

MANAGEMENT IMPLICATIONS

Results of our study have significant long-term and near-term implications for management of natural resources in DEWA. Because ambystomatid salamanders require relatively undegraded terrestrial and aquatic habitats to complete their life cycles, they make excellent indicators of overall ecosystem health (Vitt et al. 1990, Welsh and Ollivier 1998). Consequently, should DEWA management decide to include pond-breeding salamanders into their future long-term monitoring programs (e.g., Vital Signs), we believe the assessments of current status and recent trends generated from this study will be invaluable in terms of baseline data. In addition to baseline information on the salamanders themselves, this study added considerably to what was previously known about wetland habitats in DEWA. Although a significant number of wetlands in the park had been mapped prior to this study, the location of many had not been field validated and most had little if any biological and habitat data attributed to them. During the course of this study, we visited over 250 wetlands, including many that had never been mapped, and measured and linked over 30 variables to 200 site locations including information on landscape position, wetland size and hydroperiod, inpond habitat, and amphibian occurrence and abundance patterns. The relevant GIS layers and associated database is included with the attached compact disk. As a result, DEWA management has an excellent baseline data layer on wetland resources in the park that they can use as a basis to infer status and trends of this important component of the ecosystem.

This study also generated data useful for near-term management of ambystomatid salamanders in the park. In particular, information on breeding site selection provided support for certain management prescriptions that would aid in the preservation of these species. Specifically, although there was substantial variation in wetland habitat use by the three ambystomatid salamander species, there were also several important habitat preferences that they shared. In particular, all three species tended to avoid breeding in natural wetland sites that contained primary roads within 1-km of wetland edges. *A. opacum* occurrence was negatively correlated with secondary (low traffic) paved roads as well. Currently, about a third of natural wetlands within the park are within 1-km of primary roads, and over 90% are within 1-km of either a primary or secondary road. More importantly are recent trends in population growth and associated development in the area surrounding the park. Over the last three decades, population growth rates in the four counties of DEWA (Pike and Monroe, PA; Sussex and Warren, NJ) were the fastest growing counties in their respective States (Shields 2002, Cornwall Center for Metropolitan Studies 2003). Given these trends in human population and residential development both within and adjacent to park boundaries, it is likely that pressure to build new roads or improve (i.e., pave) existing roads and trails will increase. Our data suggests that additional roads would likely have negative consequences to ambystomatid salamander populations within the park. Thus, if possible, planning for new road construction or improvement should consider the location of wetlands used by breeding salamander populations, a large number of which have now been mapped.

Hydroperiod was also an important feature of breeding sites common to all three species. Excluding impoundments and wetlands that contained fish, *A. maculatum* and *A.*

jeffersonianum preferred sites with longer hydroperiods. *A. opacum* also tended to prefer longer hydroperiod sites, as long as they were not permanent, or at least dried enough in summer to provide dry areas within the pond boundary in which to lay their eggs. Consequently, environmental factors that reduce the amount of time that wetlands contain water would likely adversely affect all three species if dry conditions persisted for multiple years.

The possible increase in air temperature and drought frequency associated with human-induced increases in concentrations of green house gasses (global warming) could cause reductions in wetland hydroperiods. Many regional climate change models predict warmer temperatures, especially in winter, for the north-eastern United States (Hansen et al. 1988, Wetherald and Manabe 1988). Monitoring of wetland hydroperiods and wetland assemblages over long periods of time could provide an early warning indicator of climate change and its effects in the park. In addition, because of the relationship between tree species and evapotranspiration rates, climate or pest-induced changes in forest composition could also affect wetland hydroperiods.

In DEWA, one of the most important management concerns in recent years has been the decline in hemlock forests due to the hemlock woolly adelgid (*Adelgis tsuga*), an exotic insect native to the Far East (Evans 1995). Hemlock forests in DEWA have been infected with the pest since 1989 and there has been considerable HWA-induced mortality over last several years (R. Evans, personal communication). We compared the hydroperiods of the 102 natural wetlands that occur on forested ridges with and without significant amounts of hemlock in 200-m pond buffers. There were a total of 64 sites that did not contain significant hemlock within their buffers and 38 sites that did contain hemlock. Although the patterns

were similar for most hydroperiod ranks, we found that the surrounding forests of sites with the shortest hydroperiods (i.e., drying scores of 0, see Methods) rarely contained a significant hemlock component (Fig. 20). Only one in twelve sites containing significant hemlock (N=3) had the lowest drying scores (i.e., shortest hydroperiods) compared to one in four sites without hemlock (N=12). This pattern suggests that pest-induced hemlock decline could reduce hydroperiod and perhaps reduce the availability of ambystomatid salamander breeding habitats in the park should hemlock forests continue to decline.

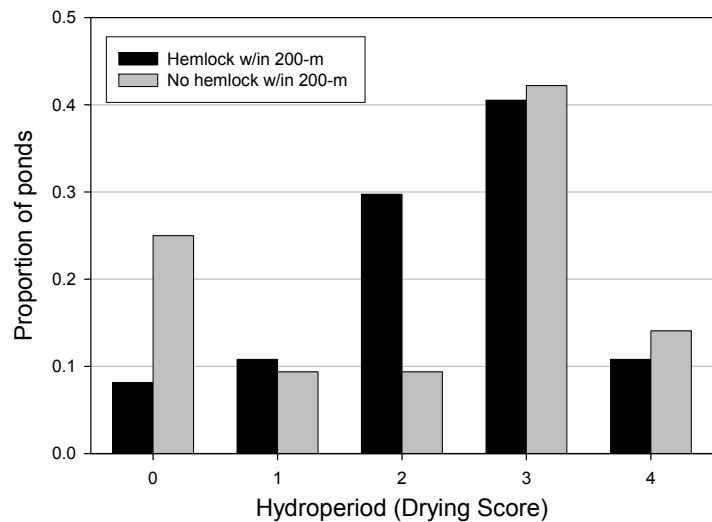
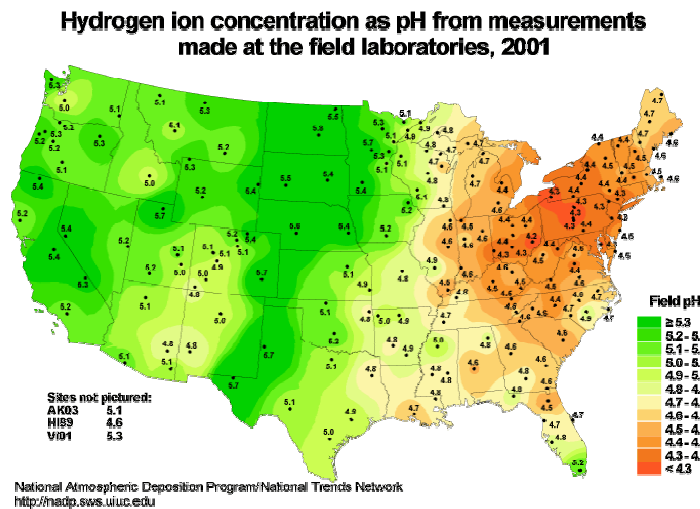


Fig. 20. Comparison of the proportion of ponds of various hydroperiods between sites that contain significant amounts of hemlock within a 200-m buffers and those that contain little or no hemlock within 200-m buffers. We used the vegetation map of Myers and Irish (1981) to infer hemlock. If hemlock comprised the primary, secondary or tertiary component of the buffer, we classified the site as having significant hemlock. See Methods for description of drying scores.

Finally, although not as important as roads or hydroperiod, the pH of ponds and wetlands was also found to be a significant predictor of whether, or to what extent, a site was used for breeding for all three species. Wetlands and temporary ponds are often naturally acidic due to the presence of sphagnum moss which is known to acidify waters, and to high concentrations of humic acids associated with the decomposition of plant matter (Freda et al. 1991). As a result, amphibians that use these habitats to breed have evolved relatively high tolerances to acidic conditions relative to other aquatic fauna such as fish. However, there is mounting evidence that acidic precipitation and deposition associated with air pollution has further increased acidity in receiving waters in some regions of the country, and may be the cause of some observed amphibian declines (Dunson et al. 1992). Acidic precipitation is particularly a problem in the northeastern United States where regional rainfall pH values frequently fall below 4.0, and the average pH of the area around DEWA in 2001 was about 4.3 (see Figure below).



It is difficult to prove the relative contributions of natural and anthropogenic sources of acidity to aquatic habitats, and we do not attempt to attribute the failure of ambystomatid salamanders to breed in sites with lower pH to acid rain. However, clearly acid rain and acid deposition continues to be a problem in the region and consequently it is very possible that the acidity of salamander breeding ponds could become higher in the future, and possibly compromise population viability if it has not already. Thus,

we believe it would be prudent for DEWA managers to include monitoring of pH in selected wetland sites as part of their long-term monitoring program.

Although the results of this study provide a wealth of information about the status and recent trends of this group of species, there are important questions that remain unanswered, especially regarding the status and likelihood of persistence of *A. jeffersonianum*. For example, why *A. jeffersonianum* is so rare in the park, and why their breeding sites and reproductive output is so clustered in the Kittitini Camp area is unclear. Our data suggest that habitat alone does not explain these observations, at least those habitat attributes we measured. Comparison of our results with those obtained in earlier surveys suggests that, although rare, *A. jeffersonianum* may be as abundant now as in years past. Nevertheless, there remains considerable uncertainty regarding factors that regulate *A. jeffersonianum* populations and consequently strategies for their preservation remain ambiguous. In addition, the ecological relationship between *A. jeffersonianum* and AJJ triploid populations is also not well understood. The ecological mechanisms for, and the long-term effects of, their coexistence are not clear from the literature or from our

data. Thus, additional research including long-term monitoring of population genetics and recruitment patterns may be needed to better inform DEWA management.

Finally, one of the main impetuses for this study was related to the management of small impoundments in the park. Specifically, there were questions about whether management plans to eliminate or cease maintenance on many of these dams might have negative biological consequences by eliminating breeding habitats of ambystomatid salamanders. Results of this study indicate that artificial impoundments do not provide significant habitat for ambystomatid salamanders. *A. jeffersonianum* and *A. opacum* almost never bred in artificial impoundments, and although *A. maculatum* used impoundments to breed, it was rarely in significant numbers and usually only when fish were absent. Even during the severe 2002 drought when a much smaller amount of natural breeding habitat was available, none of the three species increased their use of artificial impoundments. These patterns combined with the large amount of naturally occurring wetlands, swamps, and temporary ponds lead us to conclude that plans to eliminate impoundments will have little effect on pond-breeding salamanders.

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