

RESPONSES OF AMPHIBIANS TO RESTORATION OF A SOUTHERN APPALACHIAN WETLAND: PERTURBATIONS CONFOUND POST-RESTORATION ASSESSMENT

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Abstract: Although regulatory agencies in the USA typically require 3–5 yr of post-restoration monitoring of biotic responses to wetland mitigation, many researchers have argued that longer time frames are needed to assess population responses adequately. We conducted an 8-yr study to examine the demographic responses of the wood frog (*Rana sylvatica*) and spotted salamander (*Ambystoma maculatum*) to wetland creation at a mitigation bank in western North Carolina. Our primary goals were to compare juvenile output in ten reference and ten constructed ponds and to assess the overall change in breeding population size in response to site restoration. We used annual censuses of egg masses to assess changes in breeding population size and used estimates of larval population size at hatching and the initiation of metamorphosis to assess embryonic and larval survival. Adults of both species bred in most constructed ponds within a few months after filling in 1996. Estimated juvenile production from 1996 to 2002 did not differ significantly between pond types for either species. The percentage of both constructed and reference ponds that produced juveniles decreased markedly from 1996 to 1998 and remained low through 2002. The decrease in juvenile output was mostly associated with reduced larval survival rather than increased embryonic mortality across years. Drought and outbreaks of a pathogen (*Ranavirus*) were the primary causes of low juvenile production from 1998 to 2002. The overall breeding population of *R. sylvatica* increased markedly in 1999–2000 following a large recruitment of juveniles from constructed ponds in 1996–1997. With the onset of drought and ranaviral infections, the population declined to levels in 2002 that were at or below 1995 pre-restoration numbers. Despite site perturbations, the breeding population of *A. maculatum* remained relatively stable from 1995 to 2002, a phenomenon that may reflect selection for delayed reproduction and iteroparity in this species. Although we have monitored *R. sylvatica* and *A. maculatum* for seven breeding seasons after the creation of seasonal wetlands, we are still uncertain that site restoration will achieve the goal of increasing breeding populations above pre-restoration levels. Because amphibians have significant population lags and are sensitive to site perturbations, monitoring that exceeds five years may be required to assess demographic responses to site restoration adequately.

Key Words: amphibians, created ponds, drought, *Ranavirus*, North Carolina, mitigation banks

INTRODUCTION

A primary goal of wetland restoration is to renew the ecological integrity of degraded wetlands. This involves restoring physical and functional elements necessary to sustain major biotic components at viable levels (Zedler 1996). One issue is whether the success of restoration projects can be adequately assessed within the time frame (typically ≤ 5 yr) that regulatory agencies set for post-restoration monitoring (National Resource Council 2001). Researchers have primarily

assessed success using variables such as hydrology, vegetation, and invertebrates that respond quickly to site restoration. Even when using ‘rapid response’ variables such as these, many have argued that a 5-yr time frame is inadequate to assess biotic function because of population lags, the slow pace of ecological succession, and the influence of stochastic events on hydrology and population dynamics (e.g., D’Avanzo 1990, Confer and Niering 1992, Mitsch and Wilson 1996, National Resource Council 2001).

Vertebrates are important components of many sea-

sonal freshwater wetlands, but their response to wetland mitigation has been examined less frequently and typically for shorter durations than plants or other biotic indicators (Pechmann *et al.* 2001). Although researchers have primarily examined the responses of birds and fishes to wetland mitigation, amphibians are increasingly being used because they are highly sensitive to environmental pollutants, altered hydrology, and degradation of surrounding forest cover (Pauli *et al.* 2000, Semlitsch 2000, Pechmann *et al.* 2001). Hydroperiod affects the likelihood of amphibians completing metamorphosis (Pechmann *et al.* 1989, Semlitsch *et al.* 1996, Semlitsch 2000, Paton and Crouch 2002), and the distribution and abundance of egg and larval predators such as fish, insects, and tadpoles (e.g., Heyer *et al.* 1975, Woodward 1983, Schneider and Frost 1996, Petranka and Kennedy 1999). Predation, competition, and evolutionary constraints prevent most amphibian species from occupying the entire hydrologic gradient (Kats *et al.* 1988, Smith and Van Buskirk 1995, Wellborn *et al.* 1996, Skelly 1995, 1997). Consequently, amphibian assemblages vary predictability with pond duration and may change with altered site hydrology (e.g., Skelly *et al.* 1999, Snodgrass *et al.* 2000a, b).

Studies of amphibian responses to created or restored wetlands are in their infancy and have focused primarily on documenting colonization rates and communities that assemble within a few years after construction (e.g., Mierzwa 2000, Pollet and Bendell-Young 2000, Lehtinen and Galatowitsch 2001, Stevens *et al.* 2002). These attributes could be poor predictors of ecosystem function if created wetlands attract adults but act as population sinks due to high premetamorphic mortality (e.g., Turner and Fowler 1981). In addition, short-term studies may be insufficient to assess restoration success if amphibian habitat quality changes in association with forest succession, stochastic events, or metapopulation dynamics (e.g., Hecnar and M'Closkey 1996, Semlitsch *et al.* 1996, Skelly *et al.* 1999).

Because restored or created wetlands can potentially act as population sinks, measures of juvenile output and changes in adult population size over several generations may be better indicators of restoration success. This goal is rarely attainable for amphibians because many species require ≥ 2 years to reach sexual maturity, whereas funding for post-restoration monitoring rarely exceeds five years. The 8.5-yr study by Pechmann *et al.* (2001) is noteworthy in being the most comprehensive analysis of the long-term demographic responses of amphibians to compensatory mitigation in the southeastern USA. The primary goal of our study was to assess the long-term reproductive success and population responses of two amphibians

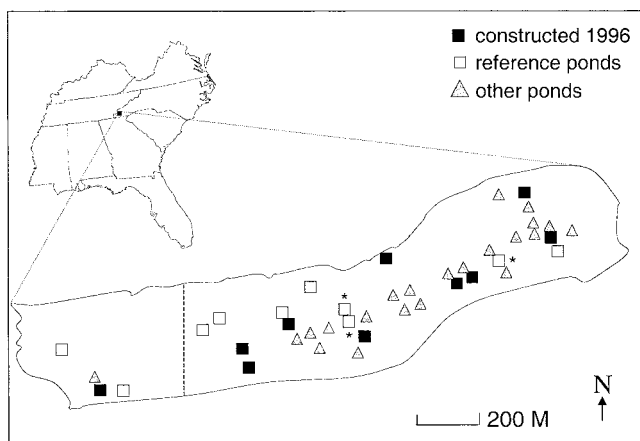


Figure 1. Location of the study site and ponds. Vertical hatched line separates the eastern and western sectors. Reference ponds with asterisks were eliminated in fall 2001 during stream reconstruction. “Other” ponds are additional major breeding habitats; small depressions that provide marginal breeding habitat are not depicted.

to wetlands restoration in the southern Appalachian Mountains, USA. Data on colonization and changes in community assemblages will be presented elsewhere.

METHODS

Site Description and History

The Tulula Wetlands Mitigation Bank is a 95-ha wetland restoration site located 4.8 km west of Topton in Graham County, western North Carolina, USA (35°17'N, 83°41'W; elevation range 784–800 msl; Figure 1). Until the mid-1980s the site supported a relatively intact floodplain of Tulula Creek, with scattered depressional fens (Moorhead and Rossell 1998) and regenerating forests dominated by red maple (*Acer rubrum* Linnaeus) and white pine (*Pinus strobus* Linnaeus). An attempt to build a golf course on site in the late 1980s failed. During this period, Tulula Creek was channelized and several drainage ditches were dug. Spoil from the drainage ditches and 11 golf course ponds was spread over portions of the floodplain to create fairways. About 40% of the floodplain forest was removed, and cattle later grazed the site. The site was purchased in 1994, and restoration occurred from 1996 to 2002. Enhancements included restoration of the original stream channel, filling of drainage ditches, creation of ponds for aquatic organisms, removal of fill from underlying wetland soils, and revegetation (Moorhead *et al.* 2001).

When initially inventoried in 1995, the site contained 11 golf course ponds with predatory fish (bluegill, largemouth bass) and 144 small, fish-free, seasonal depressions (median depth, surface area, and volume

= 13.7 cm, 12.1 m², and 1.7 m³ of water, respectively) that were the primary breeding sites of pond-breeders. Most were artificial habitats that were prone to premature drying (e.g., water-filled tire ruts and puddles; test wells for pond sites), but a small percentage was natural seasonal depressions with longer hydroperiods.

Creation of Wetlands

Seasonal and permanent wetlands were created in 1995–1996 and 1999 to provide breeding sites for amphibians, while others were created as an indirect consequence of stream restoration. In addition to ten ponds that were specifically created in 1995–1996 for amphibian use (see below), nine golf course ponds were partially filled in 1999 to create shallower sites. Some now exist as shallow stream-fed ponds and marshes that contain small fish, while others were converted into fish-free, seasonal ponds. Sections of the restored stream channel were temporarily blocked with check dams in fall 1999 to allow revegetation of channel banks prior to initiating stream flow. Small pools formed in the deepest sections of these blocked channel segments and were used as breeding sites by resident amphibians in 2000 and 2001. These were eliminated in 2002 when flow was restored to the new channel, but other sites were created when filling the old channel bed. In February 2002, the site contained 39 primary breeding sites (Figure 1).

Seven seasonal and three permanent fish-free ponds were created from October 1995 to January 1996 to compensate for the loss of natural breeding sites. These were the primary sites that were used to monitor demographic responses to created wetlands. The average surface area and maximum depth at full capacity (± 1 SE here and throughout) were 480 ± 68 m² and 44 ± 5 cm. Ponds were placed spatially to maximize demographic independence and allow interconnectivity via dispersal (Figure 1). The distance between adjoining constructed ponds ranged from 70 to 513 m and averaged 190 m.

At four sites, small pools previously existed where new ponds were constructed. These were used as breeding sites by the spotted salamander (*Ambystoma maculatum* (Shaw)) and wood frog (*Rana sylvatica* Le Conte) in 1994 and 1995. At the remaining sites, small pools were < 100 m from the pond sites and supported source populations for colonization. Tracts of second growth forest that provide adult habitat occur within 100 m of ponds. Although construction during the restoration phase caused significant site disturbance in the immediate vicinity of Tulula Creek, disturbance to the study ponds, adjoining forest, and interconnecting habitats was minimal, with the exception of three reference ponds that were eliminated in 2001 (see below).

Within one year of formation, the constructed ponds developed complex assemblages of invertebrates and amphibians, as well as a dense growth of macrophytes (e.g., *Eleocharis*, *Juncus*, *Scirpus*, *Sparganium*) along their margins and bottoms. Although some shrubs and saplings are established along the margins, the ponds have remained as open, sunny habitats (forest canopy cover < 10%) since 1996.

Selection of Reference Ponds

We initially planned to compare the population responses of amphibians in the ten constructed ponds to reference ponds off site but were unable to locate a sufficient number of seasonal ponds in the immediate vicinity. As an alternative, we selected the ten largest and most natural seasonal depressions on site as reference ponds. Six of the sites were natural habitats, while the remainder was semi-natural (e.g., stream channel cut-offs or vegetated seasonal depressions). Reference ponds were smaller and shallower than the constructed ponds (mean surface area at full capacity = 82 ± 21 m²; mean depth = 24 ± 3 cm) and more shaded. Estimated mean canopy cover from tag alder (*Alnus serrulata* (Aiton) Willdenow) or mixed pine-hardwoods ranged from 0–100% and averaged 60%.

Reference ponds were monitored from 1996 to 2002, but three were unavoidably destroyed in fall 2001 in conjunction with the reconstruction of Tulula Creek. Because reference ponds generally were of lower quality than undisturbed natural wetlands, our goals in 1996 were to create larger breeding habitats with longer hydroperiods, to increase juvenile production, and to increase adult population sizes to levels that would enhance the long-term viability of resident species.

Sampling Design

We selected *A. maculatum* and *R. sylvatica* as focal species because they are important members of vernal pond communities in the southern Appalachians and lay large egg masses that can be accurately censused to assess changes in the size of female breeding populations (Petranka and Smith 1995, Crouch and Paton 2000). Ponds constructed in the autumn of 1995 filled with water before these species began breeding in February 1996. We censused egg masses in constructed and reference ponds annually from 1996 to 2002 to estimate breeding population size.

Every 1–3 weeks during the breeding season (February–April), we systematically waded through all areas of each pond and counted masses. We identified masses deposited between visits by either higher tallies or the presence of embryos in relatively early devel-

opmental stages. We censused seasonally until no new masses were found and used the cumulative number of egg masses laid each year to estimate the size of the female breeding population. Since each female *R. sylvatica* deposits a single mass, the number of egg masses in a pond is an accurate estimate of the total breeding population (Crouch and Paton 2000). Each *A. maculatum* typically deposits 1–4 egg masses (Petranka 1998), and egg mass counts provide a relative index of annual changes in the breeding population.

To obtain estimates of the overall population response to restoration efforts, we conducted a complete count of egg masses on the eastern sector of the site from 1995 to 2002 (Figure 1). Time constraints prevented censusing the western sector. The eastern sector encompasses about 70% of the breeding habitats, including nine constructed ponds, eight reference ponds, and numerous other breeding sites. The latter included shallow sites that existed prior to 1996, habitats that were created in 1999 from the partial filling of golf course ponds, and ponds created in 2001–2002 in association with stream restoration.

We visited ponds at 1–3 week intervals from February to August to document premature pond drying, termination of hatching, and initiation of metamorphosis in each pond. Metamorphosis in most local ponds occurs in May and June for *R. sylvatica* and June and July for *A. maculatum*. With respect to reproductive success as measured by juvenile production, we scored ponds that did not fill seasonally or that dried before the initiation of metamorphosis as being failures.

We estimated the number of larvae in each pond immediately after hatching and at the initiation of metamorphosis using open-bottomed samplers constructed of 114-L galvanized trashcans with the bottoms removed (approximate area of can bottom = 0.12 m²). The sampler was pushed into the pond substrate to trap larvae, and repeated sweeps were made inside the can with either 15 × 20 cm or 17 × 25 cm aquarium nets until no larvae were captured for five consecutive sweeps.

A pond was sampled by walking a zigzag path from bank-to-bank and taking samples at approximately equidistant points along the transect. The number of samples increased with pond size and varied from approximately 10 to 85. If a pond was not at full capacity, pond surface area was estimated at the time of sampling based on 1–5 measurements of length and width using a meter tape. We estimated population size using data on the mean number of larvae per sample (typically based on 30–60 samples per pond), the surface area enclosed by the sampler (0.12 m²), and the surface area of the pond at the time of sampling.

We obtained an initial sample of hatchlings within two weeks after > 95% of the egg masses had hatched

in a pond. In almost all instances, all masses had hatched at the time of sampling. If unhatched masses were present, adjustments to the estimate were made based on the number of masses and estimated embryonic survival per egg mass. These data were expressed as hatchlings per egg mass and were used to assess changes in embryonic survival across years. The estimates of embryonic survival are probably biased because some mortality undoubtedly occurred between the time of hatching and sampling. In addition, some tadpoles may have evaded capture when pushing the sampler into the substrate or sweeping the sampler with nets.

We intensively dip-netted ponds as larvae approached metamorphosis and obtained a final estimate of population size from samples taken immediately after the first metamorphosing larva was observed in each pond. Criteria used to recognize metamorphosing larvae were the emergence of front legs for *R. sylvatica* and the partial or complete atrophy of gills and dorsal fins for *A. maculatum*. We used this sample to approximate the number of juveniles that were recruited into the terrestrial population each year. This estimate is also presumed to be biased because some larvae evaded capture when sampling and additional mortality occurred between the initiation and termination of metamorphosis. We estimated larval survival (hatching to initiation of metamorphosis) using estimates of population size at the time of the first and last seasonal samples.

Statistical Analyses

We analyzed data using Statistical Analysis System (SAS Institute 1989). Because data collected from the same ponds across years may not be statistically independent, we used repeated measures ANOVA to compare survival and juvenile production in constructed and reference ponds. Analyses were performed on ranked data (Proc Rank) because larval survival and juvenile production in many ponds often was zero and the assumption of normality was violated. We examined between-subject effects (whether overall responses differed significantly between reference and constructed ponds for the monitoring period), within-subject effects (year effects), and their interactions. The former tested whether the magnitude of a response variable was constant across years, while the interaction term tested whether patterns across years were similar for constructed versus reference ponds. We gained additional insights into variation among years by using single factor ANOVA to test for year effects within species and either an unpaired *t*-test or Wilcoxon Two-Sample Test (normal approximation) to compare pond types within years. The latter was used when assump-

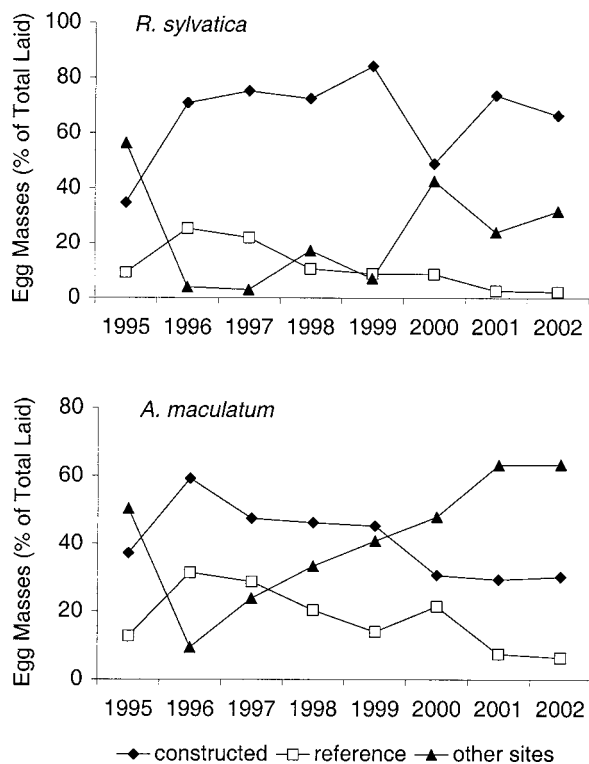


Figure 2. Shifts in relative habitat use based on number of egg masses laid on the eastern sector of the site. Values are expressed as a percentage of all masses in the eastern sector. 'Other' includes all sites other than reference and 1996 constructed ponds. Data for 1995 'constructed' are masses laid in sites that preexisted where ponds were constructed.

tions of parametric tests were violated. We used least-squares linear regression to examine shifts in overall breeding responses to created wetlands.

RESULTS

Use of Constructed Ponds

Rana sylvatica and *A. maculatum* rapidly colonized constructed ponds that first filled in 1996 (occupancy = 100% of all ponds for *R. sylvatica* and 90% of both constructed and reference ponds for *A. maculatum*). During 1995, 35% of *R. sylvatica* egg masses and 37% of *A. maculatum* egg masses in the eastern sector were deposited in the small pools that existed *in situ* prior to pond construction. During 1996, an estimated 71% of *R. sylvatica* and 59% of *A. maculatum* in the eastern sector bred in the constructed ponds (Figure 2). The mean number of *A. maculatum* egg masses in constructed ponds was significantly higher where breeding sites existed *in situ* prior to construction (124 ± 44 and 29 ± 12 masses per pond; $t_8 = -2.51$; $P = 0.04$). A similar trend occurred for *R. sylvatica* (124 ± 0 versus 74 ± 18 masses per pond), but the difference

was not significant ($t_8 = -1.29$; $P = 0.23$). Relative use of reference ponds by both species also increased between 1995 and 1996, but a marked decrease in breeding effort from 56% to 4% occurred in the remaining smaller depressions that were prone to premature drying (Figure 2).

The percentage of females that bred in constructed ponds remained relatively constant from 1996 to 2002 for *R. sylvatica* ($F_{1,6} = 0.45$; $P = 0.53$; $r^2 = 0.08$) but decreased for *A. maculatum* ($F_{1,6} = 38.4$; $P = 0.002$; $r^2 = 0.88$; slope = -4.96). However, a moderate decrease in the use of ponds constructed in 1996 occurred after 1999, as animals shifted to new breeding sites that were formed during stream reconstruction and the partial filling of golf course ponds. In 2000, approximately 42% of *R. sylvatica* and 26% of *A. maculatum* in the eastern sector bred in 21 newly created habitats that first filled in February 2000, a trend that paralleled shifts into constructed ponds in 1996. Pre-existing breeding pools were not present at any of these sites in 1999. A weak continuation of this trend occurred in 2002 when 15% of *R. sylvatica* and 6% of *A. maculatum* bred in several shallow ponds created during the filling of the channelized section of Tulula Creek in 2001.

Premetamorphic Survival and Juvenile Output

For both species, the percentage of constructed and reference ponds that successfully produced juveniles generally decreased over the 6-yr interval (Figure 3; upper panels). The percentage of ponds that produced juvenile *R. sylvatica* decreased greatly from 1996 to 1998 and remained below 35% thereafter. The decrease for *A. maculatum* was more incremental.

The percentage of constructed versus reference ponds that produced juveniles did not differ significantly for *R. sylvatica* over the seven breeding seasons (respective grand means = 34% and 26%; $t_6 = -1.09$; $P = 0.32$). However, a greater percentage of constructed ponds produced juvenile *A. maculatum* compared with reference ponds (respective grand means = 47% versus 24%; $t_6 = -2.83$; $P = 0.03$), in part due to the greater vulnerability of reference ponds to premature pond drying (see below).

Total juvenile production based on estimates of the number of larvae that survived to the initiation of metamorphosis was exceptionally high in constructed ponds during 1996 ($N = 253,696$ for *R. sylvatica*; 30,831 for *A. maculatum*). However, average annual juvenile production of both species decreased markedly from 1996 to 1998 and generally remained low thereafter (Figure 3; lower panels). A similar trend occurred in reference ponds, and in 1999 and 2000, none of the reference ponds produced juveniles.

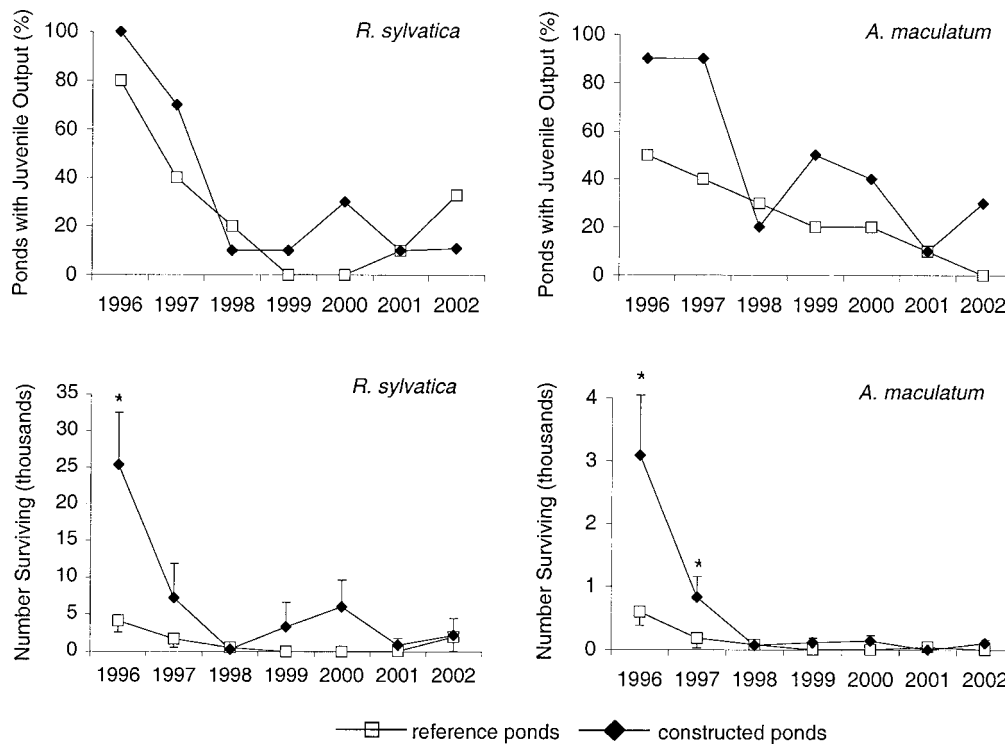


Figure 3. Changes in the percentage of ponds that produced juveniles and estimates of mean juvenile recruitment from 10 constructed and 10 reference ponds from 1996 to 2002. Upper panels are the percentage of ponds that produced juveniles annually; lower panels (means ± 1 SE) are the estimated number of larvae surviving in thousands to the initiation of metamorphosis. Asterisks indicate means that differ significantly based on Wilcoxon tests.

Repeated measures ANOVA indicated that the overall production of juvenile *R. sylvatica* did not differ significantly between constructed and reference ponds (Table 1). The year term was significant, but the year-by-pond interaction was not, indicating that mean pre-metamorphic survival of both groups showed similar

patterns of decline. Overall juvenile production also did not differ significantly between constructed and reference ponds for *A. maculatum* (Table 1), but both the year and interaction term were significant. Within-year comparisons indicate that constructed ponds produced significantly more juveniles than reference

Table 1. Summary statistics for repeated measures ANOVA on four response variables. “Pond type” tests for overall differences (between subject effects) of response variables for constructed versus reference ponds for data collected from 1996 to 2002. “Year” tests for overall changes across years (within subject effects), regardless of pond type. “Type × year” tests whether changes across years were similar for both pond types.

Variable	Source	<i>R. sylvatica</i>		<i>A. maculatum</i>	
		F (df)	P	F (df)	P
Hatchlings per mass	Pond type	1.01 (1, 7)	0.34	16.2 (1, 10)	0.002
	Year	2.10 (6, 42)	0.07	17.8 (6, 60)	<0.0001
	Type × year	0.31 (6, 42)	0.92	3.82 (6, 60)	0.003
Larval survival	Pond type	0.76 (1, 3)	0.44	0.17 (1, 7)	0.70
	Year	1.03 (6, 18)	0.44	2.08 (6, 42)	0.08
	Type × year	0.51 (6, 18)	0.79	1.01 (6, 42)	0.43
Juveniles per mass	Pond type	0.40 (1, 5)	0.55	2.71 (1, 8)	0.14
	Year	1.13 (6, 30)	0.37	2.77 (6, 42)	0.02
	Type × year	1.41 (6, 30)	0.24	1.05 (6, 42)	0.41
Total juvenile output	Pond type	1.96 (1, 15)	0.18	2.33 (1, 15)	0.15
	Year	11.5 (6, 90)	<0.0001	6.10 (6, 90)	<0.0001
	Type × year	1.09 (6, 90)	0.13	2.23 (6, 90)	0.047

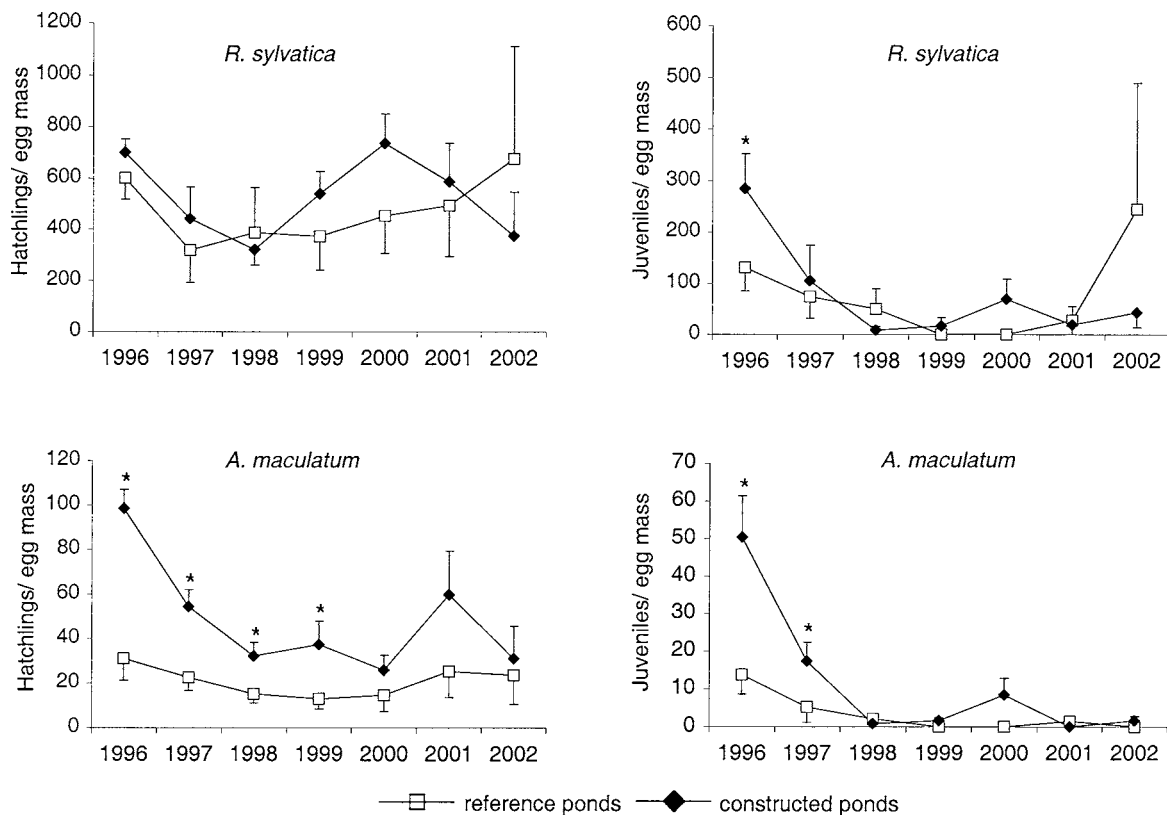


Figure 4. Estimates for number of hatchlings and juveniles produced per egg mass based on yields from open-bottom samplers. Symbols and bars are means \pm 1 SE, and asterisks indicate means that differ significantly based on either *t*-tests (hatchlings) or Wilcoxon tests (juveniles).

ponds only in 1996 ($Z = 2.77$; $P = 0.005$) for *R. sylvatica* and in 1996 ($Z = 2.09$; $P = 0.03$) and 1997 ($Z = 2.28$; $P = 0.02$) for *A. maculatum*.

For *R. sylvatica*, average hatchling production per mass did not differ significantly for constructed and reference ponds for any year (t_{8-18} ; $P > 0.32$) or for the 6-yr period overall (Table 1). The year and year-by-pond interaction terms also were not significant. In contrast, the overall mean hatchling production per mass for *A. maculatum* was greater in constructed ponds (Table 1) and differed significantly in four of seven years (Figure 4). Both the year and year-by-pond interaction were significant.

The significant interaction term for *A. maculatum* hatchling production appeared to be due to relatively large hatchling production in constructed ponds during 1996. An exploratory analysis with ANOVA showed that the difference among years was significant ($F_{6,67} = 4.49$; $P = 0.001$), primarily because of greater production of hatchlings in 1996 relative to 1998–2002 (Tukey's Studentized Range Test). In contrast, hatchling production in reference ponds did not differ among years ($F_{6,50} = 0.70$; $P = 0.83$). With the exception of *A. maculatum* in constructed ponds, these analyses suggest that the marked decrease in estimated

juvenile output from 1996 to 2002 was due primarily to increased larval mortality (Figure 4).

In contrast to hatchling production, juvenile production per egg mass (Figure 4) and larval survival (Figure 5) generally decreased between 1996 and 1999 and remained low thereafter for both species and both sets of ponds. The relatively large value for *R. sylvatica* juvenile production in 2002 for reference ponds has low reliability because of small sample size ($N = 3$ ponds; see discussion of drought below). Overall juvenile production per mass of *R. sylvatica* did not differ significantly among pond types, and neither the year or the year-by-pond interaction terms were significant (Table 1). Despite the overall lack of a significant year effect, more detailed analyses indicate that juvenile production per egg mass differed significantly among years for both constructed ($F_{6,64} = 10.15$; $P < 0.0001$) and reference ($F_{6,47} = 3.36$; $P = 0.009$) ponds. Within-year differences were significant only for 1996 ($Z = 2.02$; $P = 0.04$).

Average juvenile production per mass of *A. maculatum* did not differ significantly among pond types for 1996–2002 (Table 1), but differences were significant in 1996 ($Z = 2.35$; $P = 0.02$) and 1997 ($Z = -2.07$; $P = 0.04$). The effect of year was significant,

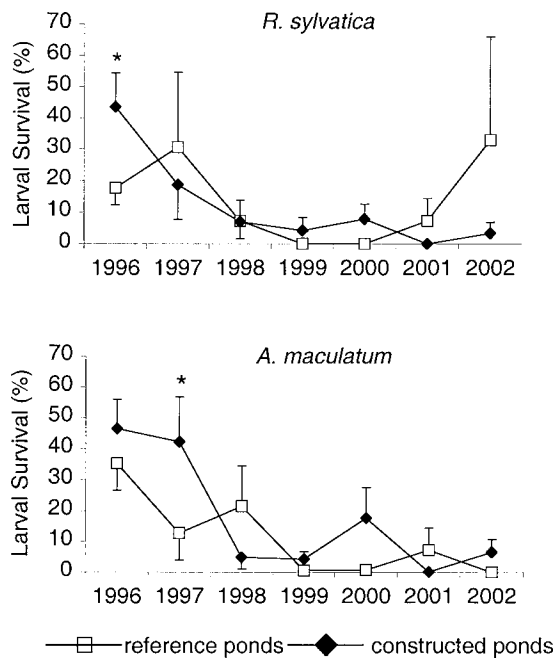


Figure 5. Larval survival for reference and constructed ponds based on population estimates from open-bottom samplers taken shortly after hatching and at the initiation of metamorphosis. Symbols and bars are means \pm 1 SE. Asterisks indicate means that differ significantly based on Wilcoxon tests.

while the interaction term was not (Table 1). Mean production per mass varied significantly among years for constructed ponds ($F_{6,65} = 2.77$; $P < 0.0001$) but not for reference ponds ($F_{6,50} = 2.19$; $P = 0.06$).

Constructed and reference ponds did not differ in overall larval survival from 1996–2002 for either species, and neither the year nor interaction terms were significant (Table 1). However, individual analyses revealed that larval survival differed significantly among years for both species (*R. sylvatica*: $F_{6,99} = 3.36$; $P = 0.005$; *A. maculatum*: $F_{6,108} = 6.11$; $P < 0.001$). Within years, differences were only significant for *R. sylvatica* in 1996 ($P = 0.04$) and *A. maculatum* in 1997 ($P = 0.05$).

Factors Affecting Juvenile Production

The general reduction in juvenile production from 1996 to 2002 is attributable to at least three factors: (1) premature pond drying and/or the failure of ponds to fill seasonally, (2) outbreaks of a pathogen that caused larval die-offs, and (3) the accumulation of predators in constructed ponds after 1996.

The study site received near normal rainfall from 1996 to 1997, but a moderate to severe drought occurred from summer 1998 to 2002. The most extreme monthly deficits occurred primarily during the summer

and fall months. Deficits were not considered to be sufficient to limit breeding activity during January–March (respective monthly averages for Tulula for January, February, and March from 1999–2002 = 12.9, 8.0, and 11.4 cm). Ponds that either did not fill or that filled and dried prematurely from 1996 to 2002 (Figure 6) resulted in either no breeding or 100% mortality of larvae. Constructed ponds filled annually and usually held water for a sufficient duration to allow metamorphosis of both species. Exceptions were 2001 and 2002, when 10–20% of ponds dried prematurely, causing catastrophic mortality.

In contrast, the more shallow reference ponds tended to deteriorate progressively with respect to hydroperiod from 1996 to 2002. During 2002, 43% and 100% of the reference ponds either did not fill or dried prematurely for *R. sylvatica* and *A. maculatum*, respectively. This pattern may reflect both altered hydrology due to ditching and stream channelization and the effects of prolonged drought.

Disease is a second factor that contributed strongly to the decrease in juvenile output from 1996 to 2002. An outbreak of a disease that causes catastrophic larval mortality was first observed in 1997. Detailed pathological studies of specimens collected during die-offs (Green et al. 2002) indicate that a *Ranavirus* (Iridoviridae) was the causal agent. Larvae of both *R. sylvatica* and *A. maculatum* were susceptible to ranaviral infections. Infected larvae were first noticed seasonally during the mid- to latter half of the larval stage, and catastrophic mortality typically occurred within 1 to 3 weeks after the first infected individuals (often with hemorrhaging on the skin) were detected. Outbreaks almost always resulted in catastrophic mortality of larvae in a pond, as indicated by the failure to capture live larvae in bottom samplers or when intensively dip-netting following outbreaks (typically with a team of 2–4 individuals dip-netting for 30–60 min).

Catastrophic die-offs from pathogens were not observed from 1994 to 1996 despite extensive work in the field. Die-offs occurred in two ponds in 1997 and have been a major source of larval mortality in most ponds on site from 1998 to 2002 (Figure 6; lower panel). The smaller percentage of reference ponds with die-offs from 1998 to 2002 reflects the fact that many dried prematurely and caused catastrophic mortality of larvae before the time when the disease normally manifests.

Egg and larval predation was a third source of pre-metamorphic mortality that contributed to the decrease in juvenile output from 1996 to 2002. In particular, egg predation by overwintering green frog tadpoles (*Rana clamitans* Latreille) on *R. sylvatica*, and *R. sylvatica* tadpoles on *A. maculatum* were significant sources of mortality in certain ponds. We commonly

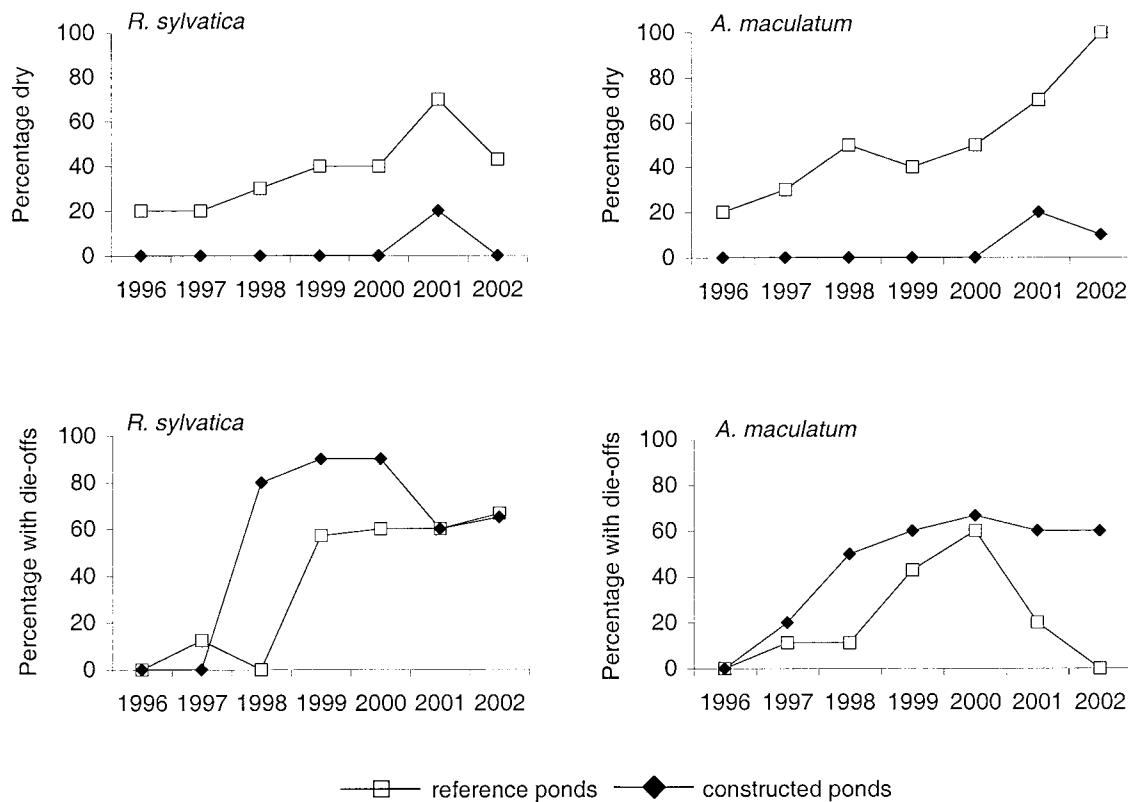


Figure 6. Changes in the percentages of reference and constructed ponds that either did not fill seasonally or that dried before larvae could metamorphose (upper panels) and the percentage of populations with catastrophic die-offs of larvae from ranaviral infections (lower panels).

observed *R. sylvatica* tadpoles preying on *A. maculatum* embryos in the shallower reference ponds but less so in the deeper constructed ponds. This may in part explain lower embryonic survival in reference ponds (Figure 4) compared with constructed ponds. Overwintering odonates and tadpoles appeared in some constructed ponds after 1996 and presumably contributed to higher larval mortality in the years after the first filling.

Overall Response of Populations to Pond Creation

Relative changes in breeding population size of *R. sylvatica* and *A. maculatum* in the eastern sector, as inferred from egg mass counts, are shown in Figure 7. Female *R. sylvatica* in Appalachian populations require 3–4 years to reach sexual maturity after metamorphosing. Thus, multiyear time lags occur between changes in annual metamorph production and corresponding changes in adult population size. The *R. sylvatica* population decreased from 1995 to 1998, increased dramatically (422%) from 1998 to 2000, and decreased thereafter to 2002 levels that are slightly below levels observed in 1995 immediately prior to pond construction. The breeding population of *A. macula-*

tum did not fluctuate as markedly. The population decreased from 1995 to 1997, slowly increased from 1997 to 2001, and decreased to 2002 levels that are slightly lower than numbers recorded in 1995.

DISCUSSION

Wetland losses in the United States are often mitigated by the creation of compensatory banks of restored or created natural wetlands. Mitigation banks allow the consolidation of mitigation for small wetland losses and enhance the monitoring and evaluation of mitigation projects (Marsh et al. 1996). However, significant failure rates and the difficulty of assessing mitigation success have caused many to question the extent to which mitigation sites adequately substitute for the loss of natural wetlands (Mitsch and Wilson 1996, Zedler 1996, Whigham 1999, Brown and Veneman 2001, National Resource Council 2001). A related issue is whether monitoring for ≤ 5 yr is sufficient to assess population responses, particularly for species with prolonged immature stages.

Amphibian ecologists have mostly used data on communities that assemble within a few years of pond construction to assess mitigation success (e.g., Mier-

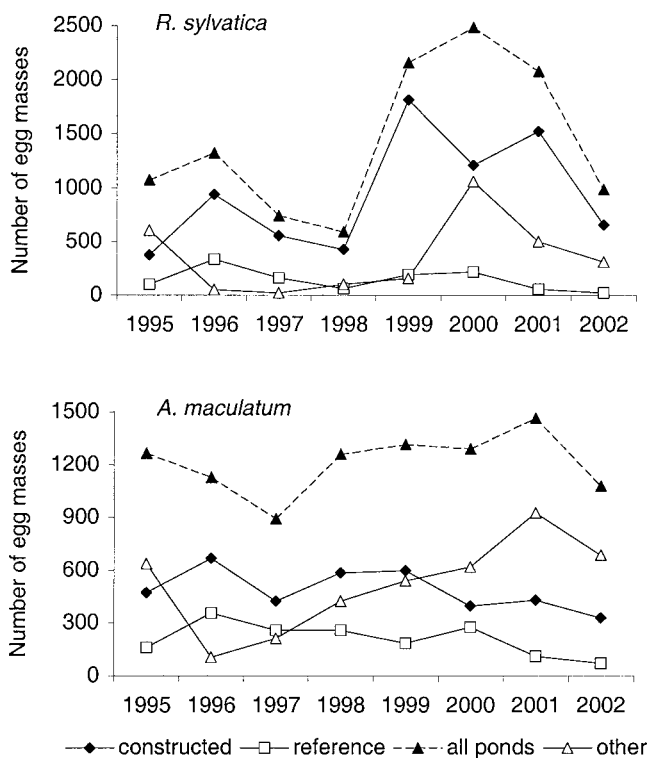


Figure 7. Changes in relative breeding population size based on egg mass counts in the eastern sector. “All ponds” = the total count of egg masses on the eastern sector; “other ponds” = all breeding sites other than reference ponds and the 1996 constructed ponds.

zwa 2000, Pollet and Bendell-Young 2000, Lehtinen and Galatowitsch 2001, Stevens et al. 2002). A limitation of these short-term approaches is that they do not directly measure demographic responses and do not provide compelling evidence that depleted populations will rebound to viable levels. At our study site, many animals abandoned their natal ponds in favor of newly constructed ponds. Because metamorphs of *R. sylvatica* and *A. maculatum* require > 2 yr to reach sexual maturity, the rapid colonization of created ponds reflected local habitat shifting rather than an increase in site-wide population size. In fact, the overall population of both species decreased during the first two years following pond construction.

Researchers have rarely conducted longer-term studies that are more likely to yield information on population resilience and viability, even though this information is critical for setting realistic time frames and criteria for assessing restoration success. Studies that exceed the 5-yr post-restoration standard may not be a panacea for resolving assessment issues, but they do provide insights into demographic responses and whether additional years of monitoring can markedly alter conclusions.

This project is a good example of how environmen-

tal perturbations can alter population trajectories and hamper assessing whether goals were achieved. Our primary goals were (1) to create larger breeding habitats with longer hydroperiods, (2) to increase juvenile production, and (3) to increase adult population sizes to levels that would enhance the long-term viability of resident species. Data collected from 1996 to 2002 indicate that the constructed ponds are far less prone to premature drying than either reference ponds or the remaining smaller habitats on site. With regard to providing breeding sites with improved seasonal hydrology, we consider goal (1) to have been met regardless of the time frame in which the response variable was measured. The extent to which the remaining goals were met was time-sensitive. Prior to the widespread occurrence of ranaviral infections in 1998, estimated juvenile output in constructed ponds was more than five times that of reference ponds for both species. The breeding population of *R. sylvatica* increased 422% from 1998 to 2000, indicating that juvenile production far exceeded replacement values. The *A. maculatum* population was more stable due to reproductive lags but increased 14% between 1996 and 2000. Based on a 5-yr analysis (1996–2000), we conclude that we successfully met goals (2) and (3).

In contrast, much uncertainty exists when the analysis is extended to seven years of post-construction monitoring. During the 7-yr interval (1996–2002), juvenile output did not differ significantly between constructed and reference ponds, and the breeding population of both species was slightly smaller in 2002 than in 1995 prior to pond creation. This reflects a general long-term decrease in larval survival and juvenile output, primarily due to the onset of ranaviral infections in 1998. When measured over this time interval, goals (2) and (3) were not achieved.

One of the most important goals of restoration ecology is to improve the structural and functional elements of ecosystems to levels that sustain viable populations of community members. Assessing this aspect of restoration is frequently difficult for vertebrates because populations are prone to stochastic events that generate variation that mask underlying population trends (Thomas 1997, Gibbs et al. 1998). We currently do not know whether pond-breeding amphibians undergo population cycles with predictable periodicity, a pattern that would further complicate assessing long-term population responses to mitigation. However, power analyses suggest that a decade or more may be required to detect moderate population trends in local pond populations of many amphibians (Gibbs et al. 1998). When planning monitoring duration for amphibians, researchers need to consider the basic demographic unit (e.g., a single pond or a cluster of local ponds) that will be used to assess population respons-

es, since pooling reduces non-trend variation. We used the grand mean from many local ponds to examine breeding population size, a procedure that should greatly increase the power of detecting true population trends.

Changes in the population size of *R. sylvatica* were attributable to changes in juvenile output between 1996 and 2002, particularly when assuming a 3-yr or 4-yr lag between the time a female metamorphoses and first returns to breed (Berven 1982, 1995). The population decline from 1995 to 1998 may reflect low juvenile recruitment due to premature pond drying in the years prior to the construction of deeper and more permanent ponds on site. The marked increase in population size in 1999–2000 corresponds to when the large output of juveniles in 1996 and 1997 first returned to breed as adults. The decline since 2000 presumably reflects low juvenile recruitment that has occurred since 1998 due to ranaviral outbreaks and premature pond drying from drought.

Adult *R. sylvatica* are short-lived and rarely breed more than twice, which may explain the abrupt changes in population size that occurred over 2–3 year intervals. Female *A. maculatum* may require 3–5 yr to reach sexual maturity, and individuals may live as long as 32 years in nature (Petranka 1998). Yearly survivorship often exceeds 70–90% in local populations, and adults may breed several times during their lifetime. The *A. maculatum* population showed far greater resilience to site perturbations than *R. sylvatica*, but damped patterns that parallel those seen in *R. sylvatica* were evident when lags of 3–5 yr between metamorphosis and first reproduction are considered. The gradual increase in breeding population size may reflect recruitment from the relatively large output of juveniles in 1996 and 1997. The decline in 2002 presumably reflects the long-term impact of egg predation from *R. sylvatica* tadpoles (Petranka et al. 1998), and drought and ranaviral outbreaks that began in 1997–1998. The overall stability of the breeding population from 1995 to 2002 may reflect selection for longevity and iteroparity as an adaptation to living in environments that result in high juvenile mortality and boom-and-bust reproductive patterns (e.g., Gill 1978, Berven 1995, Semlitsch et al. 1996, Pechmann et al. 2001).

Hydrologic and biotic perturbations occur frequently in wetlands (e.g., Berven 1995, Semlitsch et al. 1996, Babbitt and Tanner 2000) and are of particular concern at mitigation sites that tend to be geographically isolated from adjoining wetlands. For obligate wetland species with low vagility, geographic isolation may result in low population connectivity at regional scales. Of particular concern for amphibians is the fact that regional recolonization will not follow local extinction events and that isolated wetlands will undergo

progressive decline in species diversity and community complexity (Gibbs 1993). Because small populations are more vulnerable to local extinction, the creation of large mitigation banks with arrays of ponds may be the best strategy for preserving wetland biodiversity in the southern Appalachian Mountains where geographic isolation is common.

An assumption in restoring amphibian habitats at the Tulula Wetlands was that the site would have little connectivity to adjoining wetlands and must function largely as a self-sustaining unit. A major goal was to create sufficient breeding habitat to maintain populations of seasonal pond breeders at sustainable levels. Unfortunately, the critical minimum population size needed to ensure the long-term viability of amphibians inhabiting isolated wetlands is poorly resolved. Egg mass censuses from 1993 to 2002 of *R. sylvatica* and *A. maculatum* in eastern Tennessee, southeastern Kentucky, and western North Carolina (C. K. Smith and J. W. Petranka, unpublished data) indicate that annual breeding effort within isolated local clusters of vernal ponds rarely exceeds 500 masses for *R. sylvatica* and 1200 masses for *A. maculatum*. Egg mass censuses in the eastern sector of Tulula alone have equaled or exceeded these values in most years, suggesting that sufficient habitat has been created to maintain viable populations of these species, barring the continued impact of ranaviral infections.

Ranaviruses (Family: Iridoviridae) are an emerging group of pathogens that often cause catastrophic mortality in populations of fish, amphibians and reptiles. Amphibian mortality events have been investigated and reported in many areas of North America and Europe (Daszak et al. 1999), but there is limited understanding of the epizootiology of the disease (Green et al. 2002). Ranaviral infections dramatically reduced juvenile recruitment of *R. sylvatica* and *A. maculatum* from constructed and reference ponds since 1998, but the long-term impact on these species is not yet clear. One scenario is that the severity of the die-offs will decrease with time as populations evolve immunity or as the virus disappears or evolves into an attenuated, non-lethal form. A second scenario is that the virus will be a persistent source of annual die-offs in most or all ponds. The latter could eventually cause site-wide population bottlenecks or local extinctions.

We varied the depth, size, and spatial arrangement of ponds at the Tulula Wetlands in an attempt to impart a degree of demographic independence in local pond populations. Despite the widespread occurrence of ranaviral infections in constructed and reference ponds, a small percentage of ponds have successfully produced juveniles in most years. Whether our attempts to restore degraded wetlands will be sufficient to maintain populations above pre-restoration levels or

to prevent population bottlenecks can only be determined through additional years of monitoring. Regardless of the outcome, we are in agreement with Pechmann *et al.* (2001) that monitoring that exceeds five years may be required to meaningfully assess the demographic responses of amphibians to compensatory mitigation.

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