
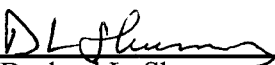


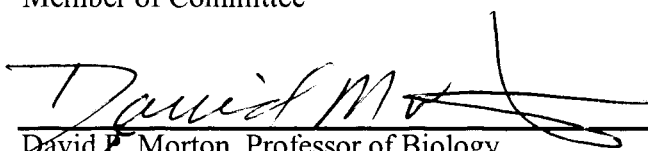
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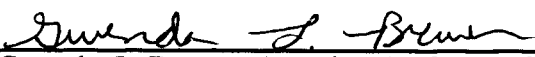
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**Colonization of an Artificial Wetland by Amphibians at Rocky Gap
State Park, Maryland**

**A Thesis in
Applied Ecology and Conservation Biology**

By

James T. Julian

**Submitted in Partial Fulfillment
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for the Degree of
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ABSTRACT

I conducted a three-year amphibian translocation and monitoring project at Rocky Gap State Park (RGSP), Allegany Co., Maryland. The study area consisted of a small artificial wetland that contained six ponds of similar surface areas but of various depths. The wetland was designed to accommodate species of anurans with a wide range of life history strategies by containing bodies of water with various hydrologic regimes. Egg masses of *Ambystoma jeffersonianum* and *Pseudacris triseriata* were translocated into ponds at RGSP in 1998 and 1999. I quantified initial aspects of the success of hatching and metamorphosis for translocated populations and examined the possible effects of pond depth and wetland characteristics on measures of success. Hatching successes for translocated *P. triseriata* and *A. jeffersonianum* were > 86% and 93%, respectively. The mean percent of translocated *A. jeffersonianum* hatchlings that completed metamorphosis was higher in 1998 (14.6%) than 1999 (1.6%). Wetland characteristics affected hatching success for both species in 1998, and an interaction between pond depth and wetland characteristics affected hatching for *P. triseriata* in 1999 and the success of metamorphosis for *A. jeffersonianum* in 1998. Measures of success for translocated populations were comparable to natural populations. Adult *P. triseriata* returned to the wetland in 1999 and laid at least two egg masses in ponds. I also monitored the wetland at RGSP for the initial stages of colonization by anurans. I predicted that anuran species that bred in the spring would use shallower bodies of water (hydrologic classes) for breeding in greater proportions than species that bred in the summer. Anuran species did not deposit significantly different densities of eggs among hydrologic classes, nor did the

total anuran egg densities differ among classes. In 1998, total anuran egg densities were higher in created wetland areas than in an adjacent, existing wetland area. Some trends suggest that *Bufo americanus* and *Rana sylvatica* (both spring-breeding species) laid higher egg densities in shallow hydrologic classes than *Rana clamitans* and *Hyla versicolor* (both summer-breeding species), but considerable variation among replicates prevented statistical analysis from detecting a hydrologic class by species interaction. The results of this research indicate that the effect of pond depth on the success of hatching and metamorphosis will differ between wetland habitats, and trends suggest a species by depth interaction could affect the choice of sites for ovoposition in anurans. Although large, permanent bodies of water can provide habitat for substantial populations of larval amphibians, these bodies of water may become dominated by relatively few amphibian species (namely large anuran species and *Notophthalmus viridescens*). Artificial wetlands with both permanent and ephemeral bodies of water would provide habitats suitable for many species of amphibians and could increase both species richness and evenness components of diversity.

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TABLE OF CONTENTS

	Page
Abstract.....	iii
Acknowledgments.....	v
List of Tables.....	viii
List of Figures.....	x
List of Appendices.....	xi
Chapter 1: Literature Review	
Introduction.....	12
Amphibian importance and declines.....	12
Amphibian colonization.....	13
Amphibian relocation, repatriation, and translocation programs.....	15
Amphibian species at Rocky Gap State Park.....	16
Research needs.....	18
Artificial/restored wetland design.....	18
Amphibian RRT programs.....	19
Rocky Gap State Park created wetland and amphibian RRT program.....	21
Literature cited.....	22
Chapter 2: Translocation of Amphibian Eggs into an Artificial Wetland	
Abstract.....	29
Introduction.....	30
Methods.....	32
Evaluation of Translocation Site	32
Design and Preparation of Study Site	32
Source Populations for Translocations.....	34
Translocation Protocol.....	34

Statistical Analysis.....	37
Results.....	40
1998 Translocations.....	40
1999 Translocations.....	41
Source Population of <i>A. jeffersonianum</i>	43
Discussion.....	44
Literature Cited.....	49
Chapter 3: Hydrologic regimes and anuran colonization in an artificial wetland	
Abstract.....	65
Introduction.....	66
Methods.....	69
Study Site.....	69
Colonization Monitoring Protocol.....	70
Statistical Analysis.....	73
Results.....	74
Discussion.....	75
Literature Cited.....	78
Chapter 4: Conclusion.....	93
Literature Cited.....	94

LIST OF TABLES

	Page
Chapter 1. Literature Review	
Table 1. Examples of amphibian relocation, repatriation, and translocation programs....	28
 Chapter 2. The Translocation of Amphibian Eggs into an Artificial Wetland.	
Table 1. Examples of amphibian relocation, repatriation, and translocation programs....	53
Table 2. Hatching success of translocated <i>Pseudacris triseriata</i>	54
Table 3. Measures of hatching success for translocated and source populations of <i>Ambystoma jeffersonianum</i> in 1998.....	55
Table 4. Hatching success of translocated <i>Ambystoma jeffersonianum</i>	56
Table 5. Measures of success of metamorphosis for translocated and source populations of <i>Ambystoma jeffersonianum</i>	57
Table 6. Measures of metamorphosis success of translocated <i>Ambystoma jeffersonianum</i>	58
 Chapter 3. Hydrologic Regimes and Anuran Colonization in an Artificial Wetland.	
Table 1. Mean egg densities of all anuran species by wetland area for 1998 and 1999.....	84

Table 2.	
Egg densities (eggs/m ²) among individual ponds in 1998 and 1999.....	85

LIST OF FIGURES

	Page
Chapter 2. The Translocation of Amphibian Eggs into an Artificial Wetland.	
Figure 1. Map of experimental wetland at Rocky Gap State Park, Allegany Co., Maryland.....	59
Figure 2. <i>Ambystoma jeffersonianum</i> metamorphosis success 1998.....	60
Figure 3. <i>Pseudacris triseriata</i> hatching success 1999.....	61
Chapter 3. Hydrologic Regimes and Anuran Colonization in an Artificial Wetland.	
Figure 1. Map of experimental wetland at Rocky Gap State Park, Allegany Co., Maryland.....	86
Figure 2. Breeding phenology of amphibians in experimental wetland at RGSP.....	87
Figure 3. Egg densities for hydrologic classes in 1998.....	88
Figure 4. Egg densities for hydrologic classes in 1999.....	89

LIST OF APPENDICES

	Page
Chapter 2. The Translocation of Amphibian Eggs into an Artificial Wetland.	
Appendix 1.	
Distribution of egg masses and egg portions into ponds at RGSP.....	62
Appendix 2.	
Depth and thermal profiles for ponds at RGSP in 1998 and 1999.....	63
Appendix 3.	
Most abundant groundcover species by wetland at RGSP in 1999.....	64
Chapter 3. Hydrologic Regimes and Anuran Colonization in an Artificial Wetland.	
Appendix 1.	
Total surface area available to amphibians by breeding season, hydrologic class, and wetland area.....	90
Appendix 2.	
Most abundant groundcover species by wetland at RGSP in 1999.....	91
Appendix 3.	
Depth and thermal profiles for ponds at RGSP in 1998 and 1999.....	92

CHAPTER 1: LITERATURE REVIEW

INTRODUCTION

Amphibian Importance and Declines

Amphibians play an integral role in both aquatic and terrestrial ecosystems and represent a majority of the biomass in some vertebrate communities (Burton and Likens 1975). Amphibians are important both as predators in controlling populations of invertebrates and as prey in moving energy to higher trophic levels. Amphibians are also excellent indicators of ecosystem health (Chovanec 1994). Amphibians are especially sensitive to environmental changes because they have highly permeable skin, biphasic life histories, and display philopatric and homing behaviors.

Declines in the abundance of amphibians have been reported worldwide (Barinaga 1990; Drost and Fellers 1996; Phillips 1990). Investigators have reported declines in the abundance of individual populations (Crump et al. 1992) and regional declines in both abundance and species richness (Drost and Fellers 1996). The proximal causes of declines for many populations of amphibians are unknown, although some declines are attributed to the development of wetlands by humans. Alteration of the hydrology of wetlands that act as dispersal corridors can result in the extirpation of some populations of amphibians (Delis et al. 1996). State and federal regulations now mandate that the loss of wetland acreage from development be mitigated through the creation of artificial wetlands, although most states have already lost a majority of their pre-existing wetlands.

Newly created and restored wetlands can provide unique opportunities for pro-active conservation of amphibians. The hydrology of created and restored wetlands can be modified to accommodate life history characteristics of desired species of amphibians if investigators possess an understanding of the natural history of amphibians. In addition, in-situ design of wetlands can be coupled with well-planned programs of amphibian relocation, repatriation, and translocation to increase the probability of colonization.

Amphibian Colonization

Investigators have examined amphibian autecology in light of principles of landscape ecology and metapopulation dynamics to predict colonization of potential breeding habitats. Amphibian colonization is strongly affected by distances to established breeding habitats and characteristics of dispersal corridors that link suitable breeding habitats to each other (Bradford et al. 1993; Delis et al. 1996; Gibbs 1998; Laan and Verbloom 1990; Petranka 1983; Rosenberg et al. 1997; Salonen and Edenhamn 1995; Sjögren 1995; Vos Claire and Stumpel 1996). Physical characteristics of a wetland will have a significant impact on the assemblage of species that breed in them (Gates and Thompson 1981; Resetarits and Wilbur 1991; Thompson et al. 1980), the survival of amphibian larvae through metamorphosis (Clark 1986; Cortwright 1997; Roth and Jackson 1987; Sadinski 1991; Skelly 1996; Smith 1983), and the survival of metamorphs to adulthood (Cortwright 1993).

The presence of aquatic predators affects selection of breeding habitats by adults, competitive outcomes among larvae, larval survival, and the persistence of populations. Laboratory and field experiments have shown that several species of amphibians clearly avoid depositing eggs in bodies of water that contain aquatic predators such as fish and

salamander larvae (Kats and Sih 1992; Petranka et al. 1987; Resetarits and Wilbur 1991). Aquatic predators affect the survival of larval amphibians not only through predation but also by altering the behavior of larvae (Holomuzki 1986; Semlitsch 1987; Stangel and Semlitsch 1987), reducing food resources, and by altering interspecific competitive outcomes among larvae (Alford 1989; Morin 1983). The cumulative effects of aquatic predators are believed to have a strong effect on amphibian assemblages because several investigators have correlated the presence of aquatic predators with the distribution of several species of amphibians (Bradford et al. 1993; Monello and Wright 1999).

Hydrologic regimes have a strong effect on assemblages of aquatic predators that, in turn, affect assemblages of larval amphibians. The presence and density of predators increases as a function of pond depth (Skelly 1996; Woodward 1983). In addition, species of predators associated with permanent bodies of water appear to be highly effective at exploiting larval amphibians (Caldwell et al. 1980; Heyer et al. 1975), especially small-bodied species that typically breed in ephemeral waters (Woodward 1983). Deep/permanent bodies of water are expected to provide conditions that favor the reproductive success of large-bodied species of amphibians because these species produce larger clutches of eggs than smaller species, and behaviors of their larvae (such as foraging strategies) make them less susceptible to predation. Furthermore, predation by large anurans (notably the bullfrog, *Rana catesbeiana* Shaw) has been implicated in the reduction and disappearance of many populations of small-bodied anurans (Christiansen and Bailey 1991; Hayes and Jennings 1986; Lanno et al. 1985; Maunder 1983; Oldham 1992).

Hydrologic regimes also control the duration for which a breeding habitat retains water. This is important in determining assemblages of larval amphibians because not all species of amphibians have larvae that develop at the same rate. The larvae of large anuran species can require at least one overwintering period in temperate climates to complete metamorphosis; therefore, adults prefer to breed in large, permanent bodies of water. The larvae of many small anuran species, however, have faster developmental rates; therefore adults prefer to breed in shallower, ephemeral bodies of water that typically have small populations of aquatic predators.

Amphibian Relocation, Repatriation and Translocation Programs

Programs of amphibian relocation, repatriation, and translocation (RRT programs) have received mixed reviews in the herpetological community. Some investigators strongly oppose RRT programs (Dodd and Seigel 1991), whereas others believe that the establishment of captive breeding populations for future reintroduction is the most appropriate way to conserve some amphibians (Goncharov et al. 1989). Relocation, repatriation, and translocation programs have been successful for many amphibian populations when individuals from early life stages are introduced (Table 1). The following criteria must be met before an RRT program is initiated for any amphibian population: selection of the release site, the appropriateness of the source population (life history and genetic considerations), transport and housing conditions for specimens, long-term monitoring of the population with clear objectives to evaluate success of the project, and an experimental design that allows for statistical analysis to test the efficacy of the project (Hein 1997; Reinert 1991).

Amphibians Species at Rocky Gap State Park

Several breeding habitats of amphibians were located within 1 km of an artificial wetland at Rocky Gap State Park (RGSP) in Allegany County, Maryland. The wood frog (*Rana sylvatica* LeConte), American toad (*Bufo americanus* Holbrook), spring peeper (*Pseudacris crucifer* Weid-Neuwied), green frog (*Rana clamitans* Latreille), and gray treefrog (*Hyla versicolor* LeConte) are anurans that were expected to colonize the artificial wetland naturally at RGSP. In addition, the Jefferson salamander (*Ambystoma jeffersonianum* Green) and the upland chorus frog (*Pseudacris triseriata* Baird) were translocated to the artificial wetland at RGSP. These two species are native to western Maryland, yet were absent from RGSP before translocations began (Conant and Collins 1991; Thompson et al. 1980). The Maryland Department of Natural Resources (1997) lists *Ambystoma jeffersonianum* as a rare to uncommon species within the state, and *P. triseriata feriarum* is thought to be in decline (S. Smith pers. comm.).

Rana sylvatica are moderately-sized anurans (snout-vent length=3.5– 7.0 cm) and are typically the first anuran species of the year to breed in western Maryland (March or April). Breeding in *R. sylvatica* is highly synchronous (Conant and Collins 1991) and egg laying for individual populations will typically last less than one week. Many populations of *R. sylvatica* breed in shallow bodies of water that dry out in the summer, although some populations use permanent bodies of water for breeding.

Bufo americanus is a moderate to large-sized anuran (5.1–8.9 cm) that breeds in April and May in western Maryland. In many populations of *B. americanus*, individuals will migrate en mass to breeding habitats on rainy spring nights and deposit eggs in depressions that temporarily fill with rainwater (Conant and Collins 1991). Larvae can

complete metamorphosis before these bodies of water dry completely because of their rapid rate of development. Other populations of *B. americanus* will breed in a variety of permanent bodies of water from which males can be heard calling for several weeks.

Pseudacris crucifer are semiariboreal, small-bodied anurans (1.9–3.2 cm) that breed in April and May in western Maryland. Males call throughout the breeding season, and populations will breed in both ephemeral and permanent bodies of water that have low-lying vegetation nearby (Conant and Collins 1991). Larvae of *P. crucifer* can complete metamorphosis in less than 10 weeks, and adults can be found in woodlots, wetlands, and overgrown fields during nonbreeding seasons.

Rana clamitans is a large anuran (5.7–8.9 cm) that breeds in the summer throughout its range. Most adult *R. clamitans* in the northern United States breed in a variety of permanent bodies of water because their larvae typically require overwintering to complete metamorphosis (Conant and Collins 1991).

Hyla versicolor is a highly arboreal anuran of moderate size (3.2–5.1 cm) that breeds throughout the summer in western Maryland. Adult *H. versicolor* breed in shallow, standing bodies of water near wooded areas (Conant and Collins 1991). Larvae that hatch early in the breeding season can complete metamorphosis in some ephemeral bodies of water, but larvae that hatch late in the season overwinter in bodies of water that persist year-round.

Ambystoma jeffersonianum is a relatively large salamander (total length 10.7 – 18.0 cm) that spends the majority of its life burrowed underground in forested habitats. Adults surface during mass breeding migrations that begin within one or two weeks of the winter's final snowmelt (usually March in western Maryland). Habitats used for

breeding are often ephemeral bodies of water adjacent to woods that contain a variety of submerged and emergent vegetation (Thompson et al. 1980). Females deposit eggs on submerged sticks, branches, or other coarse vegetation, and their carnivorous larvae complete metamorphosis in May and June (depending on local environmental conditions). Juveniles disperse into the surrounding woods upon completing metamorphosis, commence a fossorial lifestyle, and take several years to reach sexual maturity.

Pseudacris triseriata are small anurans (1.9-3.5 cm) that breed in the early spring in western Maryland (March or April). Adults typically breed in bodies of water located in wetlands, moist woodlands, temporary fields, and agricultural fields near woodlands (Conant and Collins 1991). Females typically deposit eggs in ephemeral bodies of water, although some populations will breed in permanent bodies of water (Skelly 1996; Smith 1983). Larvae of *P. triseriata* can complete metamorphosis in less than eight weeks despite developing in relatively cold, spring-time waters.

RESEARCH NEEDS

Artificial/Restored Wetland Design

Mitigation efforts that involve the creation and restoration of wetlands can provide valuable opportunities to evaluate conservation strategies aimed at ameliorating losses in amphibian biodiversity. Two conservation strategies for amphibian populations that can be accommodated through the design of created and restored wetlands are the creation of breeding habitats with heterogeneous hydrologic regimes and the implementation of well-designed amphibian RRT programs.

The abundance of aquatic predators and the duration for which a breeding habitat retains water are two important variables for assemblages of larval amphibians that are strongly influenced by hydrologic regimes. A wetland with only large, permanent bodies of water (typical of many artificial wetlands) may prevent successful colonization by many species of amphibians whose larvae survive and compete best in smaller, ephemeral bodies of water. In addition, wetlands containing only large bodies of water can further act as sink habitats for populations of small-bodied anurans by harboring substantial populations of large-bodied species of anurans or other predators. In contrast, a wetland that only contains ephemeral bodies of water can prevent successful colonization by species that breed in the late spring or summer months because aquatic habitats will not persist long enough for their larvae to complete metamorphosis.

I hypothesize that a wetland with both permanent and ephemeral bodies of water can accommodate a diverse assemblage of amphibian species because of the interaction between hydrologic regimes and the requirements of larvae from different amphibian species. Large expanses of multiple wetlands may be necessary to test this hypothesis, although projects of much smaller scale could provide valuable insight into colonization by amphibians. Wetlands less than 1 ha in size can support experiments on the effects of hydrologic regimes on preferences for breeding habitat and the relative success of several species of amphibians.

Amphibian RRT Programs

Site selection for most amphibian RRT programs can be criticized for lacking objective scrutiny. The selection of release sites often relies upon the subjective opinion of investigators because there is a lack of information available on historic geographical

distributions and autecological studies for many amphibian species. Potential release sites must be evaluated for suitable habitat and potential biotic interactions (i.e. predation and competition) that might impair the establishment of target species, regardless of the process used in release site selection. Target release sites should also be evaluated for their proximity to additional potential breeding habitats that are accessible to amphibians via dispersal corridors.

The choice of a source population needs to consider the location of that population in relation to the target release site. The source population needs to be in close geographic proximity and be subject to similar geographic conditions as the release site because some amphibian species show tremendous variation in habitat selection and breeding season chronology across their range (Conant and Collins 1991). Translocated individuals should be from early developmental stages because studies suggest a relationship between the developmental stage of amphibian individuals released and successful RRTs (Bloxam and Tonge 1995; Maunder 1983; Reinert 1991; Table 1). Introduction of individuals in either egg, larval, or juvenile stages is often successful and is attributed to the development of philopatric behavior in amphibians (Gill 1978; Berven and Grudzien 1990). In contrast, translocated adults may disperse to seek their original breeding locality. Genetic considerations for choosing a source population may prove to be difficult because there is a lack of information available on amphibian genetics. Dodd and Seigel (1991) suggest that investigators should collect baseline genetic information on populations to determine if there are populations with unique genetic material that deserve special consideration for conservation.

Precautions are necessary to prevent the spread of pathogens such as trematode eggs and water mold (*Saprolegnia* spp.). These common pathogens can cause deformities (Sessions and Ruth 1990) and lower hatching success (Blaustein and Wake 1995).

All RRT projects need to be monitored, evaluated, and documented, including small and unsuccessful ones (Reinert 1991). Translocated egg masses can be monitored for hatching success, and larvae can be monitored for their presence or absence, mortality rates, and the percentage of individuals that reach metamorphosis. Adults can be monitored for the number of individuals that return to breed as a result of translocations in previous seasons and for reproductive output.

Rocky Gap State Park Created Wetland and Amphibian RRT Program

I conducted a three-year amphibian monitoring and translocation project at Rocky Gap State Park (RGSP) in Allegany County, Maryland. The study area consisted of a small artificial wetland with six ponds of similar surface areas but of various depths. The wetland was designed to maximize amphibian diversity by providing a variety of habitats for breeding. In addition, I translocated juveniles and egg masses of *A. jeffersonianum* and *P. triseriata* into the experimental wetland to establish breeding populations. My research objectives were to determine if amphibian species differentially colonized bodies of water in the experimental wetland according to depth characteristics and to evaluate the success of the amphibian RRT program. Ultimately, I hoped that the design of the wetland, coupled with the RRT program, would facilitate colonization by a diverse assemblage of amphibian species, and colonizing species would establish self-perpetuating populations.

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Table 1: Examples of amphibian relocation, repatriation, and translocation programs.

Species	Location	Life Stage	Results	Investigator
<i>Rana sylvatica</i>	Missouri	Eggs	Large breeding colony established within 5 years	Sexton and Phillips 1986
<i>Ambystoma tigrinum</i>	New Jersey	Eggs	Successful repatriation of breeding site	Zappalorti unpubl.
<i>Ambystoma maculatum</i>	Missouri	Eggs and Larvae	Individuals observed in almost all subsequent years (1965-1986)	Sexton and Phillips 1986
<i>Bufo calamita</i>	England	Larvae	Failure – Unknown reasons	Beebee 1983
<i>Pseudacris triseriata</i>	Newfoundland	Larvae	Multiple breeding populations established with single translocations of 75 or fewer tadpoles	Maunder 1983
<i>Pelobates syriacus</i>	USSR	Juveniles	Recaptured individuals in one of three locations of release.	Goncharov et al. 1989
<i>Triturus vittatus</i>	USSR	Juveniles	Successful reproduction by released individuals	Goncharov et al. 1989
<i>Peltophyrne lemur</i>	Puerto Rico	Juvenile and Adults	No follow-up reports after initial release	Miller 1985
<i>Plethodon idahoensis</i>	Montana	Adults?	Unknown	Anonymous 1990

CHAPTER 2. TRANSLOCATION OF AMPHIBIAN EGGS INTO AN ARTIFICIAL WETLAND

ABSTRACT

Egg masses of *Ambystoma jeffersonianum* and *Pseudacris triseriata* were translocated into a small artificial wetland at Rocky Gap State Park, Allegany Co., Maryland in 1998 and 1999. I quantified initial aspects of the success of hatching and metamorphosis for translocated populations and examined the possible effects of pond depth and wetland characteristics on measures of success. Hatching successes for translocated *P. triseriata* and *A. jeffersonianum* were > 86% and 93%, respectively. The mean percent of translocated *A. jeffersonianum* hatchlings that completed metamorphosis was higher in 1998 (14.6%) than 1999 (1.6%). Wetland characteristics affected hatching success for both species in 1998, and an interaction between pond depth and wetland characteristics affected hatching for *P. triseriata* in 1999 and the success of metamorphosis for *A. jeffersonianum* in 1998. Measures of success for translocated populations were comparable to natural populations. Adult *P. triseriata* returned to the wetland in 1999 and laid at least two egg masses in ponds.

INTRODUCTION

Declines in amphibian abundance have been reported worldwide (Barinaga 1990; Drost and Fellers 1996; Phillips 1990), although the proximal cause(s) of many of these declines are unknown. Some investigators have taken pro-active measures to ameliorate these declines through relocation, repatriation, and translocation (RRT) programs, even though this approach has received mixed reviews in the herpetological community (Burke 1991; Dodd and Seigel 1991; Goncharov et al. 1989; Reinert 1991). Relocation, repatriation, and translocation programs have been successful for some amphibian populations (Table 1), but may not be an answer for the recovery of many amphibian species. The following issues need to be considered before an RRT program is initiated for any amphibian population: release site selection, the appropriateness of the source population (physio-geographical, genetic, and life history considerations), transport and housing conditions for specimens, and long-term monitoring of the population with clear objectives to evaluate project success.

Wetlands constructed for mitigation purposes can provide additional opportunities for amphibian RRT programs. Constructed wetlands can be designed to accommodate the reproductive requirements of a diverse assemblage of amphibian species and facilitate experimental designs in which statistical tests can be applied to test the efficacy of the RRT project. Amphibian RRT programs could, in turn, improve the function of wetlands by accelerating colonization by amphibians.

A small artificial wetland was constructed in the summer of 1997 in a small valley below a newly constructed golf course at Rocky Gap State Park (RGSP) in Allegany County, Maryland. Pairs of artificial ponds were placed in two created wetland areas and

a pair was placed in an adjacent, existing wetland area. Egg masses from two species of amphibians, native to western Maryland yet absent from RGSP (Conant and Collins 1991; Thompson et al. 1980), were translocated into ponds at RGSP in order to establish breeding populations. Translocated species were the upland chorus frog (*Pseudacris triseriata* Baird) and the Jefferson salamander (*Ambystoma jeffersonianum* Green). The objectives of this study were to develop a protocol that would quantify initial aspects of the success of hatching and metamorphosis for translocated populations, compare these measures of success to those of natural populations, and examine the possible effects of pond depth and wetland characteristics on measures of success.

I predicted that hatching success would not be affected by pond depth and wetland area because aspects of water chemistry that affect hatching success (Clark 1986; Sadinski 1991) were assumed to be homogeneous throughout ponds in the experimental wetland. I predicted that hatching success for translocated populations of *A. jeffersonianum* would be similar to that in existing populations. I expected to observe relatively high success of metamorphs in populations translocated in 1998 because the recent construction of the site would limit the abundances of populations of predatory aquatic invertebrates. I predicted that the success of metamorphs of translocated individuals would be lower in deep ponds compared to shallow ponds because the abundance and density of aquatic invertebrate species that prey on amphibian larvae increase as a function of pond depth (Skelly 1996; Woodward 1983).

METHODS

Evaluation of Translocation Site

The translocation site was evaluated for suitable *A. jeffersonianum* and *P. triseriata* habitat before construction of the experimental wetland at RGSP began. This evaluation was performed by one of the principal investigators (J. Howard) and a regional ecologist (E. Thompson) from the Maryland Department of Natural Resources. The subjective nature of this evaluation was typical of most amphibian RRT programs (Dodd and Seigel 1991), although both investigators have extensive experience with regional herpetofauna, and one of them (E.T.) has published on the selection of breeding sites by ambystomatid salamanders in western Maryland. The evaluation concluded that the design of the experimental wetland at RGSP could provide suitable breeding and terrestrial habitats for *A. jeffersonianum* and *P. triseriata*. Furthermore, additional breeding habitats for amphibians could be found less than 1 km from the experimental wetland. Surveys of those habitats detected adults, larvae, egg masses, and/or calling males of six species of amphibians: *Rana sylvatica* LeConte (wood frog), *Pseudacris crucifer* Weid-Neuwied (spring peeper), *Bufo americanus* Holbrook (American toad), *Hyla versicolor* LeConte (gray treefrog), *Rana clamitans* Latreille (green frog), and *Notophthalmus viridescens* Rafinesque (red-spotted newt). Forested corridors link these habitats to the experimental wetland, and subsequent colonization of these habitats by *A. jeffersonianum* and *P. triseriata* could result in the establishment of a metapopulation in and around RGSP.

Design and Preparation of Study Site

Work was completed on the 0.2-ha artificial wetland at RGSP on 7 June 1997. The study was limited to two created wetland areas and a portion of an adjacent existing

wetland (Fig. 1). By 1999, naturally colonizing sedges, primarily *Carex* and *Scirpus* spp., and rushes, *Juncus* spp., dominated groundcover in the southern created wetland area. Rice-cut grass, *Leersia oryzoides* L., and *Scirpus* spp. dominated the northern created wetland area. The dominant groundcover species were miscellaneous grasses and scouring rush, *Equisetum arvense* L., in the portion of the existing wetland used in the study (EW).

Pairs of ponds were excavated in the three wetland areas (Fig. 1). The surface areas of each pond were approximately 17 m² (4.1 m x 4.1 m) and were arranged so that each pair included one shallow pond (< 35 cm deep) and one deep pond (> 40 cm deep). Shallow ponds were designed to dry out during particularly dry summers (i.e. ephemeral ponds) and deep ponds were designed to remain filled year-round (i.e. permanent ponds). Nominally ephemeral ponds were designed to exclude colonization by some predators of larval amphibians, namely *N. viridescens*, fish, and large odonate (dragonfly) species. In addition, ephemeral ponds should prevent colonization of larger, predatory species of amphibians (i.e. *R. clamitans* and *Rana catesbeiana* Shaw, the bullfrog) whose larvae require overwintering in ponds.

I graded all ponds to include a 1-m wide shelf on one side, and I planted native emergent vegetation (*Carex* and *Glyceria* spp.) on shelves in the fall of 1997. Emergent vegetation was intended to provide refugia for larval amphibians and structure for oviposition. Larval salamanders use shallow littoral zones during daylight hours to hide from predators (Holomuzki 1986; Stangel and Semlitsch 1987), and the presence of emergent vegetation has a significant impact on the selection of breeding sites for some species of amphibians (Monello and Wright 1999).

Drift fences encircled each pond and were erected 1 m from the water's edge (modified from Dodd and Scott 1994). Drift fences were made from 1-m-tall silt fencing (with 30 cm buried into the ground) and were lined with pitfall traps constructed from #10 tin cans. Cans were fitted with 3-cm-wide lips around their open end to prevent individuals from climbing or jumping out. Pitfall traps were spaced 2 m apart and placed on alternating sides of each fence. Small funnel traps were dispersed where necessary because some pitfall traps were continually flooded. Funnel trap dimensions were 15 cm x 15 cm x 30 cm, funnels were shaped from aluminum flashing, frames were made from 13-mm² hardware cloth wrapped in window screening, and the number of funnel traps/pond remained constant.

Source Populations for Translocations

Egg masses and metamorphs of *A. jeffersonianum* were obtained from an ephemeral pond in a small wetland located in the Frostburg State University Arboretum, Frostburg, Maryland. This site is approximately 32 km due west of RGSP and is approximately 244 m higher in elevation.

Egg masses of *P. triseriata* were obtained from ephemeral pools located between an agricultural field and a small, overgrown woodlot near Oldtown, Maryland. This breeding site is approximately 16 km due south of RGSP and is approximately 122 m lower in elevation. This is the closest known breeding site of *P. triseriata* to RGSP (E. Thompson, pers. comm.).

Translocation Protocol

Egg masses of *A. jeffersonianum* and *P. triseriata* were field-collected, processed in the lab, and translocated to ponds in the experimental wetland at RGSP in the early spring

(late March/early April) of 1998 and 1999. Eggs were individually inspected under laboratory conditions, and egg masses found with bacterial or fungal growths, and eggs in developmental stages beyond neural plate development (Harrison stage 13 for *A. jeffersonianum* and Gosner stage 13 for *P. triseriata*), were excluded from translocations. Egg masses of *A. jeffersonianum* were divided into portions of 15-40 eggs/portion with dissecting scissors and placed into a common container of de-chlorinated water. Egg mass portions were assigned at random to each of the six experimental ponds at RGSP such that each pond was assigned an approximately equal number of eggs with no fewer than 100 eggs and four egg mass portions per pond (Appendix 1). Whole egg masses of *P. triseriata* were randomly assigned to ponds because individual egg masses typically contained fewer than 20 eggs. Each pond was assigned an approximately equal number of *P. triseriata* eggs, with no fewer than 100 eggs and five egg masses per pond (Appendix 1). Eggs were distributed in this manner to insure that each pond contained offspring from multiple females.

Eggs were placed into enclosure bags (made from window screening in 1998 and Log Cabin Designs brand head netting in 1999) upon translocation to their respective ponds. Enclosure bags protected eggs and newly hatched, sessile larvae from predators and allowed me to determine the proportion of translocated eggs that hatched successfully. Larvae remained in enclosure bags until 2-3 days after hatching and were then counted, removed from bags, and placed in their pond to continue development. Six additional enclosure bags containing *A. jeffersonianum* eggs were placed in the pond at the breeding site of the source population in the spring of 1998. Bags were placed throughout areas in the pond where female *A. jeffersonianum* had laid eggs.

Metamorphs of *A. jeffersonianum* were captured in pitfall and funnel traps as they dispersed from ponds (modified from Dodd and Scott 1994). Captured individuals were brought back to the lab and given toe-clips and freeze-brands to indicate the year, wetland area, and pond class (ephemeral or permanent) of emergence and each individual's mass and snout-vent-length (SVL) were recorded. Metamorphs, after being processed, were returned at night to the outside of the drift fencing that surrounded the pond from which they emerged. The proportion of hatchlings that completed metamorphosis per pond was determined by the total number of metamorphs captured at each pond. In addition, mass and SVL measurements provided a measure of the relative health of metamorphs from each pond.

A total of 54 recently metamorphosed *A. jeffersonianum* (captured from the source population and raised in captivity) was released into ponds at RGSP (9 metamorphs/pond) in the fall of 1997. These individuals were toe-clipped to identify the year of emergence and the wetland area and pond class in which they were released.

Pitfall and funnel traps were not effective at capturing metamorphs of *P. triseriata* due to their ability to climb over drift fences. I did not calculate the proportion of *P. triseriata* hatchlings that completed metamorphosis because high rates of trespass would have negatively biased my results. I was able to capture some metamorphs of *P. triseriata* with time-constrained searches. Those captured were given toe-clips to signify the year, wetland area, and pond class of emergence, and their snout-to-urostyle lengths were recorded.

I monitored the experimental wetland for the return of translocated *A. jeffersonianum* and *P. triseriata* in the spring of 1998 and 1999. I checked pitfall and funnel traps daily

for returning adults, listened for chorusing male *P. triseriata*, and examined ponds for egg masses deposited by either species. Captured adults were examined for evidence of toe-clips and freeze-brands to determine if colonizing adults were the result of previous translocations and if site fidelity was displayed for the wetland area or pond class from which they emerged.

Statistical Analysis

Hatching success for embryos was calculated for egg masses of *A. jeffersonianum* and *P. triseriata* that were translocated to RGSP. In addition, the hatching success of embryos was calculated for egg masses of *A. jeffersonianum* that hatched in enclosure bags at the breeding site of the source population. Percent hatching success was calculated using the following equation because the fate of all embryos placed in enclosure bags could not be determined:

$$\text{Percent hatching success} = \frac{\# \text{hatchlings}}{(\# \text{hatchlings}) + (\# \text{dead eggs})} \times 100 .$$

Counts of hatchlings and dead eggs of both species were analyzed with separate three dimensional (three-way) contingency chi-square analysis to test the hypothesis that an interaction(s) existed between the variables of hatching success, wetland area and nominal pond class (permanent and ephemeral ponds) (Zar 1996). If a wetland area by pond class interaction was not detected for hatching success, then 2 x 3 and 2 x 2 contingency chi-square analyses were used to determine if hatching success was independent of wetland area or pond class. Samples were pooled for wetland areas in 2 x 3 contingency tables and for pond classes in 2 x 2 contingency tables (Zar 1996). If a wetland area by pond class interaction was detected, then 2 x 2 contingency chi-square analyses were used to determine where significant differences existed between individual

ponds. The criterion for statistical significance was set at $\alpha = 0.05$, all pooled samples met the assumption of homogeneity (Zar 1996), and non-pooled samples were corrected for continuity (Yates 1934).

I compared hatching success between enclosure bags of *A. jeffersonianum* translocated to RGSP in 1998 and enclosure bags of *A. jeffersonianum* at the source population in 1998. I used multiple, two-sample t-tests to test the null hypotheses that the proportion of *A. jeffersonianum* eggs that hatched within enclosure bags did not differ between translocation years and they did not differ between eggs translocated to RGSP in 1998 and eggs at the source population in 1998. Critical values for multiple t-tests were adjusted as recommended by Zar (1996). A two-sample t-test was used to test the hypothesis that the proportion of *P. triseriata* eggs that hatched within enclosure bags were the same for eggs translocated in 1998 and 1999. Criterion for statistical significance was $\alpha = 0.05$, all proportional data were treated with an arcsine square root transformation, and Bartlett's tests (Zar 1996) confirmed that variances were homogeneous. T-tests were calculated using Statistical Analysis System (SAS) version 6.12 (SAS Institute Inc. 1990).

I calculated the percent success of metamorphosis for embryos and hatchlings of *A. jeffersonianum* translocated to RGSP in 1998 and 1999 using the following equation:

$$\text{Percent metamorphosis success} = \frac{\# \text{metamorphs captured}}{\# \text{hatchlings (or embryos)}} \times 100 .$$

Counts of metamorphs captured and hatchlings that were presumed to have died were analyzed with three-dimensional contingency chi-square analysis to test the hypotheses an interaction(s) existed between the variables of wetland area, nominal pond class, and

success of metamorphosis. If a wetland area by pond class interaction was not detected, 2 x 3 and 2 x 2 contingency chi-square analyses were used to determine if success of metamorphosis was independent of wetland area or pond class. If an interaction was detected, 2 x 2 contingency chi-square analyses were used to determine where significant differences existed between individual ponds. The criterion for statistical significance was set at $\alpha = 0.05$, all pooled samples met the assumption of homogeneity, and non-pooled samples were corrected for continuity. I used a two-sample t-test to determine if the proportion of hatchlings that completed metamorphosis was different at RGSP in 1998 and 1999. Criterion for statistical significance was $\alpha = 0.05$, proportional data were treated with an arcsine square root transformation, a Bartlett's test confirmed that variances were homogeneous, and calculations were performed using Statistical Analysis System (SAS) version 6.12 (SAS Institute Inc. 1990).

I quantified the percent success of metamorphosis for embryos of *A. jeffersonianum* at the breeding habitat of the source population in 1999, although lack of replication prevented hypothesis testing. The number of eggs at the source population in 1999 was estimated from collections made for a study initiated that spring. For that study, approximately 1/6 of all *A. jeffersonianum* eggs found at the breeding site were collected for genetic analysis. The total number of eggs remaining in the source population was estimated by the following formula:

$$\text{Eggs remaining} = [5 \times (\# \text{ eggs for genetic analysis})] - [\# \text{ eggs translocated}].$$

RESULTS

1998 Translocations

A total of 670 *P. triseriata* eggs was field-collected, examined, and translocated to ponds at RGSP on 27 March 1998. Embryos commenced hatching three days after translocation and the last embryos hatched seven days after translocation. Hatching success for individual ponds ranged from 88.9 to 100.0% ($\bar{X} = 93.9\%$, S.E. = 1.7%) and hatching success for all translocated embryos combined was 93.3% (Table 2). Hatching success was higher in CW2 than other wetland areas ($\chi^2 = 9.31$, d.f. = 2, $P < 0.05$), pond class had no effect on hatching success ($\chi^2 = 0.01$, d.f. = 1, $P > 0.05$), and a wetland area by pond class interaction was not detected ($\chi^2 = 9.87$, d.f. = 5, $P > 0.05$). A total of 15 metamorphosed *P. triseriata* was captured during time-constrained searches between 27 May and 16 June 1998.

A total of 798 *A. jeffersonianum* eggs was field-collected, examined, and translocated to ponds at RGSP on 22 March 1998. Embryos began to hatch 14 days after translocation and the last of the translocated embryos hatched within 21 days of translocation (Table 3). Hatching success for all translocated *A. jeffersonianum* embryos was 93.9%, and hatching success for individual ponds ranged from 96.2 to 98.6% ($\bar{X} = 96.9\%$, S.E. = 0.9%) (Table 4). Hatching success was lower in EW than other wetland areas ($\chi^2 = 7.69$, d.f. = 2, $P > 0.05$), pond class had no effect on hatching success ($\chi^2 = 0.58$, d.f. = 1, $P > 0.05$), and there were no interactions between hatching success, pond class, and wetland area ($\chi^2 = 10.89$, d.f. = 7, $P > 0.05$).

Metamorphs of *A. jeffersonianum* began to emerge from ponds at RGSP 69 days after translocation and 90% of all emerging metamorphs were captured within 92 days of

translocation (Table 5). A total of 118 metamorphosed *A. jeffersonianum* was captured in traps while dispersing from ponds at RGSP in 1998, and the number of emerging metamorphs captured at individual ponds ranged from 4 to 42 metamorphs/pond ($\bar{X} = 19.7$, S.E. = 5.6). Of all hatchlings that were accounted for, 16.3% completed metamorphosis, metamorphosis success for individual ponds ranged from 3.2% to 30.7% ($\bar{X} = 15.8\%$, S.E. = 3.9%) (Table 6) and a wetland area by pond class interaction was detected ($\chi^2 = 44.21$, d.f. = 5, $P < 0.05$) (Fig. 2). Metamorphosis success was higher in permanent ponds than ephemeral ponds in CW1 ($\chi^2 = 12.80$, d.f. = 1, $P < 0.05$) and CW2 ($\chi^2 = 16.05$, d.f. = 1, $P < 0.05$), but not in EW ($\chi^2 = 0.66$, d.f. = 1, $P > 0.05$). The mean SVL for all metamorphs captured was 37.3 mm (S.E. = ± 0.3) and mean SVLs for individual ponds ranged from 32.8 to 40.5 mm ($\bar{X} = 37.8$, S.E. = 1.1). The mean mass for all metamorphs captured was 1.487 g (S.E. = 0.035) and mean masses for individual ponds ranged from 0.896 to 2.027g ($\bar{X} = 1.547$, S.E. = 0.152) (Table 6).

1999 Translocations

A total of 680 *P. triseriata* eggs was field-collected, examined, and translocated to ponds at RGSP on 3 April of 1999. Embryos began hatching 15 days after translocation and the last of the translocated embryos hatched within 24 days of translocation. Hatching success was 86.6% for all translocated embryos, hatching success values for individual ponds ranged from 70.5 to 100.0% ($\bar{X} = 86.4\%$, S.E. = 5.3%) (Table 2), and hatching success was affected by a wetland area by pond class interaction ($\chi^2 = 61.09$, d.f. = 5, $P < 0.05$) (Fig. 3). Hatching success was higher in the permanent pond than the ephemeral pond in CW1 ($\chi^2 = 27.08$, d.f. = 1, $P < 0.05$), while hatching success was lower in permanent ponds than ephemeral ponds in CW2 ($\chi^2 = 12.10$, d.f. = 1, $P < 0.05$)

and EW ($\chi^2 = 16.43$, d.f. = 1, $P < 0.05$). Hatching success for *P. triseriata* did not differ between populations translocated to RGSP in 1998 and 1999 ($t = 0.94$, d.f. = 10, $P > 0.05$). Four *P. triseriata* metamorphs were captured during time-constrained searches between 9 and 12 June 1999.

Several *P. triseriata* that were translocated to RGSP as embryos in 1998 returned to the experimental wetland in the spring of 1999. Individuals that returned bore patterns of missing or re-grown fingers that resembled patterns consistent with toe-clippings given to *P. triseriata* metamorphs that were captured in the spring of 1998. Four adult *P. triseriata*, one female and three males, were captured alive at RGSP. Additional individuals returned because on several occasions, four to five males could be heard calling simultaneously. Two *P. triseriata* egg masses were found at RGSP in the ephemeral pond located in EW. Each mass contained 15-20 larvae that presumably hatched (upon later examination neither undeveloped eggs nor larvae could be found in either mass).

A total of 632 *A. jeffersonianum* eggs was collected, examined, and translocated to ponds at RGSP on 8 April 1999. Embryos commenced hatching 19 days after translocation, and the last of the translocated embryos hatched 23 days after translocation. Hatching success for individual ponds ranged from 89.5 to 96.9% ($\bar{X} = 93.9\%$, S.E. = 1.2%) and 93.8% of all translocated embryos hatched (Table 4). Hatching success was not affected by wetland area ($\chi^2 = 0.44$, d.f. = 2, $P > 0.05$) or pond class ($\chi^2 = 1.16$, d.f. = 1, $P > 0.05$) and no interactions were detected between hatching success, wetland area, and pond class ($\chi^2 = 9.44$, d.f. = 7, $P > 0.05$). Hatching success did not differ between

translocated populations of *A. jeffersonianum* in 1998 and 1999 ($t = 2.11$, d.f. = 10, $P > 0.05$).

A total of 10 *A. jeffersonianum* metamorphs was captured in traps while dispersing from ponds at RGSP in 1999, and the number of emerging metamorphs captured at individual ponds ranged from 0 to 5 ($\bar{X} = 1.5$, S.E. = 0.8). Metamorphs of *A. jeffersonianum* at RGSP began to emerge from ponds 66 days after translocation, and 90% of all emerging metamorphs were captured within 86 days after translocation (Table 6). Success of metamorphosis for all hatchlings was 1.7%, success of metamorphosis for individual ponds ranged from 0.0% to 5.0% ($\bar{X} = 1.5\%$, S.E. = 0.8%) (Table 6). Metamorphosis success was not affected by wetland area ($\chi^2 = 2.75$, d.f. = 2, $P > 0.05$) or pond class ($\chi^2 = 1.29$, d.f. = 1, $P > 0.05$), and no interactions were detected between metamorphosis success, wetland area, and pond class ($\chi^2 = 13.15$, d.f. = 7, $P > 0.05$). Success of metamorphosis at RGSP was significantly lower in 1999 than in 1998 ($t = 4.26$, d.f. = 10, $P < 0.05$). The mean SVL for all metamorphs collected was 31.1 mm (S.E. = 1.1) and the mean SVLs for individual ponds ranged from 29.8 to 37.0 mm ($\bar{X} = 32.6$, S.E. = 2.2). The mean mass for all captured metamorphs was 0.971g (S.E. = 0.128) and mean masses for individual ponds ranged from 0.787 to 1.635 g ($\bar{X} = 1.143$, S.E. = 0.254) (Table 6).

Source population of A. jeffersonianum

A total of 809 *A. jeffersonianum* eggs was collected and distributed into six enclosure bags that were placed in the pond at the breeding habitat of the source population on 22 March 1998. Embryos began to hatch 16 days after collection, and the last of the embryos hatched within 30 days of being collected (Table 3). The mean hatching success

for all embryos was 99.7% (Table 3), and individual enclosure bags ranged from 99.1 to 100.0% ($\bar{X} = 99.7\%$, S.E. = 0.2%). Hatching success was lower in embryos translocated to RGSP in 1998 than in embryos placed in the breeding habitat of the source population ($t = 4.51$, d.f. = 10, $P < 0.05$).

A total of 76 *A. jeffersonianum* metamorphs was captured while dispersing from the breeding habitat of the source population in 1999. Metamorphs of *A. jeffersonianum* began to emerge 55 days after translocation (Table 6), 90% of all emerging metamorphs were captured within 63 days of translocation, and all standing water at the breeding habitat had dried within 65 days of translocation. Of an estimated 4200 eggs at the source population in 1999, 2.13% completed metamorphosis. The mean SVL for metamorphs was 36.0 mm (S.E. = 0.2), and the mean mass was 1.573 g (S.E. = 0.031) (Table 6).

DISCUSSION

Hatching success for *P. triseriata* did not decrease during the second year of translocations, but wetland area did affect hatching success in 1998 and a wetland area by pond class interaction was detected in 1999. Wetland areas at RGSP differed in hydrology (Appendix 2) and vegetation (Appendix 3), both of which could have affected aspects of water chemistry that are important to hatching success. Fertilizer and pesticide runoff from the adjacent golf course would have had its greatest impact on the water chemistry of ponds in CW1 because a small stream located between the course and CW2 and EW could have buffered runoff from the course. However, since chemical analyses were not performed on water samples from ponds, these conclusions remain speculative.

I found it considerably more difficult to capture *P. triseriata* than *A. jeffersonianum*, and species-specific trespass rates have been documented previously (Dodd 1991). Such problems need to be identified because they can result in an inaccurate measurement of important indicators such as success of metamorphosis and breeding adults. The initial results from translocations of *P. triseriata* seem promising in light of previous studies on *Pseudacris* breeding and translocation attempts. Although relatively few adult *P. triseriata* were captured at RGSP in 1999, these numbers are consistent with those of Caldwell (1987) who rarely captured more than five individuals per season of *Pseudacris ornata* Holbrook (the ornate chorus frog) and *Pseudacris nigrita* LeConte (the southern chorus frog) that were marked as metamorphs from the previous season. Furthermore, metamorphs that emerged late in the 1998 season at RGSP may not have reached sexual maturity until the spring of 2000. Where as adults returning to RGSP laid only two egg masses in 1999, Maunder (1983) documented the establishment of several populations of chorus frogs in Newfoundland, Canada, from single translocations of 75 or fewer tadpoles.

Hatching success of *A. jeffersonianum* was not significantly different between translocation years, but differed between wetland areas in 1998, and differed between translocated and source populations in 1998. The biological significance of these differences, however, is questionable. Even in a season in which 20% of all hatchlings complete metamorphosis, a decrease in hatching success of 10% would decrease the total number of embryos that complete metamorphosis by only 2%.

Metamorphosis success rates as low as those at RGSP in 1999 are not uncommon for ambystomatid species. Semlitsch (1987a) observed that metamorphosis success for

Ambystoma talpoideum Holbrook (the mole salamander, a species with a life history very similar to *A. jeffersonianum*) ranged from 0 to 4.9% during a six-year study of three established populations. High success of metamorphosis during the first year of translocations to RGSP was likely due to relatively small populations of predatory invertebrate species. Populations of predatory invertebrate species probably became more established by the second season, especially odonate (dragonfly) species whose larvae overwinter in ponds. However, this is speculative because sampling for invertebrates was not performed, and important environmental conditions were different between the two years of the study. Western Maryland experienced a severe drought in 1999 that caused the breeding habitat at the source population and the ephemeral pond in EW to dry completely by mid-June. In addition, pond depths were relatively low and water temperatures were relatively high at RGSP in 1999 compared to 1998 (Appendix 2). Such high temperatures (especially in the month of June when most larvae completed metamorphosis) might have exceeded the upper limits of tolerance for most larvae in 1999 and increased mortality rates among them. We have no reference to determine the extent to which drought conditions affected success of metamorphosis at RGSP in 1999 because we have no data on success of metamorphosis at the source population during non-drought conditions.

The wetland area by pond class interaction detected for metamorphosis success in 1998 might also be explained by populations of predators. In the summer 1997, EW was the only wetland area with dense vegetation surrounding its ponds, and its ephemeral pond was the only pond without standing water. Therefore, it is likely that the permanent pond of EW contained the highest populations of odonates in 1998, while the ephemeral

pond of EW contained none. In addition, the permanent pond in EW and the ephemeral pond in CW2 were the only ponds that contained the predator *Notophthalmus viridescens* in the spring and summer of 1998 (unpubl. data). Higher success of metamorphosis in permanent ponds than in ephemeral ponds in CW1 and CW2 could have been due to the capacity of permanent ponds to harbor higher populations of invertebrate prey due to higher volumes of water.

Metamorphs of *A. jeffersonianum* at RGSP in 1998 appeared to be more robust than metamorphs in 1999. This might be explained by possible increases in populations of predatory aquatic invertebrates and *N. viridescens*. Other aquatic predators (fish) have been found to reduce the size of larval salamanders at metamorphosis by restricting larval salamanders to benthic regions where food resources are less abundant (Stangel and Semlitsch 1987; Semlitsch 1987b). An increase in predatory insects and *N. viridescens* between years could have also reduced available prey for larval salamanders since these three taxa share some of the same food resources. Metamorphs from the source population also appeared to be more robust than the metamorphs from RGSP in 1999. This may be correlated with the truly ephemeral nature of the breeding pond at the source population. The hydrologic regime of this pond could reduce the abundance of predatory species of invertebrates by preventing colonization by species of invertebrates whose larvae overwinter.

For investigators to evaluate amphibian RRT projects objectively they must quantify measures of success of translocated populations and compare them to such attributes of natural populations. Investigators who do this can evaluate translocation success and ensure that resources are not wasted by translocating individuals into habitats that act as

population sinks. Unfortunately, few natural history studies (Caldwell 1987; Douglas 1979; Thompson et al. 1980) are available on amphibian reproductive biology at the population level aside from those concerning common species that typically are not candidates for RRT programs. Therefore, to obtain this information investigators may need to expand their efforts by monitoring measures of success for both translocated and source populations. The translocation project at RGSP has attempted to obtain and document such information for the initial stages of colonization. The breeding activities of several generations of adults will need to be monitored at RGSP to determine if translocated species have established self-perpetuating populations.

The herpetological community has expressed several concerns over protocols used in reptile and amphibian RRT programs (Bloxam and Tonge 1995; Burke 1991; Dodd and Seigel 1991; Hein 1997; Reinert 1991), and the translocation project at RGSP attempted to address some of them. The process of site selection used for this project could be criticized for its subjectivity, although I did not find an amphibian RRT protocol that has described objective, quantitative methods to evaluate habitats for target species. Egg masses were translocated in 1998 and 1999 because studies suggest that translocating individuals in early developmental stages (egg and larval stages) increases the likelihood that individuals will return to breed there upon reaching sexual maturity (Bloxam and Tonge 1995; Maunder 1983; Reinert 1991). The source populations for translocations were in close geographic proximity to RGSP to increase the probability that individuals released would possess genotypes suited to environmental conditions at the release site and that egg masses for translocations represented progeny from multiple females. Care was taken to minimize the risk of spreading disease into populations of amphibians at

RGSP, but examinations could have been more rigorous by using a more sophisticated histopathological technique. I performed a more detailed evaluation of success than most amphibian RRT protocols (most programs only evaluate the activities of returning adults), although the monitoring of aquatic invertebrate communities might prove informative. Investigators have called for more RRT protocols with experimental designs that allow for statistical tests to be applied to data (Hein 1997; Reinert 1991). The design of the artificial wetland at RGSP did allow for some statistical analysis and hypothesis testing, although differences in hydrology and vegetation prevented me from using wetland areas as true replicates of this experiment.

The results of this experiment suggest that habitat heterogeneity, even within a relatively small translocation site, can result in differential survival of larvae among bodies of water. Investigators need to closely evaluate survival after initial translocations have been made to determine which habitats within a translocation site are best suited to receive individuals from future translocations.

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Table 1: Examples of amphibian relocation, repatriation, and translocation programs.

Species	Location	Life Stage	Results	Investigator
<i>Rana sylvatica</i>	Missouri	Eggs	Large breeding colony established within 5 years	Sexton and Phillips 1986
<i>Ambystoma tigrinum</i>	New Jersey	Eggs	Successful repatriation of breeding site	Zappalorti unpubl.
<i>Ambystoma maculatum</i>	Missouri	Eggs and Larvae	Individuals observed in almost all subsequent years (1965-1986)	Sexton and Phillips 1986
<i>Bufo calamita</i>	England	Larvae	Failure – Unknown reasons	Beebee 1983
<i>Pseudacris triseriata</i>	Newfoundland	Larvae	Multiple breeding populations established with single translocations of 75 or fewer tadpoles	Maunder 1983
<i>Pelobates syriacus</i>	USSR	Juveniles	Recaptured individuals in one of three locations of release.	Goncharov et al. 1989
<i>Triturus vittatus</i>	USSR	Juveniles	Successful reproduction by released individuals	Goncharov et al. 1989
<i>Peltophyrne lemur</i>	Puerto Rico	Juvenile and Adults	No follow-up reports after initial release	Miller 1985
<i>Plethodon idahoensis</i>	Montana	Adults?	Unknown	Anonymous 1990

Table 2: Hatching success of translocated *Pseudacris triseriata*

Year	Wetland area	Pond class	Total eggs	Hatched embryos	Unhatched embryos	Unaccounted embryos	Hatching success (%)
1998	CW1	Permanent	108	80	10	18	88.9
	" "	Ephemeral	114	101	10	3	91.0
	CW2	Permanent	114	92	2	20	97.9
	" "	Ephemeral	109	41	0	68	100.0
	EW	Permanent	115	39	3	73	92.9
	" "	Ephemeral	110	79	6	25	92.9
Totals			670	432	31	207	93.3
Pond means			111.7	72.0	5.2	34.5	93.9
1999	CW1	Permanent	112	96	2	14	98.0
	" "	Ephemeral	111	55	23	33	70.6
	CW2	Permanent	103	70	13	20	84.3
	" "	Ephemeral	118	71	0	47	100.0
	EW	Permanent	121	63	25	33	71.6
	" "	Ephemeral	115	91	6	18	93.8
Totals			680	446	69	165	86.6
Pond means			113.3	74.3	11.5	27.5	86.4

Table 3: Measures of hatching success for translocated and source populations of *Ambystoma jeffersonianum* in 1998. {Different letters indicate a significant difference ($P \leq 0.05$).}

	RGSP'98	Source '98
Days to initiate hatching	14	16
Days to complete hatching	21	30
Mean (\pm SE) hatching success of enclosure bags	96.9% (\pm 0.9%) ^b	99.7% (\pm 0.2%) ^a
Total hatchlings	725	594

Table 4: Hatching success of translocated *Ambystoma jeffersonianum*

Year	Wetland area	Pond class	Total eggs	Hatched embryos	Unhatched embryos	Unaccounted embryos	Hatching success (%)
1998	CW1	Permanent	139	137	2	0	98.6
	" "	Ephemeral	128	117	3	8	97.5
	CW2	Permanent	145	132	3	10	97.8
	" "	Ephemeral	139	125	2	12	98.4
	EW	Permanent	133	114	9	10	92.7
	" "	Ephemeral	114	100	4	10	96.2
Totals			798	725	23	50	93.9
Pond means			133.0	120.8	3.8	8.3	96.9
1999	CW1	Permanent	106	95	7	4	93.1
	" "	Ephemeral	112	88	3	21	96.7
	CW2	Permanent	100	77	9	14	89.5
	" "	Ephemeral	101	93	3	5	96.9
	EW	Permanent	102	85	4	13	95.5
	" "	Ephemeral	111	100	9	2	91.7
Totals			632	538	35	59	93.8
Pond means			105.3	89.7	5.8	9.8	93.9

Table 5: Measures of success of metamorphosis for translocated and source populations of *Ambystoma jeffersonianum* (d = day of the year).

	RGSP'98	RGSP'99	Source '99
Translocation date	22 March (d81)	08 April (d127)	08 April (d127)
First metamorph captured	30 May (d150)	13 June (d164)	02 June (d153)
50% metamorphs captured	09 June (d160)	18 June (d169)	06 June (d157)
90% metamorphs captured	22 June (d173)	01 Aug (d213)	10 June (d161)
Last metamorph captured	09 July (d190)	15 Aug (d227)	11 June (d162)
Total embryos completing metamorphosis	14.8%	1.5%	2.1%
Total metamorphs	118	10	76
Mean SVL (mm) - all metamorphs	37.3 ± 0.3	31.1 ± 1.1	36.0 ± 0.2
Mean mass (g) - all metamorphs	1.487 ± 0.035	0.971 ± 0.128	1.573 ± 0.031
Mean mass/SVL (g/mm) - all metamorphs	0.0395 ± 0.001	0.031 ± 0.003	0.045 ± 0.001

Table 6: Measures of metamorphosis success of translocated *Ambystoma jeffersonianum*

Year	Wetland area	Pond class	Total eggs	Hatched embryos	Metamorphs captured	%Hatchlings metamorphosed	%Eggs metamorphosed	Mean SVL (mm)	Mean Mass (g)
1998	CW1	Permanent	139	137	42	30.7	30.2	39.0	1.724
	" "	Ephemeral	128	117	14	12.0	10.9	37.6	1.492
	CW2	Permanent	145	132	30	22.7	20.7	32.8	0.896
	" "	Ephemeral	139	125	4	3.2	2.9	40.5	2.027
	EW	Permanent	133	114	13	11.4	9.8	38.5	1.597
	" "	Ephemeral	114	100	15	15.0	13.2	38.1	1.546
Totals			798	725	118	16.3	14.8	37.3	1.487
Pond means			133.0	120.8	19.7	15.8	14.6	37.8	1.547
1999	CW1	Permanent	106	95	2	2.1	1.9	31.0	1.007
	" "	Ephemeral	112	88	0	0.0	0.0	na	na
	CW2	Permanent	100	77	0	0.0	0.0	na	na
	" "	Ephemeral	101	93	2	2.2	2.0	na	na
	EW	Permanent	102	85	1	1.2	1.0	37.0	1.635
	" "	Ephemeral	111	100	5	5.0	4.5	29.8	0.787
Totals			632	538	10	1.7	1.4	31.1	0.971
Pond means			105.3	89.7	1.7	1.7	1.6	32.6	1.143

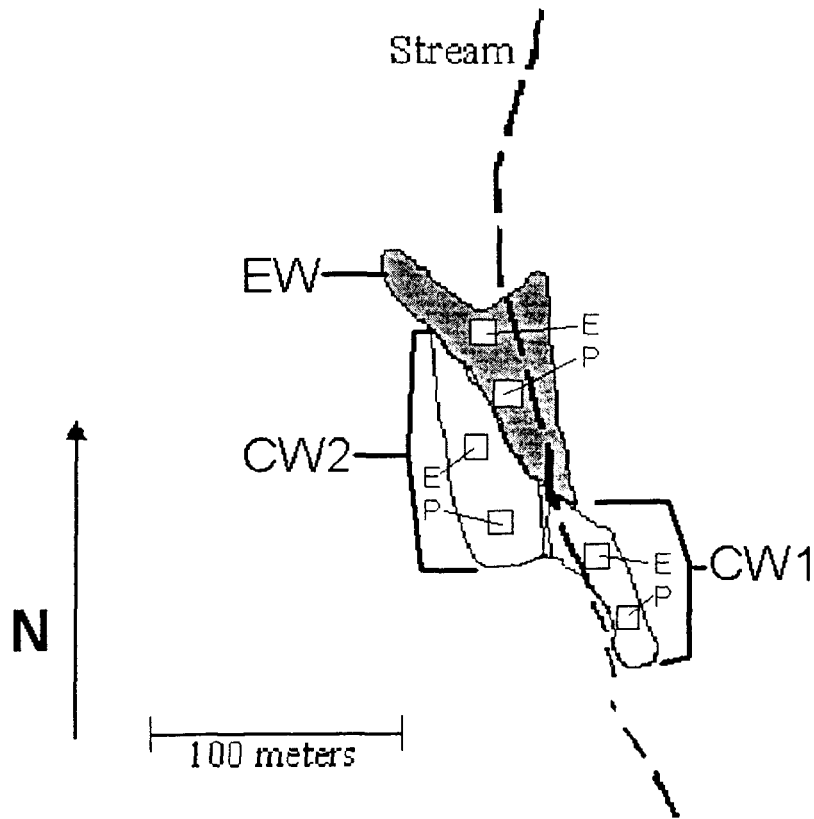


Figure 1. Map of experimental wetland at Rocky Gap State Park, Allegany Co., Maryland. EW = existing wetland habitat. CW = created/mitigated wetland habitat. P = permanent pond. E = ephemeral pond.

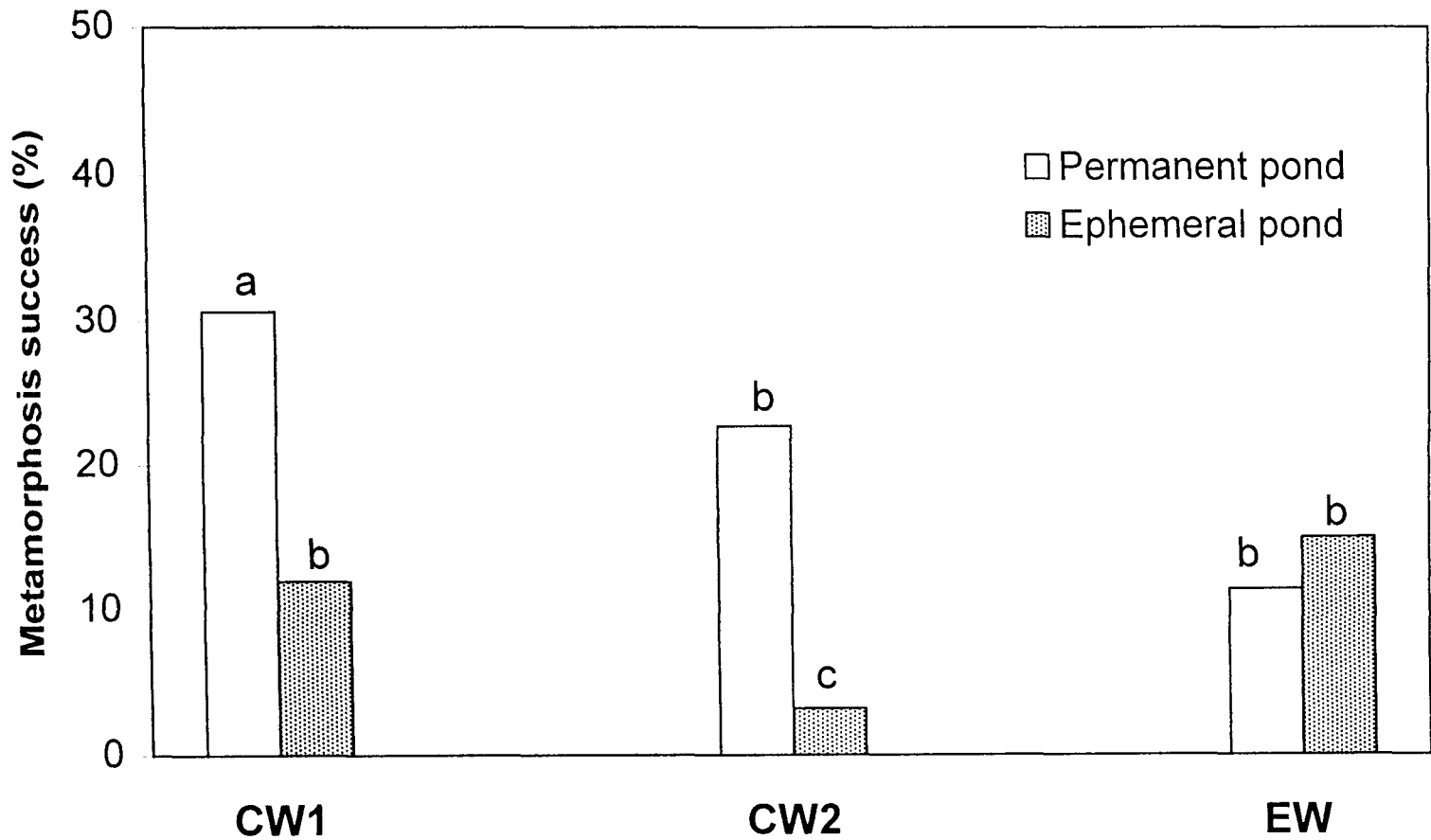


Figure 2. *Ambystoma jeffersonianum* metamorphosis success 1998. [Different letters indicate a significant difference ($P \leq 0.05$)].

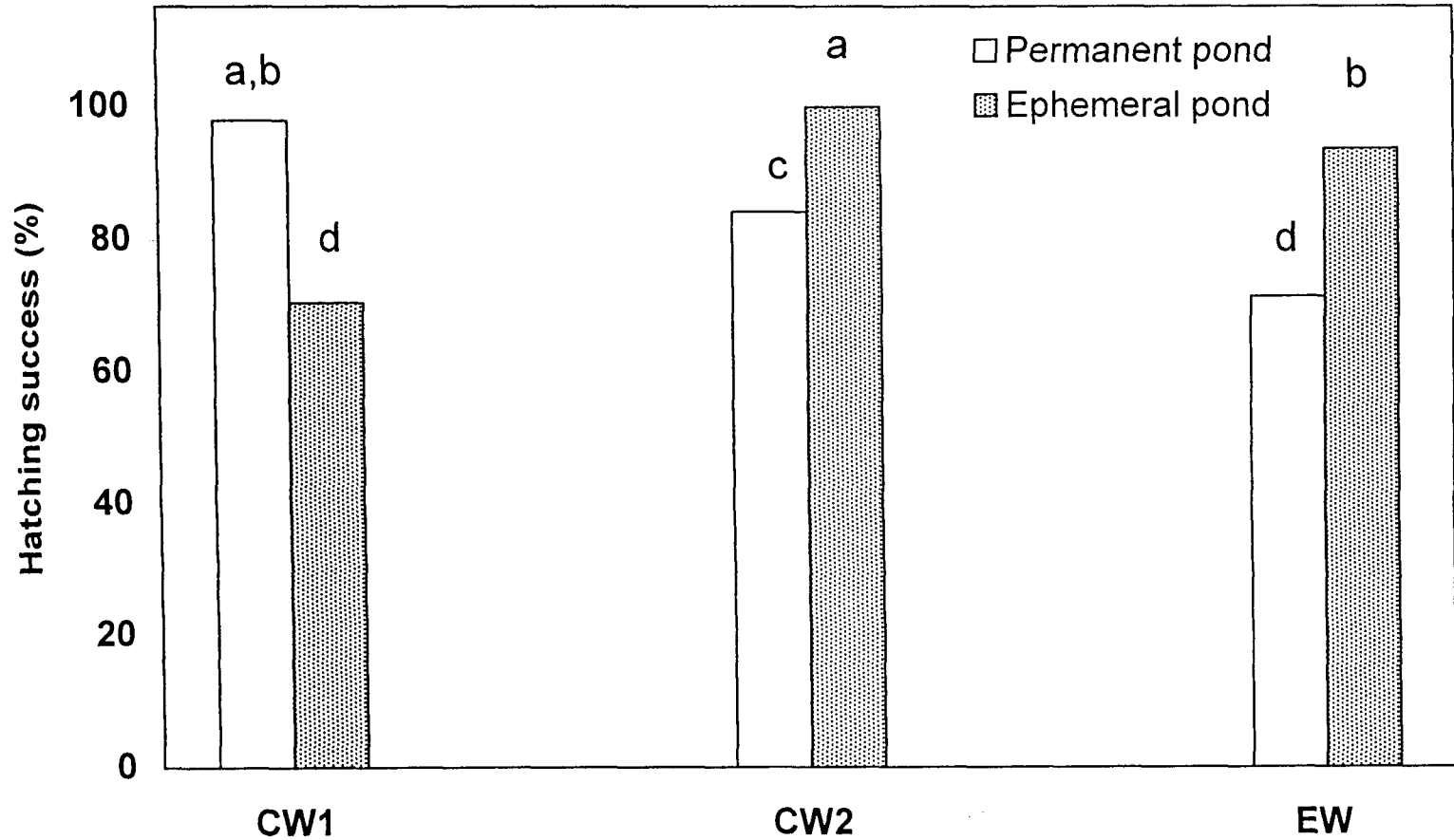


Figure 3. *Pseudacris triseriata* hatching success 1999. [Different letters indicate a significant difference ($P \leq 0.05$).]

Appendix 1: Distribution of egg masses and egg portions into ponds at RGSP

Species	Year	Pond	Number of egg masses (portions)	Mean eggs per mass (portion)
<i>A. jeffersonianum</i>	1998	CW1-P	4	35
" "	" "	CW1-E	4	32
" "	" "	CW2-P	4	36
" "	" "	CW2-E	4	35
" "	" "	EW-P	4	33
" "	" "	EW-E	4	29
<i>P. triseriata</i>	" "	CW1-P	7	15
" "	" "	CW1-E	8	14
" "	" "	CW2-P	8	14
" "	" "	CW2-E	7	16
" "	" "	EW-P	7	16
" "	" "	EW-E	8	14
<i>A. jeffersonianum</i>	1999	CW1-P	6	18
" "	" "	CW1-E	6	19
" "	" "	CW2-P	5	20
" "	" "	CW2-E	7	14
" "	" "	EW-P	6	17
" "	" "	EW-E	8	14
<i>P. triseriata</i>	" "	CW1-P	6	19
" "	" "	CW1-E	8	14
" "	" "	CW2-P	5	21
" "	" "	CW2-E	5	24
" "	" "	EW-P	6	20
" "	" "	EW-E	7	16

Appendix 2: Depth and thermal profiles for ponds at RGSP in 1998 and 1999

Variable	Time period	Year	CW1-P	CW1-E	CW2-P	CW2-E	EW-P	EW-E
Average depth (cm)	Translocation date through August	1998	53.6	37.1	34.1	25.1	37.5	19.3
Max Depth Change (cm)	" "	" "	35	12	18	9	18	27
Pond dried completely	" "	" "	No	No	No	No	No	Yes
Average water temp. (°C)	" "	" "	21.3	20.5	20.8	20.6	20.6	20.9
Average water temp. (°C)	Month of June	" "	22.6	21.9	22.2	21.4	21.6	22.4
Average depth (cm)	Translocation date through August	1999	30.6	18.8	28.5	19.1	32.4	10.5
Max Depth Change (cm)	" "	" "	53	30	17	9	18	25
Pond dried completely	" "	" "	No	Yes	No	No	No	Yes
Average water temp. (°C)	" "	" "	23.8	21.3	21.5	20.1	21.5	21.2
Average water temp. (°C)	Month of June	" "	29.5	25.5	25.5	23.5	25.8	30.5

Appendix 3: Most abundant groundcover species by wetland area in 1999

CW1		CW2		EW	
Species	% Groundcover	Species	% Groundcover	Species	% Groundcover
<i>Carex</i> spp.	24.5	<i>Leersia oryzoides</i>	53.4	Misc. grass spp.	37.2
<i>Juncus</i> spp.	12.3	<i>Scirpus</i> spp.	13.9	<i>Equisetum arvense</i>	31.5
<i>Mentha</i> spp.	8.6	<i>Carex</i> sp. 1	8	<i>Filipendula ulmaria</i>	11.5
Misc. grass spp.	8.3	Misc. grass spp.	5.4	<i>Eupatorium</i> spp.	5.4
<i>Eupatorium</i> spp.	7.6	<i>Juncus</i> spp.	1.9	<i>Mentha</i> spp.	3.1
<i>Scirpus</i> spp.	4	<i>Dipsacus laciniatus</i>	1.5	<i>Carex</i> spp.	2.7
<i>Cephalanthus occidentalis</i>	1.4	<i>Cephalanthus occidentalis</i>	1	<i>Dipsacus laciniatus</i>	1.1

CHAPTER 3. HYDROLOGIC REGIMES AND ANURAN COLONIZATION IN AN ARTIFICIAL WETLAND

ABSTRACT

I monitored the initial stages of colonization by anurans in a small, artificial wetland in Allegany County, Maryland that contained bodies of water with various depths (hydrologic classes). I predicted that anuran species that bred in the spring would use shallower bodies of water for breeding in greater proportions than species that bred in the summer. Anuran species did not deposit significantly different densities of eggs among the hydrologic classes, nor did the total anuran egg densities differ among classes. In 1998, total anuran egg densities were higher in created wetland areas than in an adjacent, existing wetland area. Some trends suggest that *Bufo americanus* and *Rana sylvatica* (both spring-breeding species) laid higher egg densities in shallow hydrologic classes than *Rana clamitans* and *Hyla versicolor* (both summer-breeding species), but considerable variation among replicates reduced the likelihood that statistical analysis would detect a hydrologic class by species interaction.

INTRODUCTION

Mitigation efforts for wetlands should emphasize the creation of habitats that facilitate colonization by a diverse flora and fauna in order to preserve biodiversity. To promote colonization by amphibians, planners must design artificial wetlands that are linked to local populations of amphibians by landscape characteristics that act as dispersal corridors. In addition, planners will need to design wetlands that provide appropriate habitats for adults to breed, for larvae to complete metamorphosis, and for metamorphs to survive to adulthood. Artificial wetlands should also provide an array of habitats for breeding that will accommodate the life histories of all target species of amphibians that could potentially colonize the wetland. Wetlands with these features increase the potential for the establishment of a diverse assemblage of amphibian species.

Investigators have examined amphibian autecology in light of principles of landscape ecology and metapopulation dynamics to predict colonization of potential breeding habitats by amphibians. Studies suggest that amphibian colonization is strongly affected by distances from established habitats for breeding and characteristics of dispersal corridors that link these habitats (Bradford et al. 1993; Delis et al. 1996; Gibbs 1998; Laan and Verbloom 1990; Petranka 1983; Rosenberg et al. 1997; Salonen and Edenhamn 1995; Sjögren 1995; Vos Claire and Stumpel 1996). Characteristics of a wetland itself, however, will have a significant impact on the suite of species that breed in them (Gates and Thompson 1981; Resetarits and Wilbur 1991; Thompson et al. 1980), the survival of amphibian larvae through metamorphosis (Clark 1986; Cortwright 1997; Roth and Jackson 1987; Sadinski 1991; Skelly 1996; Smith 1983), and the survival of metamorphs to adulthood (Cortwright 1993).

The presence of aquatic predators is an important variable in assemblages of larval amphibians and it is strongly influenced by hydrologic regimes. The presence of aquatic predators is important to many populations of amphibians because it affects adult selection of breeding habitats (Kats and Sih 1992; Petranka et al. 1987; Resetarits and Wilbur 1991), competitive outcomes among larvae (Alford 1989; Morin 1983), survival rates of larvae (Caldwell et al. 1980; Heyer et al. 1975; Woodward 1983), and the persistence of populations (Bradford et al. 1993; Monello and Wright 1999). The presence and density of predators are strongly affected by hydrologic regimes and are a positive function of pond depth (Skelly 1996; Woodward 1983). Furthermore, the larvae of amphibian species that typically breed in ephemeral ponds appear to be more susceptible to predation than the larvae of species that breed in permanent ponds (Woodward 1983).

Hydrologic regimes also control the duration in which a breeding habitat retains water. This is another important variable in determining assemblages of larval amphibians because not all species of amphibians have larvae that develop at the same rate. The larvae of large anuran species can require at least one overwintering period in temperate climates to complete metamorphosis; therefore, adults often breed in large, permanent bodies of water. The larvae of many small anuran species, however, have faster developmental rates; therefore adults often breed in shallower, ephemeral bodies of water that typically have small populations of aquatic predators.

Artificial wetlands with only large, permanent bodies of water (typical of many artificial wetlands) may prevent successful colonization by amphibian species whose larvae survive and compete best in smaller, more ephemeral bodies of water. In contrast,

a wetland that only contains ephemeral bodies of water can prevent successful colonization by species that breed in the late spring or summer months because aquatic habitats will not persist long enough for their larvae to complete metamorphosis. A wetland that contains bodies of water with heterogeneous hydrologic regimes (a wetland with both permanent and ephemeral bodies of water) can accommodate a diverse assemblage of amphibian species because there is an interaction between hydrologic regimes and the requirements of larvae from different amphibian species.

A small artificial wetland was created in the summer of 1997 at Rocky Gap State Park (RGSP), Allegany County, Maryland, as part of a mitigation effort associated with the construction of a golf course within the park's boundary. As part of the wetland's design, six ponds of similar surface areas but of various depths were created to encourage colonization by a diverse assemblage of amphibian species. The goal of this study was to describe anuran colonization in an artificial wetland that contained habitats for breeding with a variety of hydrologic regimes. Ponds were monitored for nearly three years to record adult anuran migrations, egg mass deposition, and metamorph emergence. Species that bred at RGSP included *Rana sylvatica* LeConte (the wood frog), *Bufo americanus* Holbrook (the American toad), *Pseudacris crucifer* Weid-Neuwied (the spring peeper), *Hyla versicolor* LeConte (the gray treefrog) and *Rana clamitans* Latreille (the green frog). The primary research objective was to determine if anuran species would differentially colonize bodies of water in the experimental wetland according to characteristics associated with depth. I predicted that anurans that breed in the spring (*R. sylvatica*, *B. americanus*, and *P. crucifer*) would use shallower bodies of water for breeding in greater proportions than species that breed in the summer (*H. versicolor*

and/or *R. clamitans*) because the former typically lay eggs in ephemeral waters, and deeper bodies of water pose a greater risk to the survival of their larvae.

METHODS

Study Site

One of the principal investigators (J. Howard) and a regional ecologist (E. Thompson) from the Maryland Department of Natural Resources evaluated the area surrounding the artificial wetland at RGSP for suitable habitats for amphibians and concluded that the proposed wetland design could provide suitable breeding and upland habitat for local amphibian species. Furthermore, each amphibian species detected at the experimental wetland was found to have bred in at least one body of water within 1 km of the experimental wetland.

Work was completed on the 0.2-ha artificial wetland at RGSP on 7 June 1997. The study was housed in two created wetland areas and a portion of an adjacent existing wetland (Fig. 1). By 1999, naturally colonizing sedges, primarily *Carex* and *Scirpus* spp., and rushes, *Juncus* spp., dominated groundcover in the southern created wetland area (CW1), and rice-cut grass, *Leersia oryzoides* L., and *Scirpus* spp. dominated the northern created wetland area (CW2). The dominant ground cover species were grasses and scouring rush, *Equisetum arvense* L., in the portion of the existing wetland used in the study (EW).

Pairs of ponds were excavated in the three wetland areas (Fig. 1). The surface areas of each pond were approximately 17 m² (4.1 m x 4.1 m) and were arranged so each pair included one shallow pond (< 35 cm deep) and one deep pond (> 40 cm deep). Shallow

ponds were designed to dry out during particularly dry summers (i.e. ephemeral ponds), and deep ponds were designed to remain filled year-round (i.e. permanent ponds). Nominally ephemeral ponds were designed to exclude colonization by some larval amphibian predators, namely *Notophthalmus viridescens* Rafinesque (red-spotted newts), fish, and large odonate (dragonfly) species. In addition, ephemeral ponds should prevent colonization of larger, predatory species of amphibians (i.e., *R. clamitans* and *R. catesbeiana*) whose larvae require overwintering in ponds.

I graded all ponds to include a 1-m-wide shelf on one side, and I planted native emergent vegetation (*Carex* and *Glyceria* spp.) on shelves in the fall of 1997. Emergent vegetation was intended to provide refugia for larval amphibians and structure for oviposition. Larval salamanders use shallow littoral zones during daylight hours to hide from predators (Holomuzki 1986; Stangel and Semlitsch 1987), and the presence of emergent vegetation has a significant impact on the selection of breeding sites for some species of amphibians (Monello and Wright 1999).

Colonization Monitoring Protocol

Drift fences encircled each pond and were erected 1 m from the water's edge. Drift fences were made from 1-m-tall silt fencing (with 30 cm buried into the ground) and were lined with pitfall traps constructed from #10 tin cans (modified from Dodd and Scott 1994). Cans were fitted with 3-cm-wide lips around their open end to prevent individuals from climbing or jumping out of pitfalls. Pitfall traps were spaced 2 m apart and placed on alternating sides of each fence. Small funnel traps were dispersed where necessary because some pitfall traps were continually flooded. Funnel trap dimensions were 15 cm x 15 cm x 30 cm, funnels were shaped from aluminum flashing, frames were

made from 13-mm² hardware cloth wrapped in window screening, and the number of funnel traps/pond remained constant.

The experimental wetland at RGSP was monitored for amphibian colonization from July through November in 1997, from February through November in 1998, and from February through August in 1999. I conducted surveys for egg masses and checked traps daily. I identified egg masses to species, mapped their locations, estimated the number of eggs in each mass, and categorized locations of masses as either permanent pond, ephemeral pond, or puddle. I was not able to locate eggs laid by *P. crucifer* (spring peepers) because females deposit single eggs on submerged vegetation. Therefore, the estimated number of eggs laid by *P. crucifer* was estimated as the number of gravid females captured in traps associated with a pond multiplied by the average clutch size for the species (Wright 1917).

Reproductive output for each species was expressed in egg densities because the surface area of ephemeral ponds, permanent ponds, and puddles differed between wetland areas (Appendix 1). Egg density was defined as the total number of eggs laid in an experimental unit per unit surface area. Pond and puddle surface areas used in the calculation of egg densities were determined by measurements taken in the fall of 1999. I assumed that the surface area of each pond remained constant throughout the year (regardless of changes in depth) because ponds were excavated to have edges perpendicular to their floor. Puddle surface areas within wetland areas were estimated by measuring the dimensions of depressions that had filled with water during the spring months. Most depressions contained water during the beginning of the summer months, although all puddles dried by the end of the summer. To account for changes in puddle

surface area due to drying, I assumed that amphibian species that bred in the summer months had half of the total puddle surface area available to them as compared to amphibian species that bred in the spring.

Adult amphibians captured in traps were given individual toe-clips, sexed when possible, had their snout-to-vent length recorded, and then were released on the opposite side of the drift fencing. Newly metamorphosed amphibians that were captured were given toe-clips to indicate the year, wetland area, and pond class (ephemeral or permanent) of emergence, had their snout-to-vent lengths recorded, and then were placed on the opposite side of the drift fencing. Time-constrained searches were conducted around pond perimeters to increase the number of metamorphs captured because the metamorphs of some species were difficult to trap.

I believe that the capture of adult amphibians in this study did not produce reliable data because rates of trespass for ponds were high (> 70 % per species), and most anuran species could potentially visit all ponds within daily movements. As a result, anuran colonization of the experimental wetland was evaluated solely by analyzing data collected during surveys for egg masses and the trapping of gravid *P. crucifer* females. I believe that egg mass and individual egg counts gave a direct and accurate measure of attempts at reproduction, and the capture of female *P. crucifer* gave a reasonable estimate of the relative number of eggs laid by *P. crucifer* in hydrologic classes (permanent ponds, ephemeral ponds, and puddles). Most female *P. crucifer* were captured and released with males amplexing them, and it is unlikely that gravid females left ponds without depositing their eggs.

Statistical Analysis

I tested the null hypothesis that individual amphibian species distributed eggs in nominally permanent ponds, nominally ephemeral ponds, and puddles in equal proportions. This experiment was a randomized complete block design in which each wetland area was considered a block that contained three classes of a hydrologic treatment (one permanent pond, one ephemeral pond, and a series of puddles). Friedman's test (Conover 1980) was performed to determine if significant differences existed between combinations of species x hydrologic classes. Friedman's test does not compute a test statistic for an interaction, therefore, a species by hydrologic class interaction could only be detected by comparing differences between hydrologic classes within species. If observed differences between hydrologic classes within at least one species are not identical to differences seen in other species, then a species by hydrologic class interaction is present. Friedman's test was used instead of a two-way ANOVA because Bartlett's test (Zar 1996) indicated that variances were not homogeneous. Two additional Friedman's tests (both blocked by species) were used to detect if anurans, collectively, distributed equal densities of eggs among wetland areas and if they distributed equal densities of eggs among hydrologic classes. The criterion for statistical significance was $\alpha = 0.05$. All descriptive statistics were calculated using Statistical Analysis System (SAS) version 6.12 (SAS Institute Inc. 1990). Analyses were calculated separately for each field season because populations of amphibians can show tremendous variation in reproductive output between years due to changes in environmental conditions (Pechmann et al. 1991).

RESULTS

Five anuran species bred in the experimental wetland at RGSP in 1998 and 1999. Breeding seasons for species were defined as the period during which multiple males of the species could be heard calling or egg masses could be found. The onset of breeding occurred later for *R. sylvatica* and earlier for *R. clamitans* in 1999 than 1998, although the order in which species bred remained consistent between years (Fig. 2).

In 1998, anurans, collectively, did not lay significantly different egg densities among hydrologic classes ($S = 0.0$, $k = 3$, $n = 4$, $P > 0.05$). The mean egg density for all anuran eggs laid in permanent ponds was 31.38 eggs/m² (S.E. = 11.26), the mean egg density for ephemeral ponds was 28.13 eggs/m² (S.E. = 17.37), and the mean egg density for puddles was 57.51 eggs/m² (S.E. = 26.94). There were significant differences between egg densities laid in wetland areas ($S = 6.70$, $k = 3$, $n = 5$, $P < 0.05$), and egg densities were significantly higher in CW1 than EW ($|R_{CW1} - R_{EW}| = 8$, $k = 3$, $n = 4$, $P < 0.05$) (Table 1). *Rana sylvatica* and *B. americanus* appeared to be the only species to lay high egg densities in puddles, but Friedman's test failed to detect differences in egg densities between hydrologic classes for any species ($S = 9.74$, $k = 14$, $n = 3$, $P > 0.05$) (Fig. 3). In addition, *R. clamitans* was the only species to lay eggs in all three hydrologic treatments and gravid *P. crucifer* females were only captured at permanent ponds. Large standard errors were associated with mean egg densities because four out of five species deposited eggs in fewer than half of all bodies of water available to them (Table 2). Both *R. sylvatica* and *H. versicolor* laid eggs in 22% of all bodies of water, *B. americanus* and *P. crucifer* each laid eggs in 33%, and *R. clamitans* laid eggs in 55% of all bodies of water.

Four of five species deposited eggs in bodies of water in CW1, all species deposited eggs in CW2, and only one species deposited eggs in EW.

In 1999, the mean egg density for all anuran eggs laid in permanent ponds was 72.14 eggs/m² (S.E. = 29.26). The mean egg density for ephemeral ponds was 126.54 eggs/m² (S.E. = 49.80), and the mean egg density for puddles was 40.76 eggs/m² (S.E. = 26.76). There were no significant differences between egg densities laid among hydrologic classes for combined species ($S = 3.50$, $k = 3$, $n = 4$, $P > 0.05$) or among wetland areas ($S = 2.80$, $k = 3$, $n = 5$, $P > 0.05$) (Table 1). Four of five species deposited eggs in bodies of water in CW1 and CW2, and all species deposited eggs in EW (Table 3). Egg densities were not significantly different among hydrologic classes for any species ($S = 12.43$, $k = 14$, $n = 3$, $P > 0.05$), although the only species to lay eggs in puddles, *R. sylvatica* and *B. americanus*, were spring-breeding species (Figure 4). In addition, *R. clamitans* and *H. versicolor* (two summer-breeding anurans) appeared to lay a majority of their eggs in permanent ponds and laid no eggs in available puddles. Large standard errors were associated with mean egg densities although two species laid eggs in more than half of the bodies of water available to them. Both *R. sylvatica* and *B. americanus* laid eggs in 33% of all bodies of water, *H. versicolor* laid eggs in 44%, *R. clamitans* laid eggs in 56%, and *P. crucifer* laid eggs in 100% of all bodies of water where they could be monitored.

DISCUSSION

Anuran species, collectively, did not deposit significantly different egg densities among permanent ponds, ephemeral ponds, and puddles. Individual anuran species did not deposit significantly different egg densities among hydrologic classes, although *B.*

americanus and *R. sylvatica* (both spring-breeders) used puddles for egg deposition and *R. clamitans* and *H. versicolor* (both summer breeders) did not in 1999. There were high amounts of variation in egg densities among hydrologic classes, within species, during both years of the study. This variation prevented me from using more powerful statistical analyses (i.e. ANOVA) and I could not reject the null hypothesis that all anuran species in the experimental wetland at RGSP deposited eggs in similar proportions among available hydrologic classes. Tradeoffs between predation pressure and the threat of desiccation due to pond drying will differ between amphibian species due to the physiology (Wilbur 1987; Woodward 1983), behavior (Alford 1989; Smith 1983), and the developmental rates of their larvae (Morin 1983; Semlitsch 1988; Semlitsch and Wilbur 1988; Skelly 1996). In light of that research, I expected to observe a species by hydrologic class interaction because individual amphibian species would lay proportionately more eggs in bodies that their larvae would survive and compete best in.

Egg densities of anurans, as a whole, were different between wetland areas in 1998. Although groundcover species that dominated wetland areas were different (Appendix 2), differences in egg densities between wetland areas in the experimental wetland may not be due entirely to differences in vegetative assemblages. Sites for oviposition in amphibians have been correlated not only with characteristics of vegetation (Gates and Thompson 1981; Mitchell and Miller 1991; Monello and Wright 1999), but also such factors as the presence of predators (Kats and Sih 1992; Monello and Wright 1999; Resetarits and Wilbur 1991), water chemistry (Gates and Thompson 1981; Rowe et al. 1993), and the presence of other amphibian larvae (Resetarits and Wilbur 1991). In addition, drought conditions appeared to have resulted in lower mean depths and higher

mean water temperatures in ponds at RGSP in 1999 compared to 1998 (Appendix 3). During both years of this study, *Ambystoma jeffersonianum* eggs were placed in ponds at RGSP as part of an amphibian translocation program. Although the presence of these predators could have affected the selection of breeding sites by anurans, these effects should have been felt evenly among all ponds since approximately equal numbers of eggs were placed in each pond.

The results of this experiment do not provide statistical support for the construction of wetlands that contain bodies of water with various hydrologic regimes because trends within most species were not consistent between years. However, these results do not justify current artificial wetland designs that frequently create only large, permanent bodies of water. Although large, permanent bodies of water could provide habitat for substantial populations of tadpoles, these bodies of water are most readily colonized by large species of anurans (notably the bullfrog, *Rana catesbeiana* Shaw) that have been implicated in the reduction and disappearance of many populations of small-bodied anurans (Christiansen and Bailey 1991; Hayes and Jennings 1986; Lanno et al. 1985; Maunder 1983; Oldham 1992). Furthermore, important population processes such as larval survival could not be measured accurately, and the duration of the study was not sufficient to document population dynamics that could result in niche partitioning among shallow and deep bodies of water. Differential survival rates of larvae, coupled with the philopatric behavior typical of many amphibian species, might result in niche partitioning among deep and shallow waters after several generations even if adults deposited eggs in deep and shallow waters in similar proportions during the initial stages of colonization.

Under this scenario, amphibian species could establish populations differentially in either deep or shallow waters within a single artificial wetland.

To test alternative wetland designs, a robust experiment would consist of wetlands grouped into habitat classes that would include multiple wetlands containing either all ephemeral bodies of water, all permanent bodies of water, or both ephemeral and permanent bodies of water. Diversity indices of each habitat class could then be compared in a pair-wise fashion to determine which class is colonized by the most diverse assemblage of amphibian species. The scale of such a project is much larger than that afforded by most individual mitigation efforts, although the construction of large and environmentally controversial “wetland banks” may afford opportunities to conduct such research.

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Table 1. Mean egg densities of all anuran eggs by wetland area for 1998 and 1999

	1998		1999	
	Mean (eggs/m ²)	Std. error	Mean (eggs/m ²)	Std. error
CW1	55.88	18.40	104.49	47.37
CW2	51.58	23.83	45.82	22.61
EW	5.59	5.59	101.87	43.67

Table 2. Egg densities (eggs/m²) among individual ponds in 1998 and 1999.

Species	Hydrologic class	1998			1999		
		CW1	CW2	EW	CW1	CW2	EW
<i>Rana sylvatica</i>	Permanent pond	0	0	0	0	0	191.1
	Ephemeral pond	0	17.4	0	0	0	43.9
	Puddles	0	269.3	0	0	30.6	0
<i>Bufo americanus</i>	Permanent pond	127.6	0	0	0	0	94.0
	Ephemeral pond	0	0	0	0	0	0
	Puddles	142.3	183.6	0	142.3	275.4	0
<i>Pseudacris crucifer</i>	Permanent pond	68.0	57.4	0	102.0	114.8	501.3
	Ephemeral pond	0	0	0	170.2	46.5	350.9
	Puddles	NA	NA	NA	NA	NA	NA
<i>Hyla versicolor</i>	Permanent pond	43.8	0	0	112.1	0	42.6
	Ephemeral pond	0	20.3	0	23.9	0	6.6
	Puddles	0	0	0	0	0	0
<i>Rana clamitans</i>	Permanent pond	95.7	0	78.3	646.3	0	94.0
	Ephemeral pond	210.1	174.2	0	266.0	174.2	0
	Puddles	94.9	0	0	0	0	0

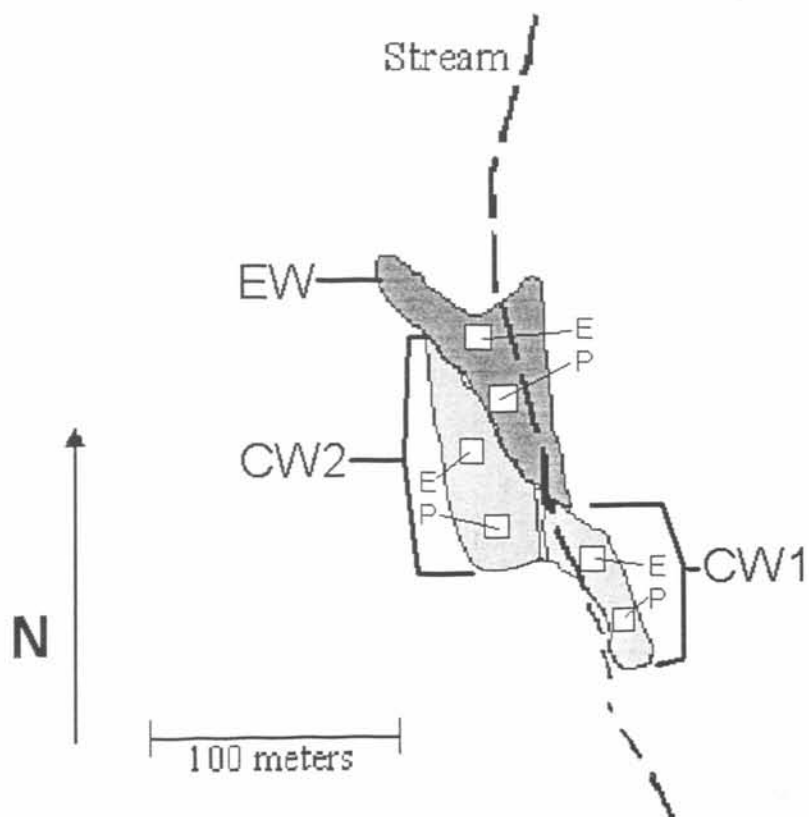


Figure 1. Map of experimental wetland at Rocky Gap State Park, Allegheny Co., Maryland. EW = existing wetland habitat. CW = created/mitigated wetland habitat. P = permanent pond. E = ephemeral pond.

		March	April	May	June	July	August	Sept.	Oct.
<i>Rana sylvatica</i>	1998		←→						
	1999		←→						
<i>Pseudacris crucifer</i>	1998		←→		←→				
	1999		←→		←→				
<i>Bufo americanus</i>	1998		←→		←→				
	1999		←→						
<i>Hyla versicolor</i>	1998				←→		←→		
	1999				←→				
<i>Rana clamitans</i>	1998				←→		←→		
	1999				←→			?	

←→ Breeding season (egg laying & males calling)

←→ When metamorphs emerged

Figure 2. Breeding phenology of amphibians in experimental wetland at RGSP.

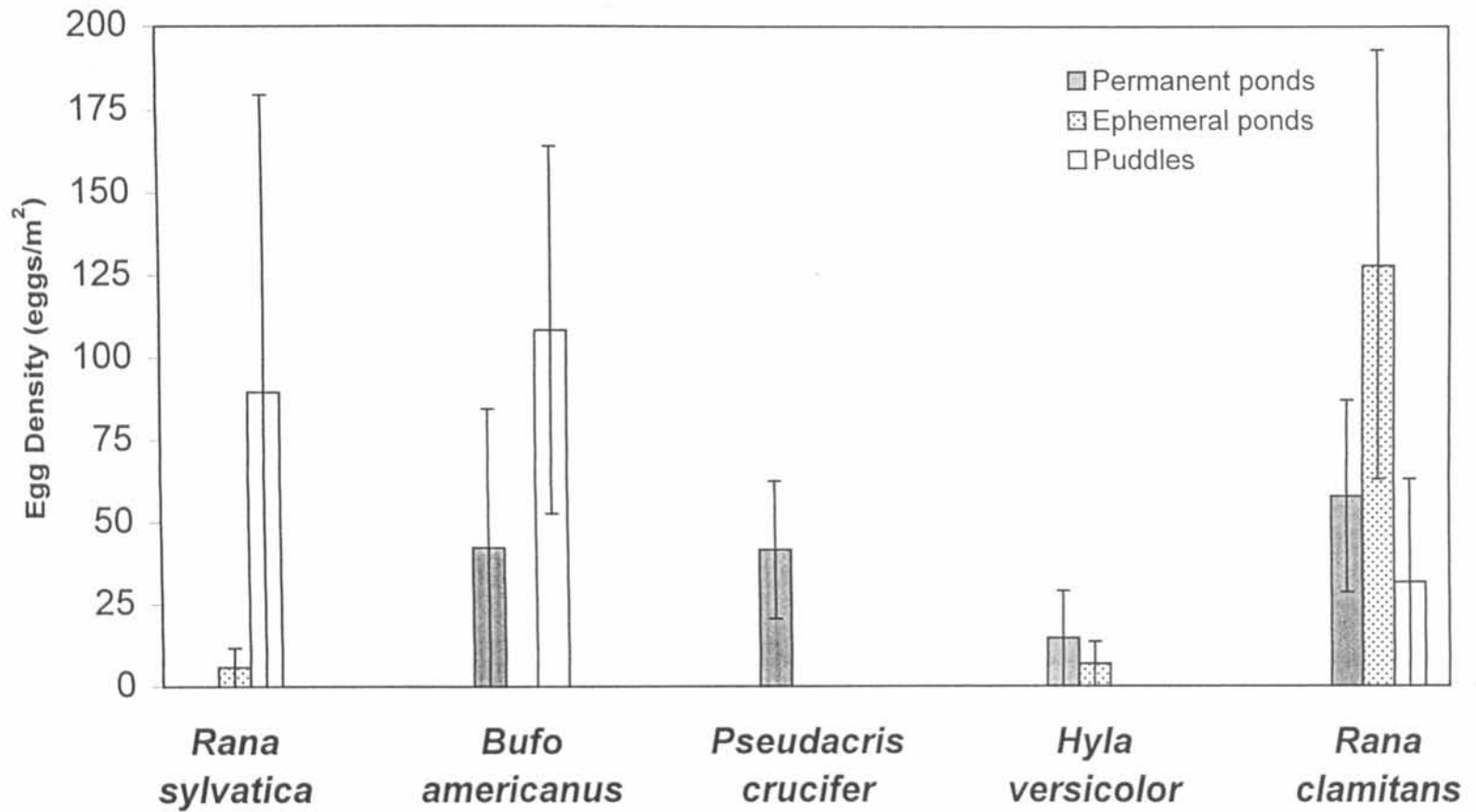


Figure 3. Egg densities for hydrologic classes in 1998.

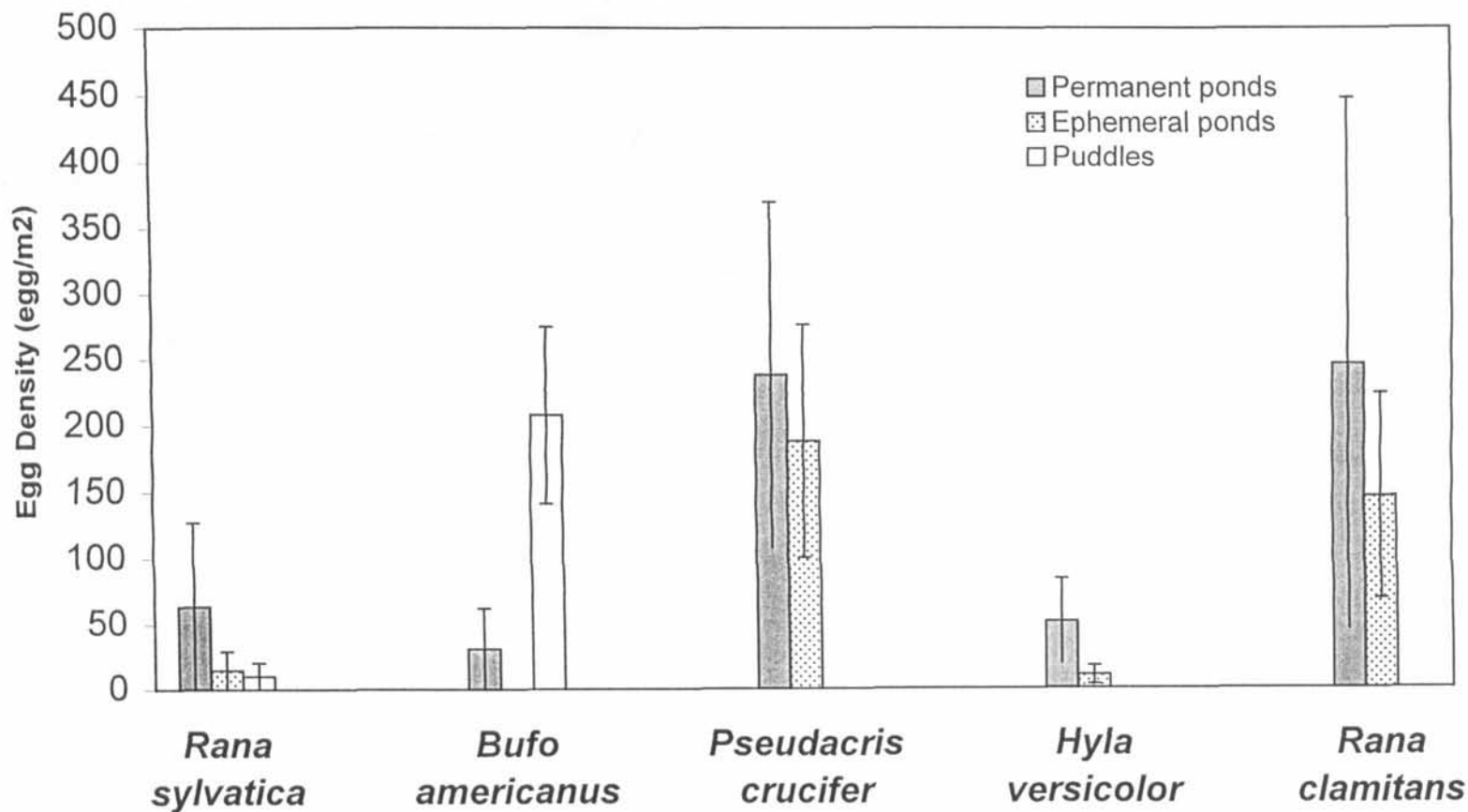


Figure 4. Egg densities for hydrologic classes in 1999.

Appendix 1: Total surface area available to amphibians by breeding season, hydrologic class, and wetland area

	Body of water	Wetland Area			Total
		CW1	CW2	EWE	
SA available for spring breeders (m²)	Permanent pond	23.5	13.9	16.0	53.4
	Ephemeral pond	18.8	17.2	13.7	49.7
	Puddles*	21.08 (6)	16.34 (8)	0.25 (1)	37.7
SA available for summer breeders (m²)	Permanent pond	23.5	13.9	16.0	53.4
	Ephemeral pond	18.8	17.2	13.7	49.7
	Puddles*	10.54 (6)	8.17 (8)	0.13 (1)	18.8

* Number of puddles in parentheses

Appendix 2: Most abundant groundcover species by wetland area in 1999

CW1		CW2		EW	
Species	% Groundcover	Species	% Groundcover	Species	% Groundcover
<i>Carex</i> spp.	24.5	<i>Leersia oryzoides</i>	53.4	Misc. grass spp.	37.2
<i>Juncus</i> spp.	12.3	<i>Scirpus</i> spp.	13.9	<i>Equisetum arvense</i>	31.5
<i>Mentha</i> spp.	8.6	<i>Carex</i> sp. 1	8	<i>Filipendula ulmaria</i>	11.5
Misc. grass spp.	8.3	Misc. grass spp.	5.4	<i>Eupatorium</i> spp.	5.4
<i>Eupatorium</i> spp.	7.6	<i>Juncus</i> spp.	1.9	<i>Mentha</i> spp.	3.1
<i>Scirpus</i> spp.	4	<i>Dipsacus laciniatus</i>	1.5	<i>Carex</i> spp.	2.7
<i>Cephalanthus occidentalis</i>	1.4	<i>Cephalanthus occidentalis</i>	1	<i>Dipsacus laciniatus</i>	1.1

**Appendix 3: Depth and thermal profiles from for ponds at RGSP in 1998
and 1999 (April through August)**

Variable	Year	CW1-P	CW1-E	CW2-P	CW2-E	EW-P	EW-E
Average depth (cm)	1998	52.8	37.2	34.1	25.0	37.3	18.1
Max Depth Change (cm)	" "	35	12	18	9	18	27
Pond dried completely	" "	No	No	No	No	No	Yes
Average water temp. (°C)	" "	21.6	20.8	21.1	20.8	20.9	21.2
Average depth (cm)	1999	31.3	19.0	28.6	18.9	32.7	11.0
Max Depth Change (cm)	" "	53	30	17	9	18	25
Pond dried completely	" "	No	Yes	No	No	No	Yes
Average water temp. (°C)	" "	23.8	21.3	21.5	20.1	21.5	21.2

CHAPTER 4: CONCLUSION

I completed a more detailed evaluation of success than most amphibian RRT protocols, although I encountered difficulty in replicating my experiment and comparing source and translocated populations. Investigators suggest that amphibian RRT protocols should have an experimental design that allows for hypothesis testing (Hein 1997; Reinert 1991). A robust experimental design proved difficult at RGSP because vegetation and hydrology were markedly different between wetland areas. To understand results more clearly, investigators should carefully measure water chemistry and invertebrate populations within experimental units because they may find it difficult to produce accurate replication of their experiment. To evaluate project success, comparisons to natural populations are necessary (Burke 1991; Dodd and Seigel 1991). Measures of success in translocated populations appeared comparable to natural populations, but sounder conclusions could have been made if hatching success for source populations of *P. triseriata* were measured and if metamorphs were trapped at the source population of *A. jeffersonianum* in 1998.

I expected anuran species at RGSP to partition breeding habitats according to hydrologic treatments. Anuran species, collectively and individually, did not deposit significantly different egg densities among hydrologic treatment classes. Again, variation in vegetation and hydrology between wetland areas made it difficult to create a robust experimental design. Wetland characteristics (such as vegetation) could have influenced the choice of ovoposition sites as much as hydrologic class and trends within most

species were not consistent between years. Furthermore, inadequate replication resulted in non-normal distributions and heterogeneous variances that limited data analysis to non-parametric tests.

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