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Constructed Ponds as Mitigated Habitat for the Wood Frog (*Rana sylvatica* LeConte) and the Spotted Salamander (*Ambystoma maculatum* Shaw) in West Virginia

Celeste Dawn Good

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Constructed Ponds as Mitigated Habitat for the Wood Frog
(*Rana sylvatica* LeConte) and the Spotted Salamander
(*Ambystoma maculatum* Shaw) in West Virginia

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Marshall University

In partial fulfillment of the
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Master of Science
Biological Sciences

By

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ABSTRACT

Constructed Ponds as Mitigated Habitat for the Wood Frog (*Rana sylvatica* LeConte) and the Spotted Salamander (*Ambystoma maculatum* Shaw) in West Virginia

By Celeste D. Good

Many forest dwelling amphibians depend upon aquatic breeding habitats, making them susceptible to habitat changes. To determine if amphibian use of temporary pools occurred, 9 ponds were constructed in 3 forested areas on the MeadWestvaco Wildlife and Ecosystem Research Forest. Studies were conducted in 6 ponds during 2004, and all 9 in 2005 using drift fences. Trapped amphibians were measured and given a pond specific mark with visible implant elastomers. A significant difference was found between low and high elevation sites for juvenile *R. sylvatica* snout-to-vent length. No significant differences were found for soil, air or water temperatures between sites at differing elevations. Low elevation *R. sylvatica* juveniles egressed and developed 2-3 weeks before those at high elevation sites. Tadpoles did not successfully metamorphose from 3 ponds in 2005. Clear-cut treatments surrounding the ponds will be applied in 2006 and results will be compared to baseline data.

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LITERATURE REVIEW

With increased land development, amphibian species have been in decline since the beginning of the last century (Dimauro and Hunter 2002). They have permeable skin, eggs and gills, which makes them sensitive to environmental changes (Dunson et al. 1992). Some amphibian life cycles require aquatic habitat for breeding and larval growth, and terrestrial habitat for foraging and hibernation, making them sensitive to alterations in both (Porej et al. 2004; Griffiths 1997; Rothermel and Semlitsch 2002). This emphasizes the importance of conservation for both types of habitat to encourage amphibian survival within timbered forests. Conservation and connectivity of these habitats must be understood, as amphibians require terrestrial habitat that is adjacent to aquatic habitat for breeding.

Rana sylvatica (Wood Frog) and *Ambystoma maculatum* (Spotted Salamander) have been documented as highly philopatric to their breeding ponds, meaning that they return to their natal ponds to breed each year (Madison 1997; Berven and Grudzien 1990). *A. maculatum* have been shown to disperse an average of 118 m away from the breeding pond ranging from between 20 m to 158 m (Madison 1997). Semlitch (1998) suggested a buffer zone of 164.3 m for pond breeding salamanders based upon their findings and previous studies. This range encompasses an average of the movement of pond breeding salamanders away from their breeding range. *Ambystoma maculatum* seemed to be most sensitive to changes in their environment within 250 m of their breeding pool (Herrmann et al. 2005). The movement of *R. sylvatica* adults and emigrating juveniles follows a non-random migration pattern of at least 300 m (Vasconcelos and Calhoun 2004), and genetic dispersal for an area populated by these

amphibians is over 1000 m (Berven and Grutzien 1990). Adult *R. sylvatica* have been found to be 100 % philopatric, while juveniles are 80 % philopatric (Berven 1990). Other studies have demonstrated differences in philopatry between sexes of *R. sylvatica*. Adult females were documented to be 88 % philopatric to the breeding pond in which they were originally marked, while adult males were 98% philopatric (Vasconcelos and Calhoun 2004). In the same study, adult *A. maculatum* of both sexes were 100% faithful to their breeding ponds. Some amphibians are so highly philopatric that they will continue to travel to their old breeding site, even after it has been removed or destroyed (Pechman et al. 2001). This emphasizes the importance of early establishment of constructed habitat before original habitat is destroyed.

Both *R. sylvatica* and *A. maculatum* use anthropogenic pools, or pools incidentally created by machinery and human land use during timbering. These can become reproductive “sinks”, meaning that reproductive energy is expended for breeding, but there is high juvenile mortality due to premature pool drying in comparison to naturally occurring pools. *R. sylvatica* juveniles emerged earlier from anthropogenic pools and were smaller in size than those emigrating from natural pools (DiMauro and Hunter 2002). In bottomland forests, ruts and skid trails created during logging have less water level fluctuation than natural vernal pools, which reach their highest water levels in winter and lowest levels in summer (Cromer et al. 2002).

Constructed ponds have been successfully colonized by amphibians for breeding in previous studies (Pauley (2005); Pechman et al. 2001). Ponds which are purposely made should be constructed with means to retain water for a sufficient period of time to prevent ecological sinks. Both *R. sylvatica* and *A. maculatum* require breeding ponds

with a hydroperiod of at least 4 to 9 months (Paton and Crouch III, 2002). A network of ponds may be best to prevent metapopulation genetic drift from occurring (Griffiths 1997). This would decrease the likelihood that populations would become isolated thereby cutting off and limiting new gene flow into the population. However, larval success and water quality factors in constructed ponds are not well known.

Burne and Griffin (2005) conducted a study which examined factors related to species richness of amphibians in temporary ponds. The length of time that temporary ponds held water was associated with a greater number of species colonizing them. Additionally, canopy cover and pond vegetation were associated with the types of species which colonized the ponds. Different species have varying habitat requirements; some have a better chance at survival with increased vegetation, utilizing it as cover, and for egg attachment.

The longer a pond holds water during the year has been correlated with a greater diversity of predators that will occupy it. Those with a brief hydroperiod, contained odonates and predatory beetles, while ponds with a longer hydroperiod had a greater number of odonates and salamanders. Created ponds that hold water for a long period of time, could potentially attract greater numbers of predators, which may prey upon breeding amphibians, eggs and larvae (Skelly et al. 2002).

Ambystoma maculatum and *R. sylvatica* require fishless ponds that hold water for a certain amount of time in order to successfully complete the aquatic stages of their lifecycles (Skidds and Golet 2005). These species utilize aquatic habitats for a span of 6 months, generally from February through July or August (Skidds and Golet 2005).

Porej et al. (2004) examined habitat characteristics associated with pond breeding amphibians. The amount of forest within 1 km of the population was positively associated with pond use of both *A. maculatum* and *R. sylvatica*, which emphasizes the importance of terrestrial buffer zones for amphibian species. Herrmann et al. (2005) noted that species richness seemed sensitive to changes surrounding breeding ponds up to 1000 m. Additionally, *R. sylvatica* were less likely to be found in areas where canopy cover was reduced to less than 40 percent, and most likely to occur where canopy was greater than 60 percent.

Movement between terrestrial and aquatic habitat is important when planning conservation measures. During the non-breeding season, male *R. sylvatica* have been found to travel further than 65 m from the breeding ponds, while females remained within 65 m of the pond (Regosin et al. 2003). Rothermel (2004) monitored juvenile movement of *A. maculatum* and *B. americanus*. The amphibians that reached forested areas successfully originated from ponds less than 50 m away. Those from ponds at greater distances were not captured as often, suggesting that dispersal distance to the forest, especially in fragmented areas, may influence juvenile success. Studies have shown movement of juvenile and adult striped newts and narrow-mouthed toads to be non-random within wetland habitat (Dodd and Cade 1998). Migration to or from wetlands does not appear to occur through narrow corridors. During a long term study, *A. maculatum* used the same entrance and exit between pond and terrestrial habitat between years (deMaydenier and Hunter 1999). In conservation, corridors might be more successful if movement patterns of the amphibians endemic to the area are understood. Emigration studies have shown that both *A. maculatum* and *R. sylvatica* choose a non-

random path of movement toward forested habitat, and avoid settling in edge habitat (deMaydenier and Hunter, 1999; Werner and Glennemeier 1999; Vasconcelos and Calhoun 2004).

Edge effects occur when species avoid natural or created vicinities along the forest edge. *Rana sylvatica* and *A. maculatum* have been found to avoid habitat 35 m from the forest edge (deMaydenier and Hunter 1998). This suggests that conservation plans which involve a buffer zone away from altered habitat would need to take edge effects into consideration. Other forest dwelling amphibians may experience these effects as well, including terrestrial species, such as *Plethodon cinereus* (Red-backed salamander) (deMaydenier and Hunter 1998). Some salamander species will not avoid older edges if plant re-growth has occurred; however they will avoid newly created edges. Plant recovery occurs over time, and more cover is available at older clear cut edges. Anuran species, such as *R. sylvatica* avoid edges along both new and old clear cuts (deMaydenier and Hunter 1998). *Rana clamitans* were trapped more often within a forested area at 50 m from a clearcut than 0 m, and *A. maculatum* were trapped more often within forested areas at 50 m and 200 m away from a clearcut than at 0 m (Renken et al. 2004). *Ambystoma maculatum* were trapped more often crossing forested areas than fields (Rothermel and Semlitch 2002).

Little is known about the hydrology of constructed ponds. Natural pools may be fed by underground springs, or first-order streams, while constructed ponds rely on sufficient precipitation to sustain through the breeding and larval period of amphibians colonizing them. These factors affect the development periods for larval amphibians, and are important when considering habitat characteristics (Freda and Dunson 1985). Low

dissolved oxygen (DO) levels in temporary pools, have been associated with high canopy cover, a contributing factor is a high amount of leaf litter that decomposes in ponds (Werner and Glennemeier 1999). Werner and Glennemeier (1999) also stated DO was commonly found to be lower for temporary closed canopy pools, than permanent open canopy ponds. Additionally, DO was higher in the earlier months and lower in the later months for temporary closed canopy pond. *Rana sylvatica* tolerate low DO levels, and are found in high numbers in closed canopy forest ponds, while other species like *Rana pipiens* are unable to fare well in low DO closed canopy forest ponds, therefore breeding in open field ponds. For both species, low DO was associated with decreased growth and survival rates, but these effects were more marked in *R. pipiens* (Werner and Glennemeier 1999).

SPECIES ACCOUNTS

RANA SYLVATICA

Rana sylvatica is a species which occurs in almost all counties of West Virginia (Fig. 1), in forested areas from low to high elevations (Green and Pauley 1987). Its range in North America (Fig. 2) extends north from Labrador to Alaska, throughout the Appalachians, south to Alabama, Georgia and South Carolina, and west to Tennessee, Kentucky, Indiana, Wisconsin and Minnesota (Green and Pauley 1987; Conant and Collins 1998). These frogs are characterized by a “robber’s mask” across the eyes and range from light pinkish tan to dark brown in color (Fig. 3). Their belly is mostly white, and the legs are marked with dark stripes (Conant and Collins 1998). Adults can reach a size of up to 7.6 cm (Green and Pauley 1987). This species exhibits sexual dimorphism in that males are smaller than females, and during mating season males are generally darker than females. Males also have larger thumbs during mating season, which aids them during amplexus (Davis and Folkerts 1986). Their call has been said to resemble the sound of squabbling ducks.

They emerge from hibernation to breed during the first rains in early spring or late winter, between February and March (Davis and Folkerts 1986; Green and Pauley 1987). Snow may still be on the ground, and they have been seen in ponds where ice is still present (Green and Pauley 1987). They breed in temporary, fishless ponds located within forested habitat. Breeding occurs later in higher elevations because of lower temperatures and inclement weather. The breeding season is explosive, lasting 1-2 weeks. Males will mount females, holding them in amplexus for up to 4 days, until eggs are fertilized. It is believed that they fast during this time. Each female will only mate

with 1 male, and may lay between 400 and 900 eggs. Females lay in communal masses around a shallow area of a woodland pond and attach their eggs to vegetation (Davis and Folkerts 1986). Eggs are clear in appearance and are clumped in masses (Fig. 4). Eggs hatch generally hatch within 2-3 weeks, depending on temperatures and weather conditions. Tadpoles develop into juvenile frogs in 1.5-2.5 months (Green and Pauley 1987). Juveniles remain near the pond for a few weeks, and then emigration generally coincides with precipitation. It has been documented that they stay within 100 m of their breeding pond. It can take 1-2 years for males to reach sexual maturity, and for females it has been known to take up to 3 years, however most females are mature by 2 years of age (Bellis 1965; Berven 1982). Sub-adults inhabit the surrounding forest areas until adulthood when they return to natal ponds to breed (Berven 1990).

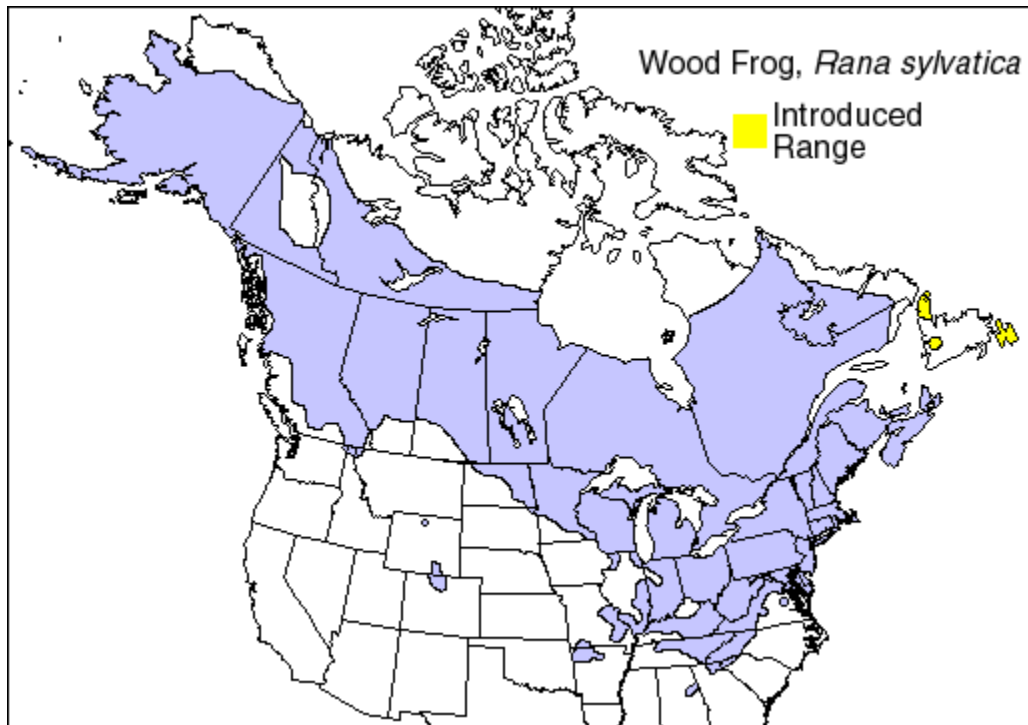


Figure 2. Range of *R. sylvatica* in North America
(USGS website: <http://www.npwrc.usgs.gov/narcam/idguide/wood1.htm>)



Figure 3. A *Rana Sylvatica* subadult found at Site 2,
80 m away from the first pond, June 11, 2005.
(Photo by: Celeste Good)

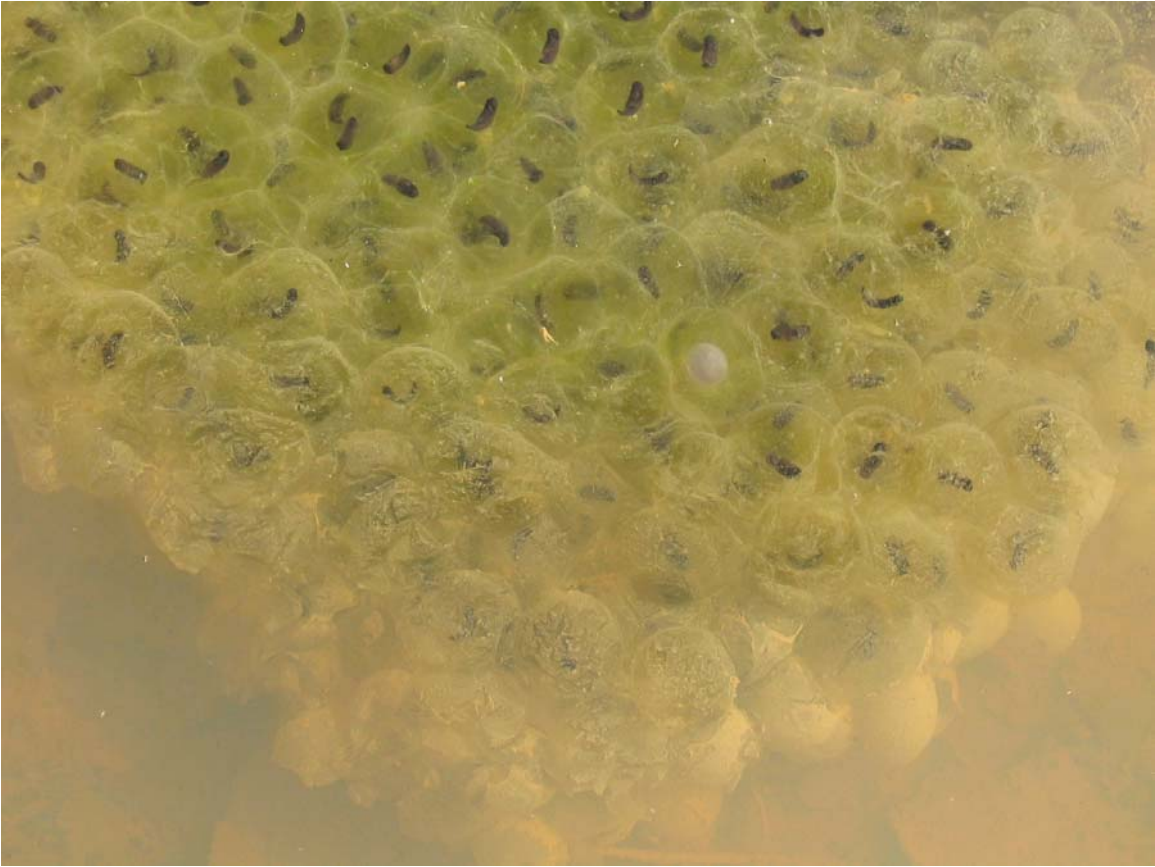


Figure 4. *Rana sylvatica* egg mass March 22, 2005 from Site 1, Pond 1.
(Photo by: Becky Murray)

AMBYSTOMA MACULATUM

The range of *A. maculatum* in West Virginia is believed to be statewide throughout forested areas (Fig. 5). In North America, (Fig. 6) it ranges from New England to northern Florida, then extends west through Illinois, Oklahoma and Texas (Green and Pauley 1987). Salamanders in the family Ambystomatidae are known to spend most of their lives underground. *Ambystoma maculatum* are dark gray to black in color with 2 irregular dorsal rows of large yellow spots (Fig. 7). They have a shovel shaped snout common to the family Ambystomatidae. Adult size can be up to 20.3 cm and 12 costal grooves are generally present along the sides (Green and Pauley 1987).

They emerge from underground burrows during the first rains between February and early March to breed in fishless, temporary ponds located within a forested habitat. Mating occurs within 1 week, and is explosive. Males deposit spermatophores which contain sperm on aquatic grasses or vegetation near the water. Females pick up the spermatophores and hold them inside their cloaca until eggs are fertilized. Egg masses may contain 12-250 eggs, are whitish in appearance, attached to vegetation, and usually laid in communal masses (Green and Pauley 1987).

Radio tracked *A. maculatum* were highly associated with short tailed shrew burrows (Madison 1997). During cold periods, and foraging, they use these burrows for shelter from freezing temperatures. As a defense, they have skin secretions which discourage predators. In the non-breeding season, they forage in the surrounding forest. The distance they traveled from the breeding ponds has been found to be between 20 m to 158 m (Madison 1997).

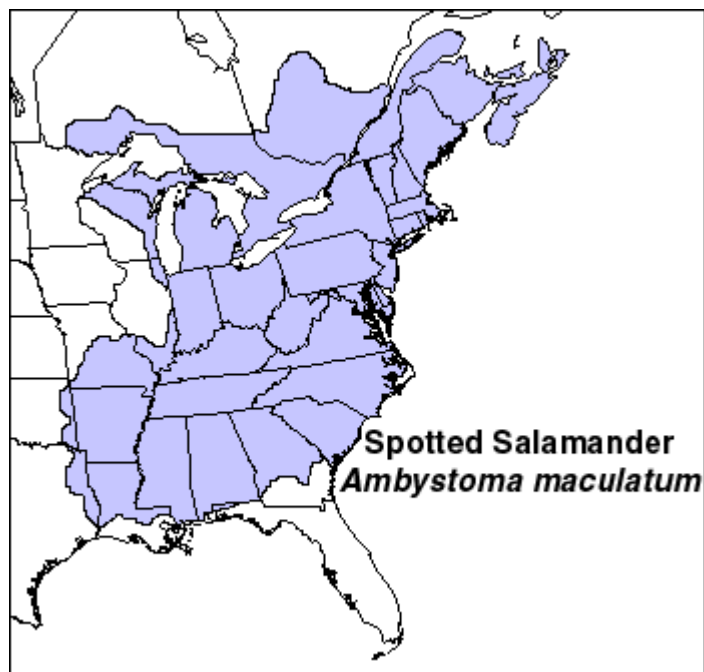


Figure 6. Range of *A. maculatum* in North America
(USGS website: <http://www.npwrc.usgs.gov/narcam/idguide/ambymacu.htm>)



Figure 7. *Ambystoma maculatum* adults

<http://www.marshall.edu/herp/Salamanders/spotted.htm>

INTRODUCTION

In the 1880's, almost all the forests of West Virginia were completely clear-cut and in many cases the land was burned in order to be used for agriculture. By the 1920's, most of the original forests had been removed (Clarkson 1997). Today, an estimated 78% of West Virginia is covered by forested habitat, and 98% of this is available for timber harvesting. Of this land, 88% is privately owned and only 12% is publicly owned (Griffith and Widmann 2003). This subjects a great amount of land to potential timber harvesting and impacts amphibians that solely breed in woodland ponds. Conservation of resources is important when obligate breeding habitat is removed during the course of timbering. Many forest dwelling species depend upon ephemeral pools for breeding and habitat, including *A. maculatum* and *R. sylvatica*. There are many other species that require this type of habitat. In the eastern United States, there are 10 species of salamander and 19 species of frogs and toads which are associated with temporary ponds (Pauley et al. 2000).

One potential conservation method involves the construction of new temporary ponds within adjacent unharvested areas for breeding purposes, along with establishment buffer zones to allow connectivity to forested terrestrial habitat. The objectives of this study were (1) to monitor constructed ponds for utilization by amphibians (*A. maculatum* and *R. sylvatica*) for breeding, (2) to monitor and mark juveniles emigrating from the constructed ponds, and (3) to determine any phenological differences in ponds due to varied elevation between sites.

METHODS

STUDY SITE

Four sites were originally created for this study within the MeadWestvaco Wildlife and Ecosystem Research Forest (MWERF) in Randolph County, WV (Fig. 8). The MWERF is a deciduous forested area in which Best Management Practices are enforced for timbering. Tree species colonizing the area include: *Liriodendron tulipifera* (Tulip poplar), *Prunus serotina* (Black cherry), *Quercus rubrum* (Red oak), *Acer sacharum* (Sugar maple), *Acer rubrum* (Red maple), *Acer pensylvanicum* (Striped maple), *Magnolia fraseri* (Fraser magnolia), and *Fagus grandifolia* (American Beech). Ponds created at the fourth site, located on Rocky Run in the MWERF, were eliminated from the study in early spring of 2005 as none of the created ponds were retaining water. This may have been due a shift of the plastic lining and erosion the pond sides during winter precipitation. Therefore 3 of the original 4 sites were used for this study. Two sites (Site 2 and Site 3) were located near Rocky Run (Fig. 9) and 1 site (Site 1) was located 16 km north on the Three Forks tract (Fig. 10). Ponds at Site 1 were between 2600 and 2700 ft in elevation, while ponds at Site 2 and Site 3 were located between 3100 and 3200 ft in elevation. UTM coordinates for ponds at each site are listed in Table 1. In December of 2003, 3 ponds were constructed at each site on Rocky Run by bulldozer, and then lined with heavy, clear DuPont® plastic which was covered with soil. The ponds were then allowed to fill with natural precipitation through the winter. The size of the ponds was approximately 2 to 3.5 m in diameter. Ponds fluctuated in depth during the course of the study, the most shallow was 2 cm and the deepest was approximately 1 m in depth. Ponds were placed on north-east facing slopes at each site

as this aspect stays cooler and retains more moisture during summer months which is a habitat preference for amphibians.

In February of 2004, drift fences were constructed with 18 inch tall silt fencing, supported by wooden garden stakes, to encircle the ponds. Fences were secured at the base with metal garden staples. This was done to ensure no holes were beneath the fence that might allow amphibians to escape beneath it, thus avoiding the traps. A funnel trap array (Fig. 11, 12) was set up on both sides of the 6 ponds. Funnel trap methods have been described as highly effective for trapping *R. sylvatica* (Buech and Egeland 2002). The funnel traps consisted of 5 gallon plastic buckets fitted with plastic lids, bungee cords were used to help secure the lid onto the buckets. A hole was cut at the base of the bucket where a wire mesh funnel was fitted. Buckets were placed beside the fence in such a way that the funnel was flush with the ground and fence to decrease chances of trap avoidance. The buckets were placed in pairs, with funnels facing opposite direction. In spring of 2005, the 3 ponds at Three Forks (Site 1) were added, using the same construction methods. Drift fences, consisting of the same type of silt fencing, and funnel traps were installed (Fig 13, 14, 15). The funnel traps here consisted of 5 gallon plastic Tupperware® containers in which both ends of the container were fitted with a wire mesh funnel, so that animals could enter from either direction. Four traps were placed inside and outside fences for a total of 8 traps per pond.

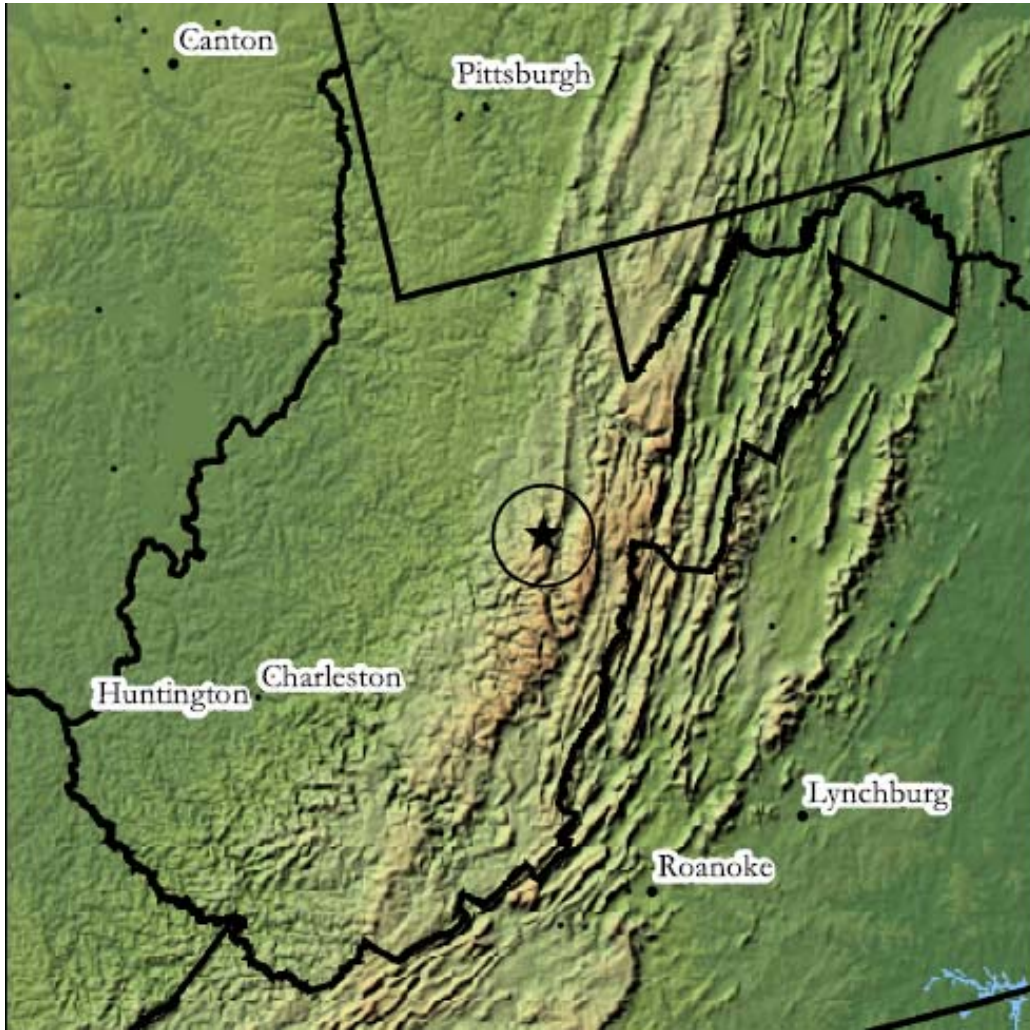


Figure 8. Location of the MWERF in Adolph, WV in Randolph County.

Rocky Run Sites (Sites 2 and 3)

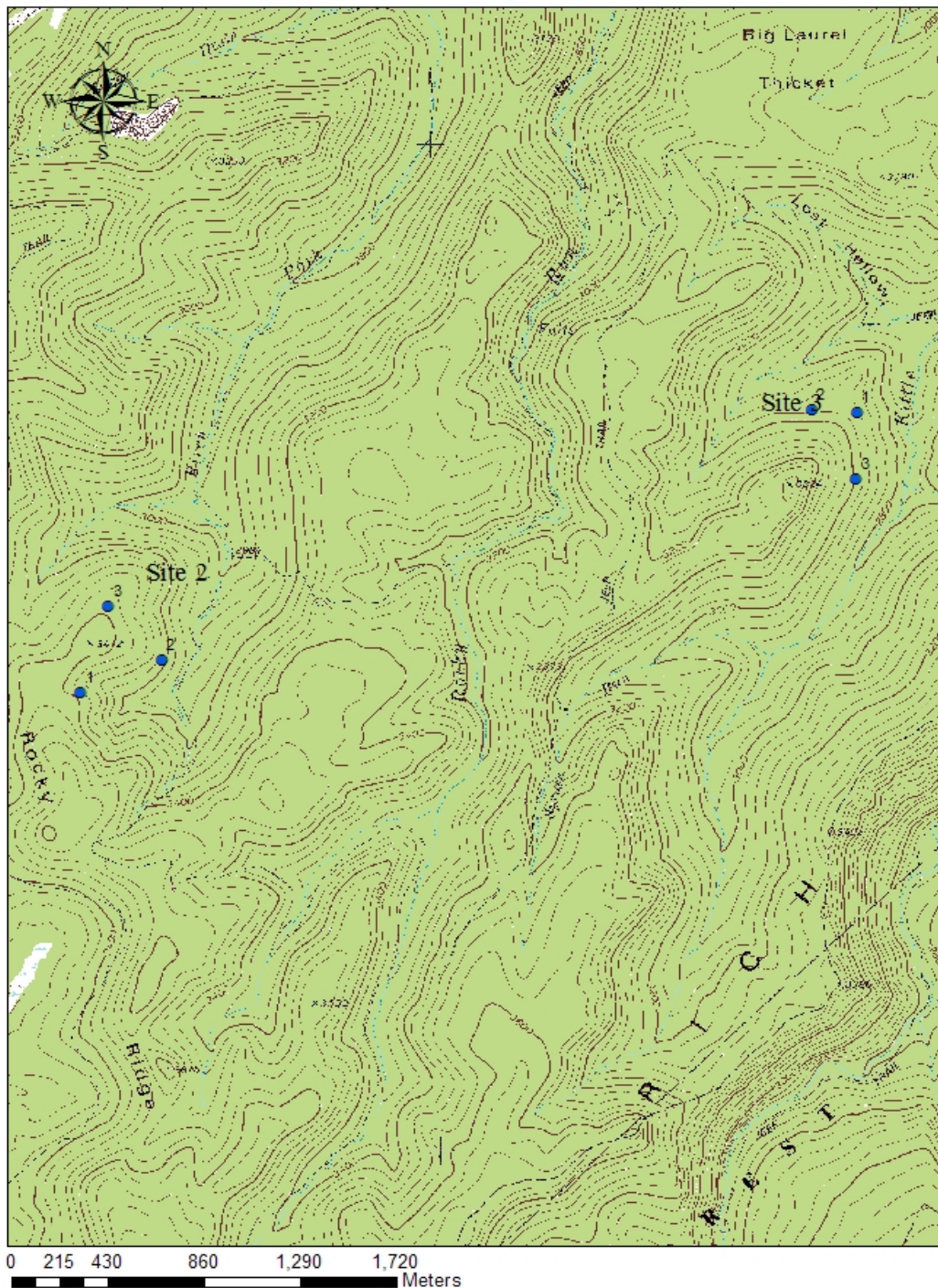


Figure 9. Location of ponds at Site 2 and Site 3 of Rocky Run.

Three Forks Sites (Site 1)

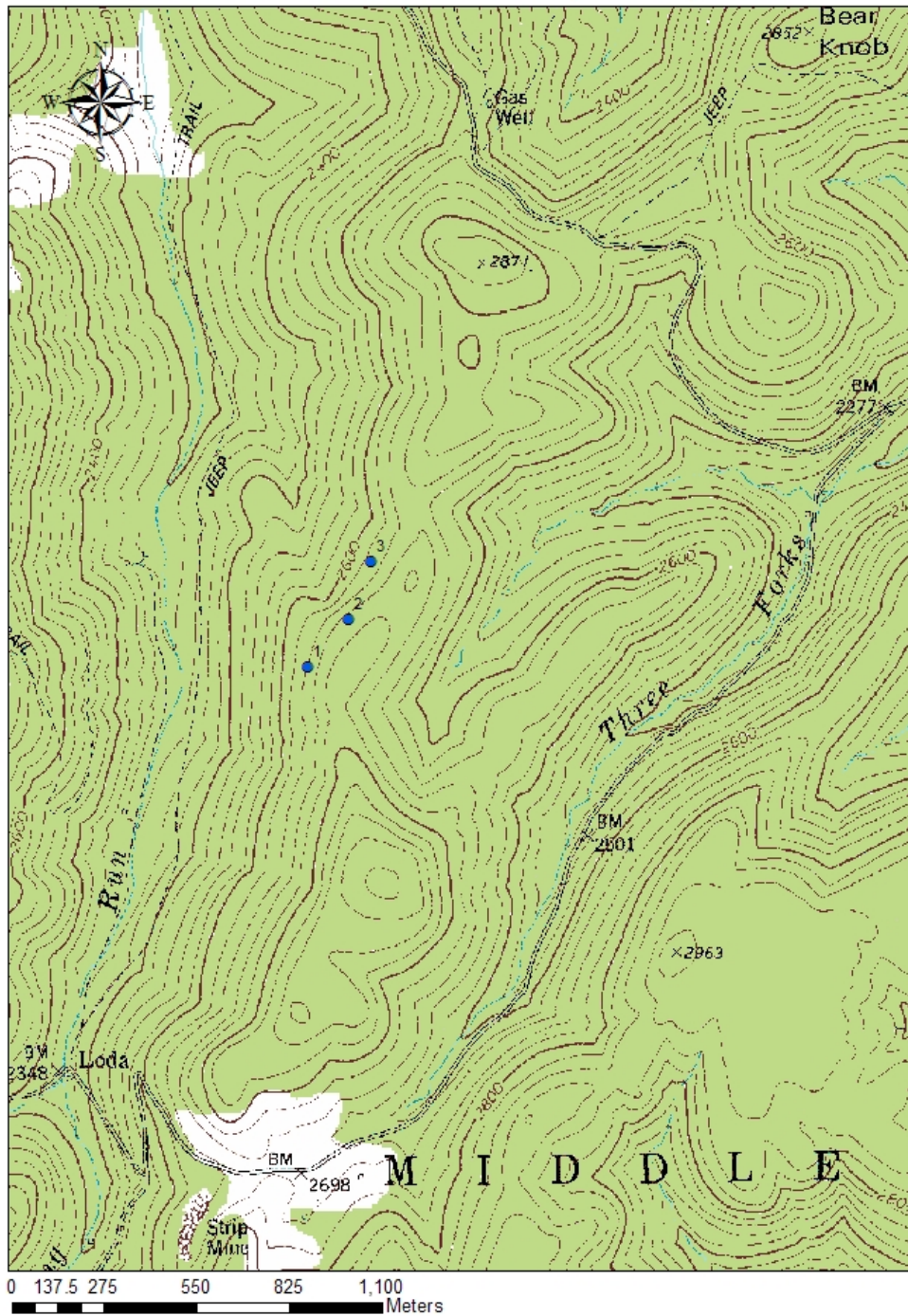


Figure 10. Location of ponds at Site 1 near Three Forks.

Table 1. Coordinates (UTM NAD 83) for all ponds at the Meadwestvaco Wildlife and Ecosystem Research Forest

Pond	Northing	Easting
1.1	580412	4296274
1.2	580533	4296414
1.3	580600	4296578
2.1	578160	4282300
2.2	578522	4282451
2.3	578285	4282698
3.1	581622	4283583
3.2	581422	4283597
3.3	581619	4283282



Figure 11. Paired funnel traps at Site 3, Pond 2 in November 2005.

(Photo by: Celeste Good)



Figure 12. Pond with drift fence and funnel trap array at Site 3, Pond 3 on June 15, 2005.
(Photo by: Celeste Good)



Figure 13. Pond 2 at Site 2 in March 2005, before drift fences were installed.
(Photo by: Becky Murray)



Figure 14. Installing ground stakes for drift fence at Site 1 (Pond 2), March 2005.

(Photo by: Becky Murray)



Figure 15. Installing silt fencing onto wooden stakes to completely encircle Pond 2 at Site 1 (March 2005).
(Photo by: Becky Murray)

AMPHIBIAN MONITORING

Ponds were monitored March through October in 2004 and 2005. Traps were checked twice weekly until eggs hatched, and tadpoles reached stage 30, as determined using the Gosner key to anuran larval development (Gosner 1979). This stage precedes toe development and is marked by limb bud growth to twice the diameter of its length. In June, traps were checked at least 5 times per week per pond to monitor any *R. sylvatica* juveniles that might egress.

Environmental information recorded consisted of air temperature, water temperature, soil temperature and weather conditions (sunny, overcast, rain). Air temperature was measured using a Kestrel 2000 wind and air temperature meter. This was used at every pond whenever pond traps were set or checked. Water temperature was taken using a water temperature thermometer, which was measured each time the traps were set or checked for every pond. Soil temperature was taken using a soil temperature probe, and was measured about half a meter from the ponds so as not to puncture the plastic lining. This was also measured when the traps were set or checked. Once a month, water depth and pH were measured at each pond. Water depth was taken as close to the center of the pond as possible using a measuring tape. pH was measured using a pH meter with probe near the pond surface. These were measured less often because it was assumed they would not be fluctuating as much as air, water and soil temperature.

A YSI Hydrolab Data Sonde was placed in 2 of the ponds (Pond 2 and Pond 3) at Site 3 between July 19, 2005 and July 23, 2005. It was planned that these would be rotated so that all ponds at all sites could be measured for Dissolved Oxygen, pH and

water temperature levels, and then compared. However, the equipment was borrowed, and had to be returned earlier than originally planned. Information was only able to be retrieved from the Data Sonde placed in Pond 2. This information is presented for DO, water temperature and pH for Pond 2 at Site 3 in Figure 24.

In April 2004, amphibian eggs (*A. maculatum* and *R. sylvatica*) were stocked in each of the 6 ponds at Rocky Run due to lack of adults naturally colonizing the ponds. The eggs were collected from a natural pond on Birch Fork Road in the MWERF. One *R. sylvatica* egg mass and 8 *A. maculatum* masses were collected and staged (Gossner 1979). Eggs and larvae were distributed through the ponds at Site 3 of Rocky Run. Ponds 1 and 3 received 300 *R. sylvatica* and 100 *A. maculatum* eggs, while pond 2 received 600 *R. sylvatica* and 200 *A. maculatum* as it was retaining twice as much water as the other two ponds. When eggs hatched, *R. sylvatica* tadpoles were distributed in all ponds at site 2. At ponds 1 and 2, one *A. maculatum* egg mass was deposited. Pond 3 did not receive eggs because it was not retaining a sufficient amount of water at the time. Egressing juveniles were trapped during the summer months using the drift fence/funnel trap array.

Amphibians trapped entering or leaving the ponds were measured using Swiss Precision Instruments 2000 calipers. Information recorded included snout-to-vent length (SVL) and vent-to-tail tip length (VT), if appropriate. Animals were given a visible implant elastomer (VIE) tag that was pond specific. VIE is a silicone based polymer which consists of a fluorescing pigment and a curing agent. The two are mixed together in a 1:10 ratio, loaded into a syringe and are injected while soft. This compound fully hardens within 24 hours. VIE was originally used for marking fish, but is now

additionally used for amphibians, reptiles and crustaceans. In 2004, each amphibian captured was given a pond specific batch mark, which consisted of 1 mark in one of the following: Pond 1: right front axillary (RF), Pond 2: left front axillary (LF), or Pond 3: right rear inguinal (RR) on the ventral side of the animal. In 2005, adults captured in the spring were marked with a batch mark, while juveniles captured in the summer/fall were given an individual specific tag. This consisted of 3 marks (yellow, red, green or orange) per animal according to an individually assigned code. This allowed for the possibility of population estimates and recapture information on initial size and location capture. VIE was injected beneath the epidermis using an insulin needle (Fig. 16). The needle was cleansed with an alcohol pad before and after each use to prevent transmission of disease. Specimens were then released on the opposite side of the fence, with the assumption that the fence intercepted the direction of movement. Elastomer tags have been shown to last for several years, making them a good tool for a long term study (Davis and Ovaska 2001; Bailey 2004). According to National Marine Technologies, the manufacturer of VIE, the silicone based polymer is not harmful to animals (MSDS, 2005).

Perimeter fences (Fig. 17) using the same silt fencing as the pond arrays, were set up in 2004 to surround pond 2 at the Rocky Run Site 3. The array consisted of four 100 m lengths of drift fence approximately 100 m away from the ponds. Funnel traps were set up 5 m apart on both sides of the fence. Funnel traps were created using 5 gallon plastic Tupperware® tubs with lift off lids. A hole was cut in either end of the tub and a wire mesh funnel was installed at both ends. This was set up in order to track movement of adults and juveniles entering or leaving the study area. In 2005, a segmented concentric circle of 4 drift fences, each segment 15 m long, were set up at approximately

12 m and 25 m from the pond. Pitfall traps were used as opposed to funnel traps, due to cost constraints. Pitfall traps along with drift fences are effective in monitoring direction of movement and the relative abundance of amphibians in an area (Gibbons and Semlitsch 1981; Gibbons and Bennett 1974). The traps consisted of 1 gallon plastic flower pots fitted with circular wooden lids with an attached rope for small mammals to escape. They were buried flush with the ground, and placed 5 m apart on the side of the fence facing the pond, for a total of 3 traps per fence segment. The purpose was to capture juveniles leaving the pond (Fig. 18). However no *R. sylvatica* or *A. maculatum* were trapped in either attempt.

STATISTICAL PROCEDURES

Statistical t-tests were performed using SAS. A Shapiro-Wilk test was used to test for data normality. Data transformations were performed to normalize air, water and soil temperatures as this data did not fall within a normal distribution according to the Shapiro-Wilk test. Cubed transformations were performed for water and air, while an inverse square transformation was performed for soil. Snout-to-vent lengths from high and low elevations passed the Shapiro-Wilk and therefore were not transformed prior to t-test analysis. For SVL, the variances of the two samples were not equal, therefore the Satterwaite t-test for unequal variances was used. A pooled t-test was used to test for air, water and soil temperatures because the variance was not significantly different between groups.



Figure 16. *Rana sylvatica* juvenile being marked with VIE (July 15, 2005).
(Photo by: Pat Keyser)

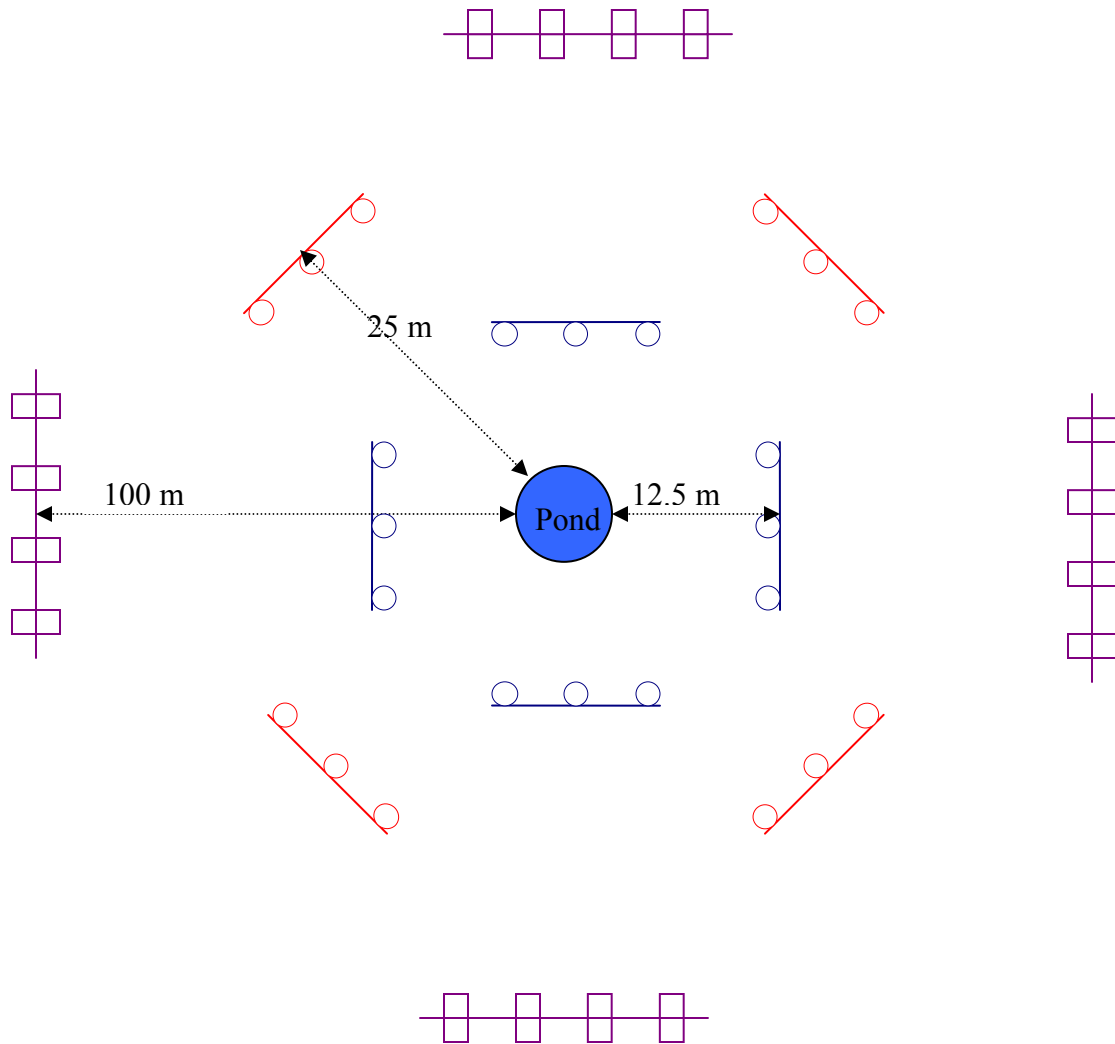


Figure 17. Diagram of the perimeter fence setup at Pond 2 on Site 3. Fences set up along the perimeter in 2004 (purple) are 100 m away from the pond and 100 m in length. Fences in the center, set up in 2005, (red) are 25 m from the pond and 15 m in length, and 12.5 m (blue) from the pond with 15 m length.



Figure 18. *Rana sylvatica* juveniles, Site 1, Pond 3 (July 15, 2005).
(Photos by: Celeste Good)

RESULTS

In 2004, no breeding amphibians were captured, however, in 2005, adult *R. sylvatica* (n=17) were captured from March 28 to April 4 and 6-8 *R. sylvatica* egg masses were found per pond. Eggs were laid by March 10, 2005 at the Three Forks site and no adults were seen. Adult *R. sylvatica* were heard calling at Site 3 on March 28, 2005, and many adults were captured April 4, 2005 at Site 2. Eggs hatched near the end of April at all sites, and tadpoles began metamorphosing in mid-June at the Three Forks site, the beginning of July at Site 2 on Rocky Run, and the second week of July at Site 3 on Rocky Run. Tadpoles remained at Site 3 in Pond 1 and Pond 3 through November, while tadpoles at all other ponds successfully metamorphosed by September 14, 2005. All juveniles had emigrated from Site 1 by mid-August and Site 2 by mid-September.

The 2004 captures, from stocked egg masses, consisted of *R. sylvatica* juveniles (n=25) captured in late June-July, and juvenile *A. maculatum* (n=4) in July. In October, 1 *A. maculatum* juvenile was captured. A pair of breeding *A. maculatum* was observed in March. Two adult *R. sylvatica* were captured, 1 in July, and 1 in October.

Captures in 2005 (Fig. 19) were believed to be amphibians naturally colonizing the area as none of the adults had been previously batch marked with VIE. However, adult males have been noted to mature after 1 year (Berven 1982), and these may have been males returning to breed that had not been marked the previous year. No *A. maculatum* were captured, however 1 adult was observed in pond 2 on Site 2 on March 15, 2005. *Rana sylvatica* juveniles (n=154) were captured from July through October. Juveniles began emigrating from the ponds at Three Forks on June 24, 2005. They did

not begin leaving the Rocky Run ponds until July 16, 2005. Most captures were made at Site 3, while there were fewer juveniles captured at Site 2 and 3 (Fig. 20).

For all sites, snout-to-vent length (SVL) ranged from 9 mm to 20 mm in juvenile *R. sylvatica* ($\mu=14.5$ mm) (Fig. 21). For adults, SVLs ranged from 48.1 mm to 69.3 mm ($\mu=55.8$ mm) (Table 2). Mean SVL was larger for juveniles egressing from Site 2 ($\mu=16.3$ mm) than from Site 1 ($\mu=14.0$ mm) or Site 3 ($\mu=11.1$ mm) (Fig. 22, 23 and 24). A significant difference was found between Site 1 and Site 2 for SVL using a pooled t test ($\alpha=.05$) (Fig. 24). Site 3 was not included in the comparison due to small sample size ($n=6$). Site 3 had the smallest SVL and juveniles egressed from this site later than the other 2 sites. The mean for low elevation (14.0 mm) and the mean for high elevation was (16.4 mm). Minimum SVL=11.4 mm for low elevation while max SVL=19.0 mm. For high elevation, min SVL= 14.6 mm, and max SVL=20.1. The bulk of the range for low elevation was between 13 mm and 15 mm, while the majority of the distribution was between 15.5 mm and 17 mm for high elevation.

Other species trapped in 2005 included *Notophthalmus v. viridescens* (Red-spotted Newts) ($n=25$), *Desmognathus ochrophaeus* (Allegheny Mountain Dusky Salamanders) ($n= 2$), *Hemidactylium scutatum* (Four-toed Salamanders) ($n=1$), *Bufo a. americanus* (Eastern American Toads) ($n=1$) and *Rana clamitans melanota* (Northern Green Frogs) ($n=75$).

Sites 1 and 2 had more juvenile egression than site 3 during the duration of the study. At site 3, very few juveniles egressed from the ponds, and tadpole development was much slower than at the other sites. Egression of juveniles began earlier for Site 1 which was at the lowest elevation of all sites.

A data sonde was placed in pond 2 at Site 3 from July 19 through July 23 to monitor pH, dissolved oxygen, and specific conductance (Fig 25). Dissolved oxygen was found to be below 1 ppm for most of the time. The only time it went higher was during rain events. pH stayed constant around 5.8, and there was little water temperature fluctuation probably due to the canopy cover. Temperature remained between 17°C and 21°C. DO had most fluctuation, ranging from -0.6 ppm to 1.5 ppm most of the time, and increased to above 5 ppm during a period of rain.

No significant differences were found in soil temperature between high (Sites 2 and 3) and low elevation (Site 1) sites ($\alpha=.05$) using a Saitherwaite t-test. No significant differences were found between elevations for water or air temperature, but these temperatures did remain lower in the higher elevation sites (Fig. 26, 27 and 28).

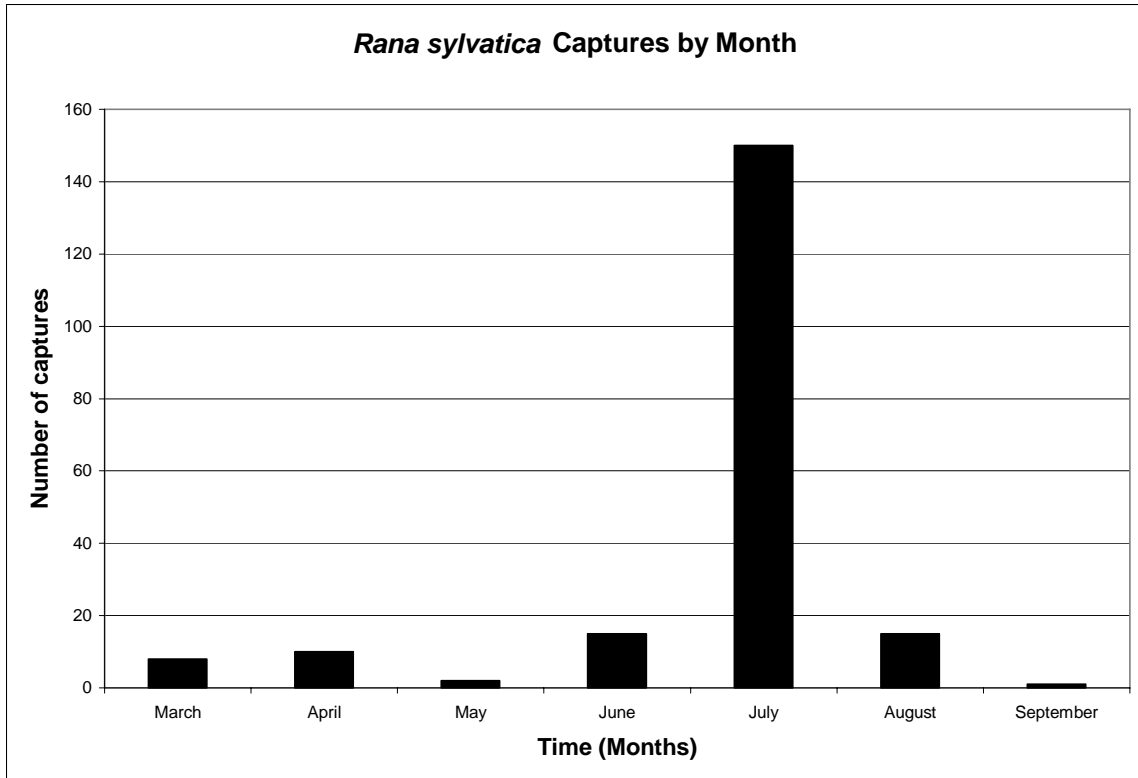


Figure 19. Total number adult and juvenile *Rana sylvatica* captures for 2005 by month. Adults were captured in March and April, while juveniles were captured June through September, with the majority captured in July.

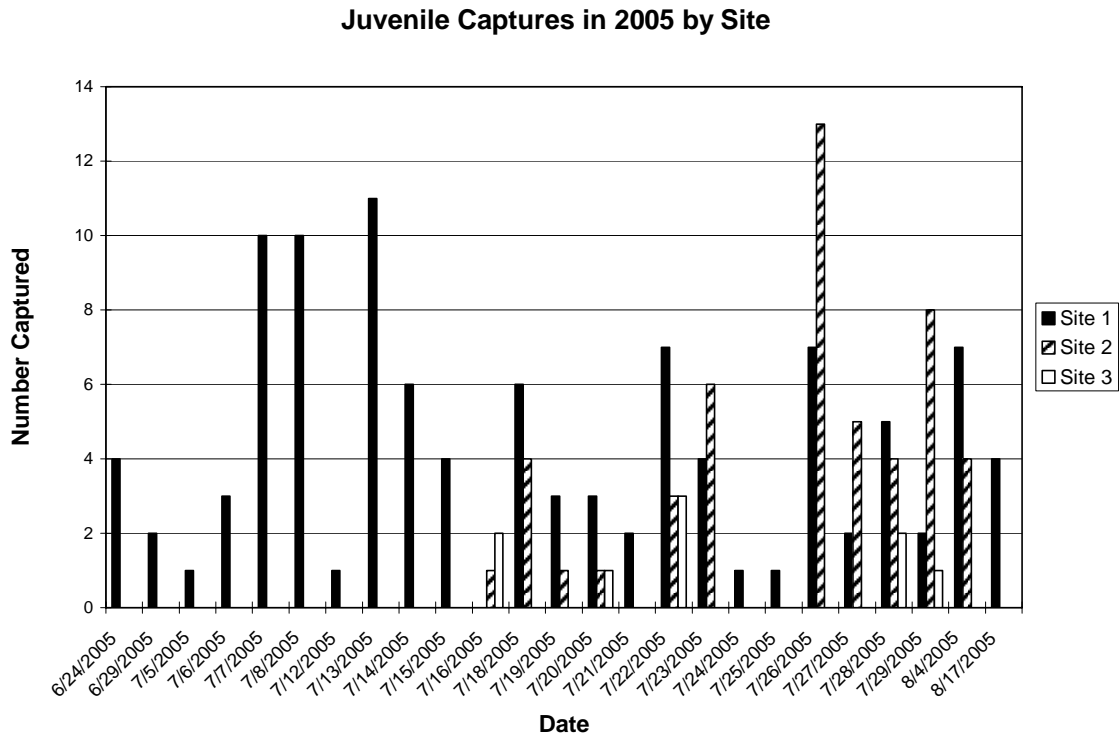


Figure 20. Comparison of *R. sylvatica* juvenile captures between sites. Site 1 emigration began first, followed by Site 2 and then Site 3. There were more total captures made at Site 1 (n=106), with Site 2 captures being (n=50) and Site 3 (n=9).

Size Distribution of All *Rana sylvatica* Juveniles Captured (2005)

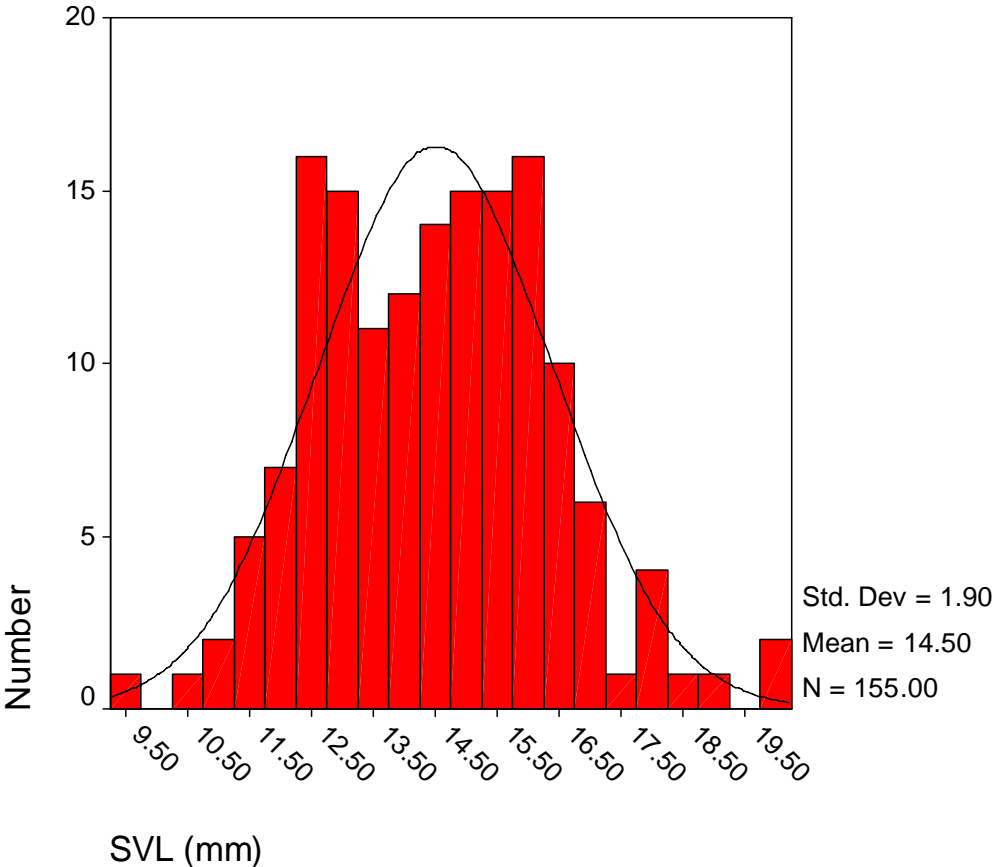


Figure 21. Histogram with normal curve of size distributions (SVL) for all *Rana sylvatica* juveniles captured in 2005. A bimodal curve occurred when all SVLs for all sites are graphed together.

Snout-to-Vent Length for *R. sylvatica* Juveniles from Site 1

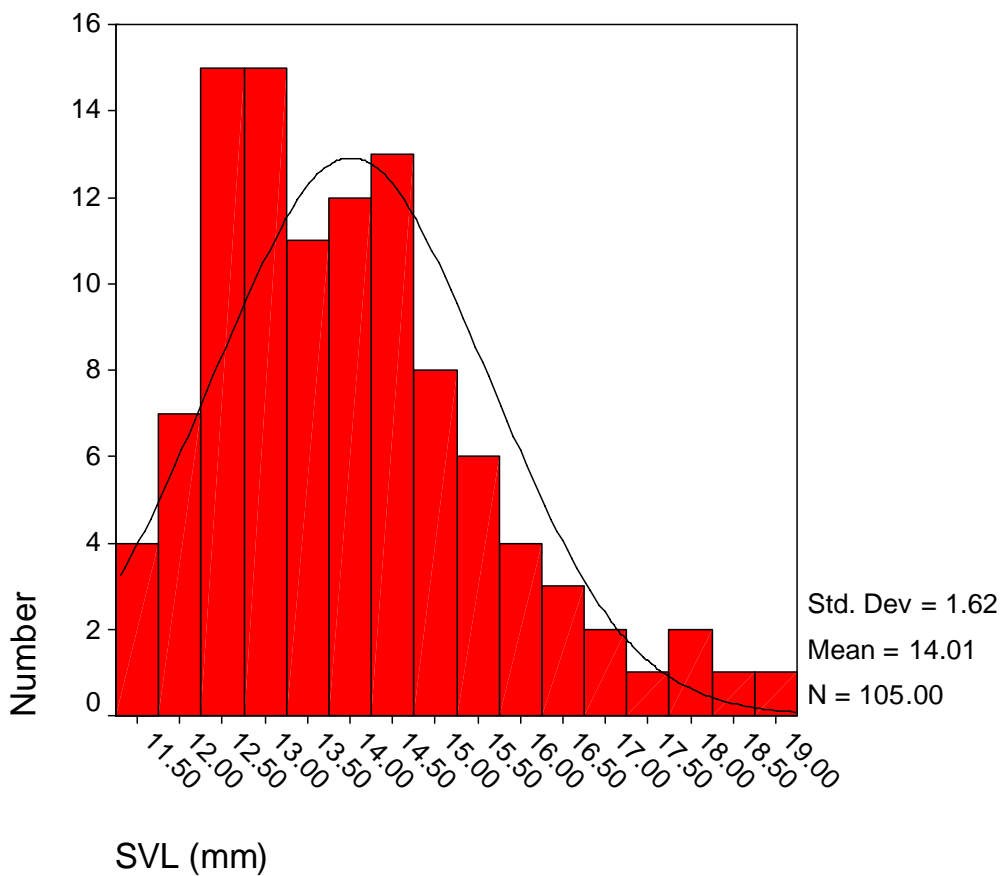


Figure 22. Histogram for *R. sylvatica* juveniles from Site 1 only (Low elevation site). Range is from 11.4 mm to 19.0 mm with the majority between 12.8 mm and 14.9 mm, the 25th and 75th percentile values.

Snout-to-Vent Length for *R. sylvatica* Juveniles from Site 2

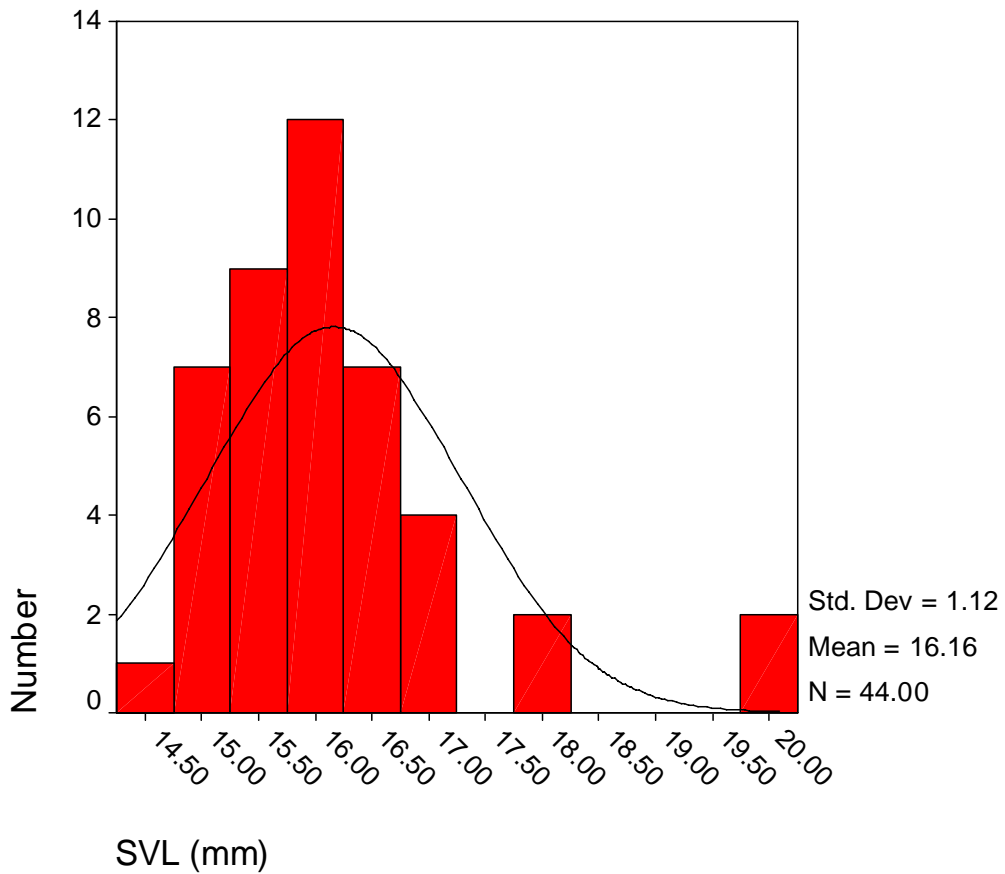


Figure 23. Histogram for *R. sylvatica* juveniles from Site 2 only (Low elevation site). The minimum value was 14.6 mm and the maximum value was 20.1 mm. The range between the 25th and 75th percentile rank is 15.5 mm to 16.5 mm.

Comparison of Size Distribution for *R. sylvatica* Juveniles Between Elevations

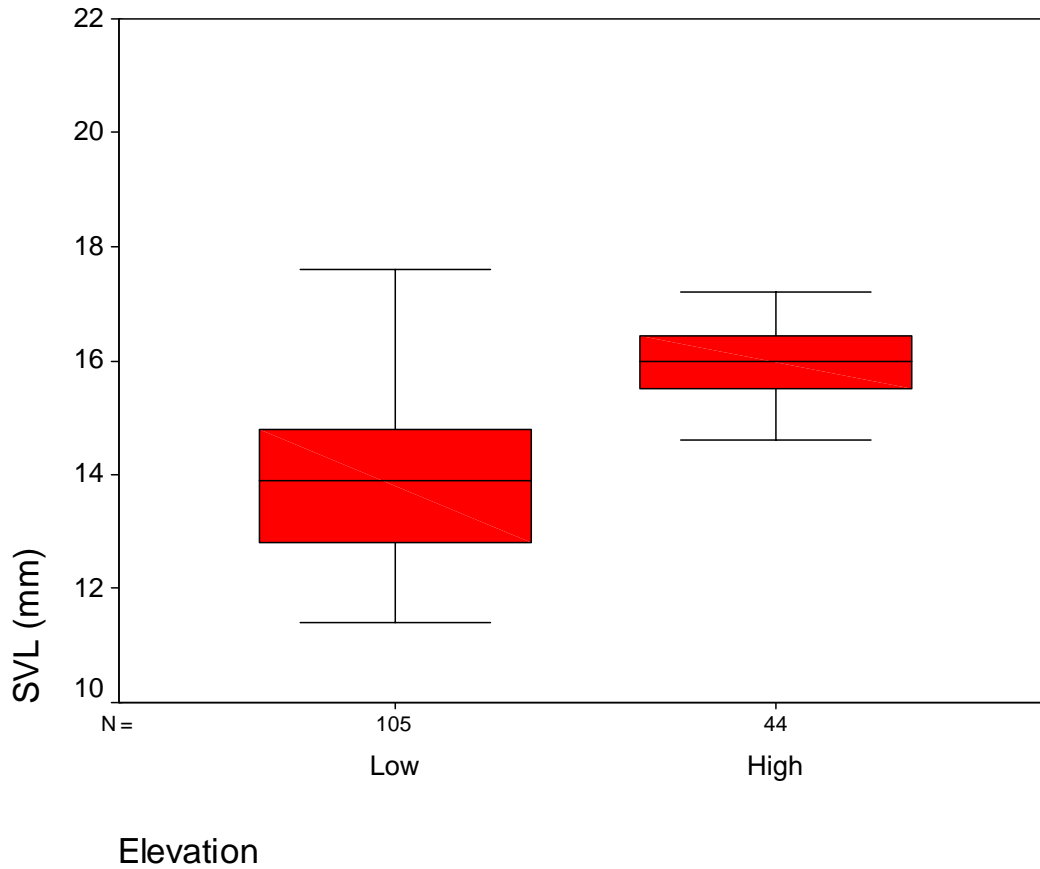


Figure 24. Comparison of distribution of *R. sylvatica* SVL for emigrating juveniles between Site 1 (low elevation) and Site 2 (high elevation). A significant difference was found between Site 1 ($\mu=14.0\pm 1.62$) and Site 2 ($\mu=16.4\pm 1.12$) for SVL using a pooled t test ($\alpha=.05$).

Table 2. Snout-to-vent lengths and sex for adult *R. sylvatica* captured between March 28, 2005 and April 4, 2005. (M=male, F=female and U=undetermined).

Site	Pond	Sex	SV Length (mm)
2	1	F	61.9
2	1	M	52.1
2	1	U	49.8
2	1	U	56.7
2	2	F	58.2
2	2	F	62.3
2	2	U	48.1
2	2	U	50.9
2	2	U	52.5
2	3	U	54.1
3	1	F	58.6
3	1	M	55.1
3	2	U	49.4
3	3	F	69.3
3	3	M	53.5
3	3	M	57.2
3	3	M	59.5

Data Sonde Measurements at Pond 2, Site 3

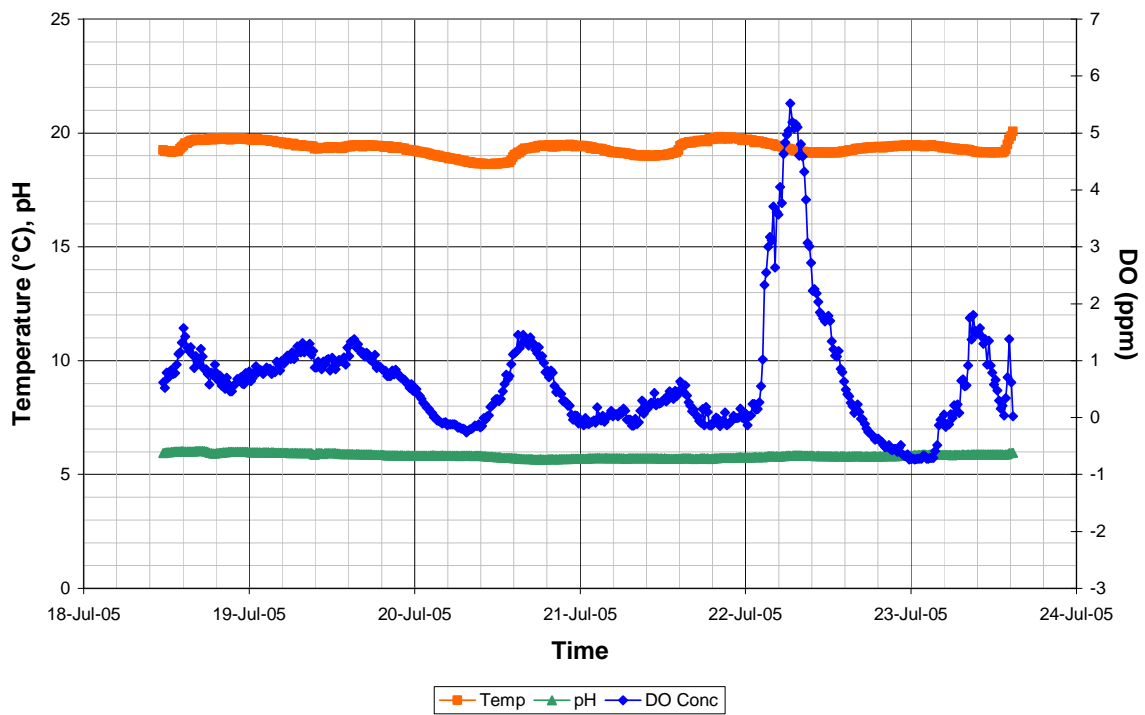


Figure 25. Dissolved oxygen (blue line), temperature (red line) and pH (green line) from July 19 through July 23, 2005.

Distribution of Water Temperature Between High and Low Elevations

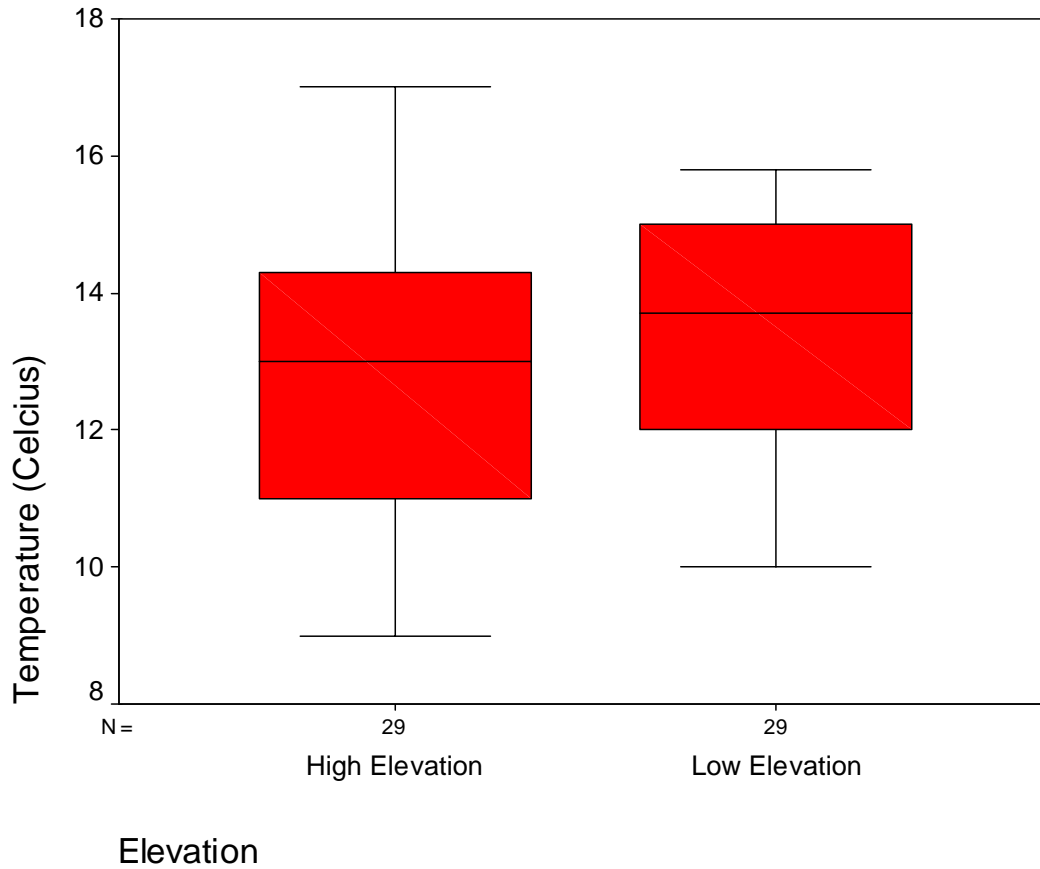


Figure 26. A comparison of the distribution of water temperature between high and low elevation sites. High elevation min temp=9.0, while the max temp=17.0 ($\mu=12.8$). Low elevation min temp=10.0, while max temp=15.8 ($\mu=13.4$). No significant difference was found for water temperature (Saiterwaite t test ($\alpha=.05$)).

Distribution of Soil Temperature for Elevation

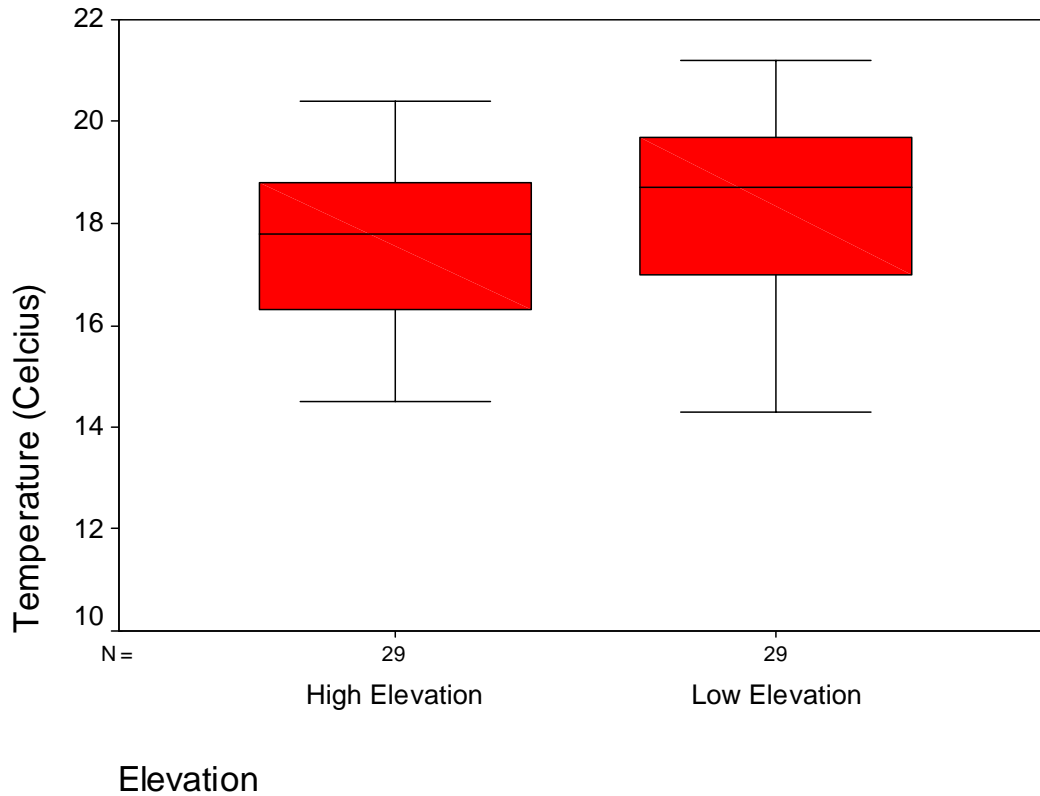


Figure 27. A comparison of soil temperature between low elevation (Site 1) and high elevation (Site 2 and 3) ponds. Min temp=11.8 for high elevation and max temp=20.4 ($\mu=17.2$). Min temp=14.3 for low elevation, while max temp=21.2 ($\mu=18.3$). There was no significant difference found for soil temperature between high and low elevation sites (Saitherwaite t test ($\alpha=.05$)).

Air Temperature Distribution for High and Low Elevation Sites

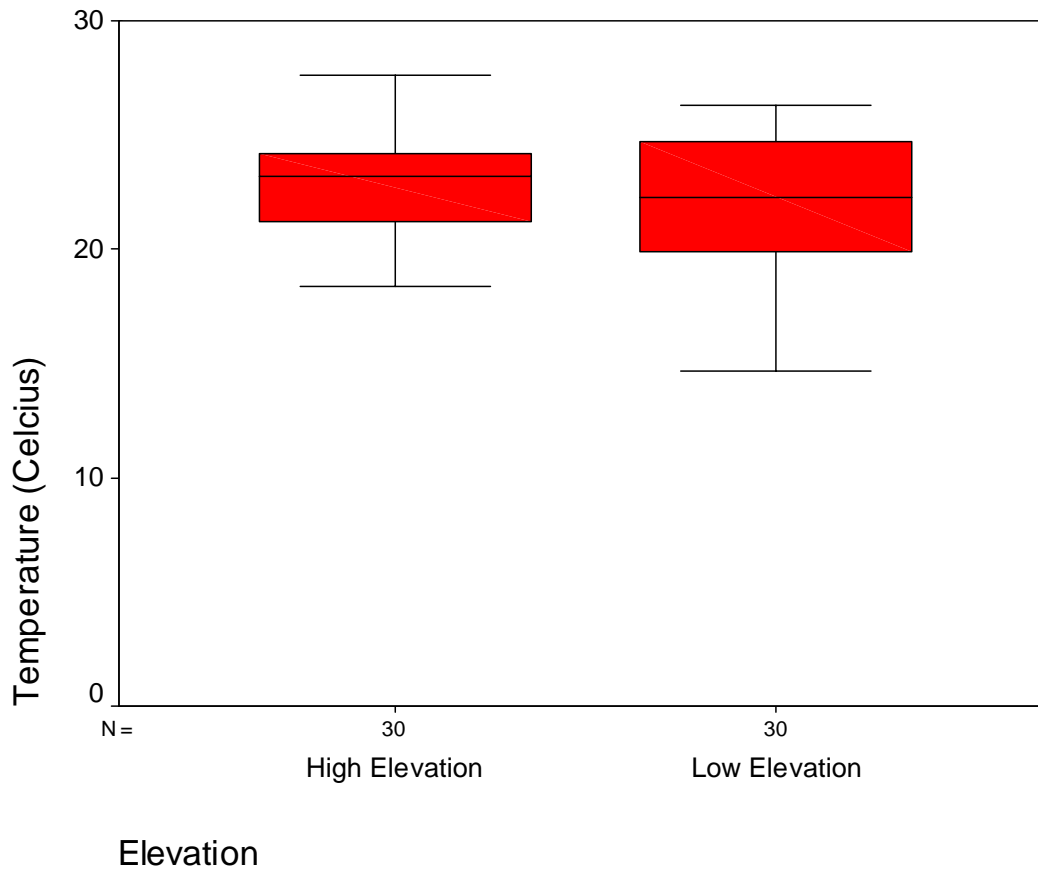


Figure 28. Temperature differences between high and low elevation sites in degrees Celsius. Min temp=10.6 for high elevation and max temp=26.3 ($\mu=21.6$). Min temp=15.0 for low elevation and max temp=27.6 ($\mu=22.6$). Air Temperature was not found to be significantly different between sites (Saitherwaite t test ($\alpha=.05$)).

DISCUSSION

The first objective of this study was to find if constructed ponds would be used voluntarily by amphibians for breeding. No adults were found to mate in the ponds during 2004. In 2005, there were 6-8 *R. sylvatica* breeding pairs per pond, as demonstrated by the number of egg masses found. It took 2 years for amphibians to utilize the constructed ponds for breeding. The ponds were seeded with egg masses in 2004 and this could be related to current amphibian colonization. Studies have shown that *R. sylvatica* females take 2-3 years to mature, while it takes only 1 year for some males to mature (Bellis 1965; Berven 1982). Some of the adults in 2005 may have been from those egg masses. Colonization of the ponds compares with Pauley (2005) and Pechman et al. (2001), in which both studies showed that created ponds were utilized with time. Pechman et al. (2001) found that salamander species took 2 years to colonize created ponds for breeding while anurans colonized the ponds in the same year that they were constructed. After their study was conducted for 8 years, species composition differed from the habitat that was destroyed. Two species of salamander that had been present at the destroyed habitat did not colonize the constructed ponds. The ponds studied by Pauley (2005) were constructed in 1993 and 4 species of anurans colonized them for breeding that same year. Pool age has been associated with species colonization in which older pools supported a greater number of amphibian species than more recently created pools (Laan and Verboom 1990). Influences upon rate in which pools are colonized for breeding include distance from existing habitats, range of the species, level of philopatry, obstacles between habitat types, and the size of the population present in existing habitats (Laan and Verboom 1990). Previous studies have shown that over time,

amphibians will colonize created ponds. In years to follow it is likely that increasing numbers of adults will move to the constructed ponds to breed, as demonstrated by adult *R. sylvatica* colonizing them 2 years after they were created.

Bufo a. americanus (Eastern American Toads) were originally included in this study because they utilize woodland ponds for breeding. No *B. americanus* were seen using the ponds for breeding in 2005. However on May 26, 2005 at Site 3, *B. americanus* were heard calling, and were often seen along the trails between ponds. Young of year *B. americanus* were encountered frequently in July at Site 1. *Rana sylvatica* are known to prey upon embryos and other larvae, and can become cannibalistic in highly competitive environments. Werner (1999) noted *R. sylvatica* preyed upon *B. americanus* and that breeding *B. americanus* will avoid laying eggs in ponds where *R. sylvatica* tadpoles are located. If eggs are laid where *R. sylvatica* are present, they are highly likely to be consumed by the larvae. It is possible that due to *R. sylvatica* larvae presence in the pools during the time *B. americanus* were mating, and that the toads avoided these pools to prevent larval predation upon toad eggs.

The lack of *A. maculatum* breeding in the ponds could be due to the high level of philopatry demonstrated by these animals (Madison 1997; Berven and Grudzien 1990; Vasconcelos and Calhoun 2004). Lehtinen and Galatowitsch (2001) noted that restored wetlands contained fewer species compared with reference wetlands. Factors that seemed to affect species colonization were habitat size and distance to source ponds. The ability of a species to disperse great distances from their breeding ponds seems to indicate the likelihood that they will utilize mitigated areas (Lehtinen and Galatowitsch 2001). Pond placement may be a factor if the constructed ponds are a great distance from

potential source ponds. Marsh et al. (1999) found that placement of artificial ponds at distances greater than 200 m from source ponds increased the time it took for them to be colonized by tungara frogs (*Physalaemus pustulosus*), which have a known range of 200 m. The distance traveled from breeding ponds for *A. maculatum* from other studies ranges from 158 m to 300 m (Madison 1997; Vasconcelos and Calhoun 2004). They are also up to 100 % philopatric to their natal ponds (Vasconcelos and Calhoun 2004). No other natural ponds were noted nearby the constructed pools and dispersal distances that *A. maculatum* may need to travel to locate constructed pools may be too great. In time the ponds may be colonized by *A. maculatum* as their natal pools dry up, and adults travel closer to the constructed ponds during foraging.

The second question this study sought to answer was if larvae would successfully metamorphose from constructed ponds. There was slow tadpole development in several ponds, however 6 of the 9 ponds in 2005 had successful egression of *R. sylvatica* juveniles into the surrounding forest. Skelly (2004) compared *R. sylvatica* embryo development between ponds that were shaded and those that received more light, and ponds with low versus high temperatures. He found slower embryo development occurred in both shaded and low temperature ponds. In my study, ponds at lower elevations (Site 1) received more light as this stand was logged more recently than the higher elevation stands. Additionally, Site 1 was at a lower elevation, and temperature was generally higher earlier in the day than the other sites, although this was not found to be statistically different. Constructed ponds studied by Pechman et al. (2001) did not yield any metamorphosing juveniles in the year they were created. Only 8 juveniles from the genus *Hyla* metamorphosed the following year due to frequent pond drying. After

plastic linings were installed in the ponds, juveniles successfully metamorphosed and emigrated from the ponds. Their four-year study showed juveniles from 10 different species eventually survived and were able to egress from the ponds.

Amphibians vary in their hydroperiod requirement. What is sufficient for one species may not be long enough for other species. This may also vary with region. Many amphibians in Rhode Island require ponds to hold water between 4 and 9 months (Paton and Crouch 2002).

Some deformities were seen on juveniles at Site 3 near Pond 2 and one adult *Notophthalmus v. viridescens* missing all phalanges was found at Site 2, Pond 2. This could signify a trematode infection, which can lead to deformities in limbs (Johnson 1999). This occurs when free swimming cercaria enter through the spiracles of tadpoles, and colonize in developing cells. They develop into metacercaria, a resting stage, within the limb buds and pelvic girdle of the tadpole. This can interfere with cell signaling during specialization and growth which leads to limb deformation (Johnson 1999).

Low DO and pH during embryo development have also been associated with deformities in *A. maculatum* as well as *R. sylvatica* (Pierce et al. 1984; Ling et al. 1986). Hydrology in Site 3 may have affected the development of tadpoles in ponds 1 and 3. Low dissolved oxygen could hinder development, and may explain why most did not survive (Werner and Glennemeier 1999). According to the data log collected from the Data Sonde, pH and temperature remained stable in pond 2 at Site 3: pH was around 5, while temperature remained stable between night and day. A pH of 5 is on the low range of tolerance for *R. sylvatica* (Pierce et al. 1984). Competition between *R. sylvatica*

tadpoles could be another factor, as the ponds that dried, or had slower tadpole development and crowding may have occurred (Pearman 1993).

The migratory distance of juvenile amphibians is important when planning conservation measures which involve 2 types of habitat. Rothermel (2004) noted that the success of juveniles was related to their ability to reach forested areas, and the distance of natal ponds to forests predicted the likelihood that they would make it to the forest. Juveniles that had further to travel were less likely to reach forested habitat. The perimeter fences used in my study were not sufficient in trapping juveniles, though many terrestrial salamanders were captured. This may be due to the fact that the fences did not completely enclose the perimeters and juveniles may have had an emigration pattern that the fences did not intersect. Also, maintenance of the fences was difficult due to time constraints in the field. The fences sometimes pulled up from the ground, which would have allowed juveniles to pass underneath.

Pitfall traps may have been faulty due to small size, and larger traps may be needed to successfully capture any egressing juveniles. Gibbons and Semlitch recommended 20 liter buckets, but stated that smaller traps, such as #8 coffee cans should be sufficient for smaller amphibians including juveniles. However, during July 2006, the time that they were monitored, there were very few juveniles egressing from ponds. Trapping a small number of juveniles is difficult as they could travel in any direction from the pond, and may have either missed the traps, or not traveled far enough from the pond to reach the traps. The pitfall traps installed captured no juveniles, but did trap many small mammals that did not survive overnight including 3 shrews and 2 moles.

These animals prey upon amphibians, and any amphibians trapped would have been consumed.

Radio tracking adult amphibians and egressing juveniles could be one way in which the distance traveled away from the breeding pond could be determined. Also a greater amount of traps or a fence completely encircling the perimeter might help. However, this would be more costly requiring more materials, time and labor.

A third question is how the differences in development and phenology vary between elevations. No significant differences were found for soil, air or water temperatures between high elevation and low elevation sites. Both soil and water temperatures remained lower at the high elevation sites compared to low elevation sites, but according to the performed t test, there was not a significant difference between these groups. Air temperature was not found to be significantly different between elevations. Low elevation sites were generally checked in the morning and high elevation sites were checked towards the afternoon when temperatures had increased. This accounts for air temperatures towards June and July beginning to increase in the high elevation greater than the lower elevation air temperatures. The low elevation temperatures remained stable throughout the duration of the study, while the high elevation temperatures gradually increased. Installing data loggers is a method which would enable temperatures to be monitored throughout the day, as opposed to only being measured once per day. It would also enable comparisons between sites for the same time of day, and it is likely that significant differences between sites would be apparent.

Tadpoles at Site 1, the lowest elevation, began metamorphosing and egressing during the last week of June. Tadpoles at Site 2 were next to transform during the first

week of July, and those in site 3 began during the second week of July. Higher elevation sites were covered in snow through most of March, while the lower elevation sites had very little snow, and may have allowed frogs to come to and breed earlier in these ponds. The juveniles at Site 2 had a larger mean SVL than juveniles which emerged from Sites 1 and 3. Site 3 had the smallest mean SVL of juveniles leaving the ponds, however these measurements were not used in statistical analysis because of low sample size. Juveniles from the high elevation site (Site 2) had significantly larger SVL than those from the low elevation site (Site 1). Sagor et al. (1998) observed that adult *R. sylvatica* found at high elevations in Québec were significantly larger than those captured at low elevations in Québec. Additionally, those at high elevations in Quebec matured later than those at lower elevations. Large size at emigration is a factor for success (Rothermel 2004) and it may be beneficial for higher elevation amphibians to have a larger size at emigration than for those at the lower elevation sites. Bergman's Rule states that ectothermic animals located in colder or higher elevations/latitudes are larger in size to aid them in heat conservation (Ashton 2002). In an analysis of previous studies, Ashton (2002) stated that 24 out of 36 species that were at high elevations or colder temperatures exhibited significantly larger body size. Larger size allows the animal to develop more fat stores, which could be utilized during times of snow cover, when food is scarce. Also, larger size in amphibians may play a role for increased survival; larger females have an increased capacity to hold eggs, and male frogs have been shown to select for a larger female mate (Sagor et al. 1998). Skelly et al. (2004) demonstrated that temperature plays an important role in rate of embryo development for amphibians, therefore some of the temperature differences found, although not statistically significant, may help to explain

why eggs at lower elevation sites hatched and larvae egressed before those at the higher elevation sites. Van Voorhies (1996) showed that cells in nematodes and fish grow slower, but nematodes reached an average of 22 % larger size at colder temperatures than at warmer temperatures, while red blood cells of cichlid fish grew 29% larger at colder temperatures than those cells at warmer temperatures. Although temperature differences were not found to be statistically significant in my study, they were lower for water and soil temperatures. It may be that slight differences in temperature, though not calculated significant through statistics, differ enough to amphibians for them to make a difference. If temperature are monitored in intervals over 24 hour periods in the future, then these results may differ.

If preferred breeding ponds are removed, eventually amphibians will colonize in other areas (Dodd Jr. and Cade 1998), and the chances of this would increase if the new pond is en route to the old breeding pond. Amphibians may encounter them while searching for their normal breeding ponds. Pauley (2005) mentioned that amphibians may happen upon new breeding habitat while foraging in the summer and fall.

In the future, individuals constructing ponds for breeding habitat should take the following under consideration when lining them with plastic to aid in water retention. Over time, compounds in plastic break down chemically depending upon the designated grade, and this may present a source of xenoestrogen such as polyphenols. For example, nonylphenol was found to inhibit tadpole growth in *R. catesbeiana* (American bullfrog) tadpoles (Christensen et al. 2005). This would be detrimental since the obligate aquatic phase of amphibians is in the egg and larvae stage, presenting the beginnings of development. Bisphenol A at the low dose of 10^{-7} M was shown to promote female

characteristics involving the expression of an estrogenic biomarker in *Xenopus laevis* (African clawed frog) tadpoles. This altered the sex ratio of offspring and skewed the majority toward developing female gonads (Levy et al. 2004). It is still being researched exactly how these compounds may affect developing larvae, but for conservation means, this should be taken into consideration.

FUTURE RESEARCH

In the future, each site will consist of 3 silvicultural treatments: a control, a clear-cut leaving a 100 m buffer zone around the pond, and a clear-cut with a corridor 20 m wide connecting the pond and woodland habitats (Fig. 29). The corridor treatment will be monitored for amphibian emigration across it, to see if amphibians will utilize corridors to return to the main forest for foraging, or if other amphibians will migrate to the ponds through the corridor. A buffer zone of 100 m² will surround the ponds to provide foraging habitat during the non-breeding months. Breeding immigration and juvenile emigration will be monitored throughout the spring and summer, and compared to baseline data.

CONCLUSIONS

In conclusion, I believe that constructed ponds are a sufficient way to provide breeding habitat for local amphibian populations when natal pools have been removed or fragmentation from original habitat has been created due to timbering practices. Ponds should be constructed and monitored for activity in advance so that adult amphibians are able to encounter them during the non-breeding season. This also allows leaf litter to collect on the bottom of pools, which provides nutrients for developing amphibian larvae. Placing several ponds within the same area creates a pond network for new populations and provides a sufficient amount of metamorphosing juveniles that would protect against mortality and pond drying. This would also help relieve genetic drift by providing breeding habitat for a variety of individuals of the same species.

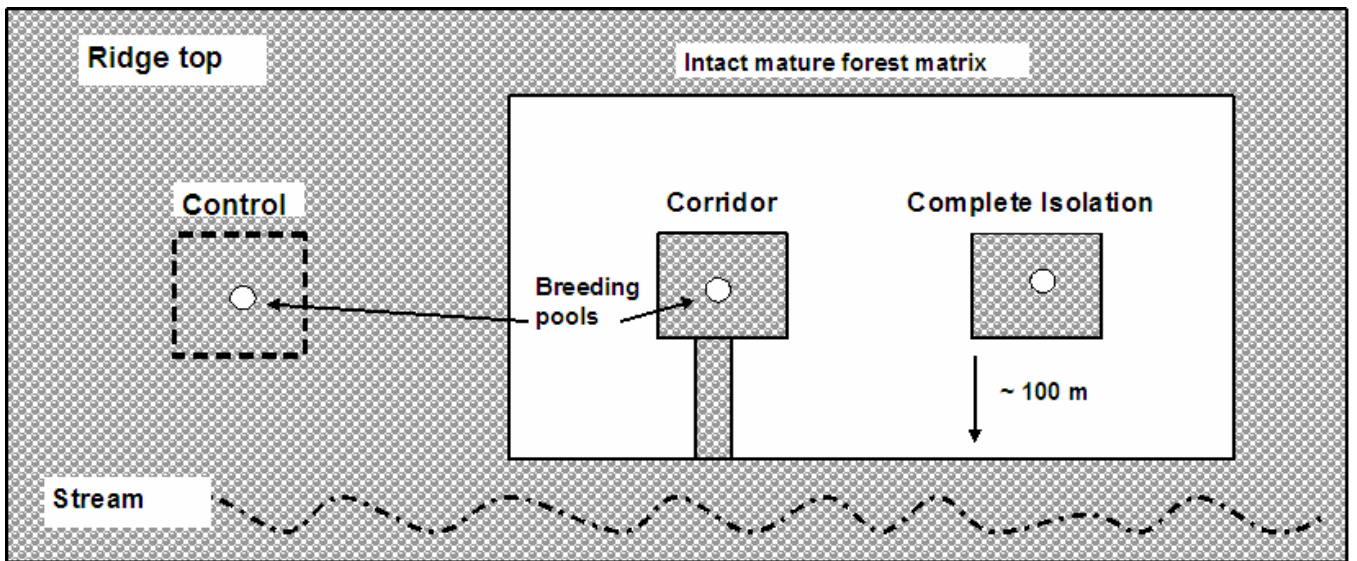


Figure 29. Proposed experimental treatments for the three constructed pond sites.

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