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A Continued Study of the Use of Created Ponds for Amphibian Breeding in Fragmented Forested Areas

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A Continued Study of the Use of Created Ponds for Amphibian Breeding in Fragmented Forested Areas

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ABSTRACT

A continued study of the use of created ponds for amphibian breeding in fragmented forested areas

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Amphibian populations are declining worldwide due to factors such as habitat degradation, fragmentation and destruction. I conducted a study to explore the use of created ponds in a forested habitat by breeding amphibians, specifically *Rana sylvatica* and *Ambystoma maculatum*. The objectives were to examine the movement of these animals after leaving the ponds, the survival and movement of juveniles, how both respond to fragmentation, and how similar the created ponds were to natural ones. Nine ponds were constructed in December 2003 in the MeadWestvaco Wildlife Ecosystem Research Forest (MWERF) in Randolph County, West Virginia. All trapped amphibians were measured and given a pond specific mark. Three silviculture treatments were cut around all ponds in August 2006. A significant difference in air temperature was found between elevations and between silviculture treatments. A significant difference in capture rates was found between elevations. A significant difference was found between created and natural ponds.

ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Dr. Thomas K. Pauley, for his support and guidance throughout my graduate school career. I feel he took a chance on me with nothing behind it but faith and the opinion of one of his past graduate students and I will always be grateful that he decided to take it. I would like to thank Dr. Tom Jones and Dr. Jayme Waldron for taking the time to serve on my committee and for the helpful suggestions and revisions on early drafts of my thesis. None of this would have been possible without the help of Celeste Good and Deborah Barry. They created and implemented this project between 2004 and 2005 and were unselfish in giving me access to all of their data. I would like to thank Dr. Laura Adkins and Dr. Zach Felix for taking the time out of their busy schedules to assist with my statistics. I would like to thank the Herpetology Lab for their friendship and support. I would like to thank all of the various graduate students and technicians that shared the field site with me over the course of this project. They provided hours of entertainment when not in the field and I will cherish their friendships for years to come. Particularly, I would like to thank Shawn Crimmins and Chris Runner for their help in locating field sites and for offering advice and support when it was sorely needed. I would like to thank MeadWestvaco for providing access to their research forest and for partial funding for this project. I would like to thank the Marshall University Graduate College for providing partial funding for this project. Finally, I would like to thank my family for always supporting and believing in me even when I was not so sure I deserved it.

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INTRODUCTION

Amphibian populations have been declining worldwide mainly because of habitat fragmentation, destruction, and degradation (Dimauro and Hunter, 2002). Amphibians are important to the ecosystem because they keep decomposition species under control by being a main predator and also offer nutrients to the ecosystem by being a prey species for many other animals in the forest (Burton and Likens, 1975). Many forest-dwelling amphibians have biphasal life cycles where they use aquatic habitats for breeding and terrestrial habitats for foraging and hibernation, including *Rana sylvatica* (wood frog) (Figure 1) and *Ambystoma maculatum* (spotted salamander) (Figure 2). Many studies have shown that both species are ~ 100 % philopatric to their natal pools, sensitive to canopy cover (needing 60-70 %), and sensitive to edge effects (deMaynadier and Hunter, 1998, Homan et al. 2004, Skelly et al. 2002). Their particular habitat requirements and the need to move between different environments make them susceptible to habitat changes that may prevent them from moving between required habitats. In West Virginia, 98% of the 78% of forested land is available for timber harvesting (Griffith and Widmann, 2003). This could impact forest-dwelling amphibians by the removal of obligate breeding habitat and movement corridors during and after the harvesting of timber.

One prospective conservation tool involves mitigation via construction of new temporary pools to replace destroyed ones in unharvested areas for amphibian breeding purposes along with the creation of corridors to allow for connectivity between forested habitats. A study was started in 2003-2004 in Randolph County, West Virginia by

Deborah Merritt and was later continued by Celeste Good (Good, 2006) to consider the effectiveness of this conservation tool. Specifically, Good (2006) found that amphibians would colonize constructed ponds for breeding purposes, that juvenile amphibians could successfully egress from constructed ponds, and that there were slight phenological differences between elevations but many questions were left unanswered. The objectives of my study were to continue where the earlier research left off and (1) monitor created ponds for initial and continued amphibian breeding activity, particularly by *R. sylvatica* and *A. maculatum*; (2) monitor juvenile egression from the created ponds; (3) investigate the affect of habitat fragmentation on adult migration and juvenile egression; and (4) compare the created ponds to natural ponds to demonstrate if they offer suitable breeding habitat for different amphibian species.

METHODS

STUDY SITE

Three sites were originally created for this study in December of 2003 within the MeadWestvaco Wildlife and Ecosystem Research Forest (MWERF) in Randolph County, WV (Figure 3). The MWERF is a privately owned deciduous forest where Best Management Practices are implemented for timbering. The management goals of the MWERF were to employ conservation-minded logging practices as well as to allow ongoing research to study how those practices affected the forest community. Common tree species in the area include: *Liriodendron tulipifera* (Tulip poplar), *Prunus serotina* (Black cherry), *Quercus rubrum* (Red oak), *Acer saccharum* (Sugar maple), *Acer rubrum* (Red maple), *Acer pensylvanicum* (Striped maple), *Magnolia frasier* (Frasier magnolia), and *Fagus grandifolia* (American beech).

In December of 2003, 3 ponds were constructed at 3 compartments in the MWERF using a bulldozer. Each pool was lined with clear DuPont plastic and covered with soil. A 4^{th} site was included at the Three Forks tract and was created in the same fashion during the spring of 2005 (Table 1). Each pond was placed on a north-east facing slope, which stays cooler and more moist during the summer months which is also a habitat preference for amphibians. The ponds were allowed to fill naturally with precipitation throughout the winter months. The size of these ponds was approximately 2 to 3.5 m in diameter. Their depths fluctuated throughout the study with the most shallow being 2 cm and the deepest 1 m. One of the compartments located along Rocky Run in the MWERF, was eliminated from the study in early spring 2005 due to it's inability to retain water. As a result 2 of the original 3 compartments on the MWERF were used for this study. Two sites (Compartments 7 and 9) were located near Rocky Run while the third site (Compartment 3) was located 16 km north on the Three Forks tract which is also owned by MeadWestvaco. Ponds at Compartment 3 are between 715 and 761 meters in elevation, while ponds at Compartments 7 and 9 are between 805 and 957 meters in elevation.

In February of 2004, drift fences were constructed around each pond with 18 inch silt fencing supported by wooden garden stakes (Figure 4). The fencing was secured at the base with metal garden stakes to eliminate any holes or gaps between the fence and the ground that might allow amphibians to escape beneath it, thus avoiding the traps. A funnel trap array was set up on both sides of the fence surrounding each pond. Funnel trap methods have been proven highly effective for trapping *R. sylvatica* (Buech and Egeland, 2002). The funnel traps were created out of 18.9 L plastic buckets fitted with plastic lids. Small bungee cords were used to help secure the lids. A hole was cut at the base of the bucket and a wire mesh funnel was fit into it and acted as a ramp for amphibians to crawl into the traps. Buckets were placed in pairs beside the fence so that the funnel was flush to the ground and the fence to decrease the chance of trap avoidance. In total, there were 16 traps (8 inside and 8 outside the fence) around each pond. When the ponds were added at Compartment 3, drift fence and funnel traps were installed using the same methods but using some different materials. These traps were created using 18.9 L plastic Tupperware containers and both ends were fitted with a wire mesh funnel. Four traps were placed inside and outside the fence for a total of 8 traps per pond (Good, 2006).

In the fall of 2006, three fragmentation treatments; a clear cut leaving a 100 m buffer zone surrounding a pond, a clear cut leaving a 100 m buffer zone surrounding a pond and a 20 m wide forested corridor which connected the pond to a nearby forested area, and a control treatment; were cut in all of the compartments (Figure 5). This was done to study what effects habitat fragmentation would have on the migration of breeding amphibians and juvenile egression. A telemetric study was also used to assess the possible silviculture treatment effects on adult amphibian movements.

Figure 1. *Rana sylvatica* adult.

(Photo by: Amy Schneider)

Figure 2. *Ambystoma maculatum* adult.

(Photo by: Amy Schneider)

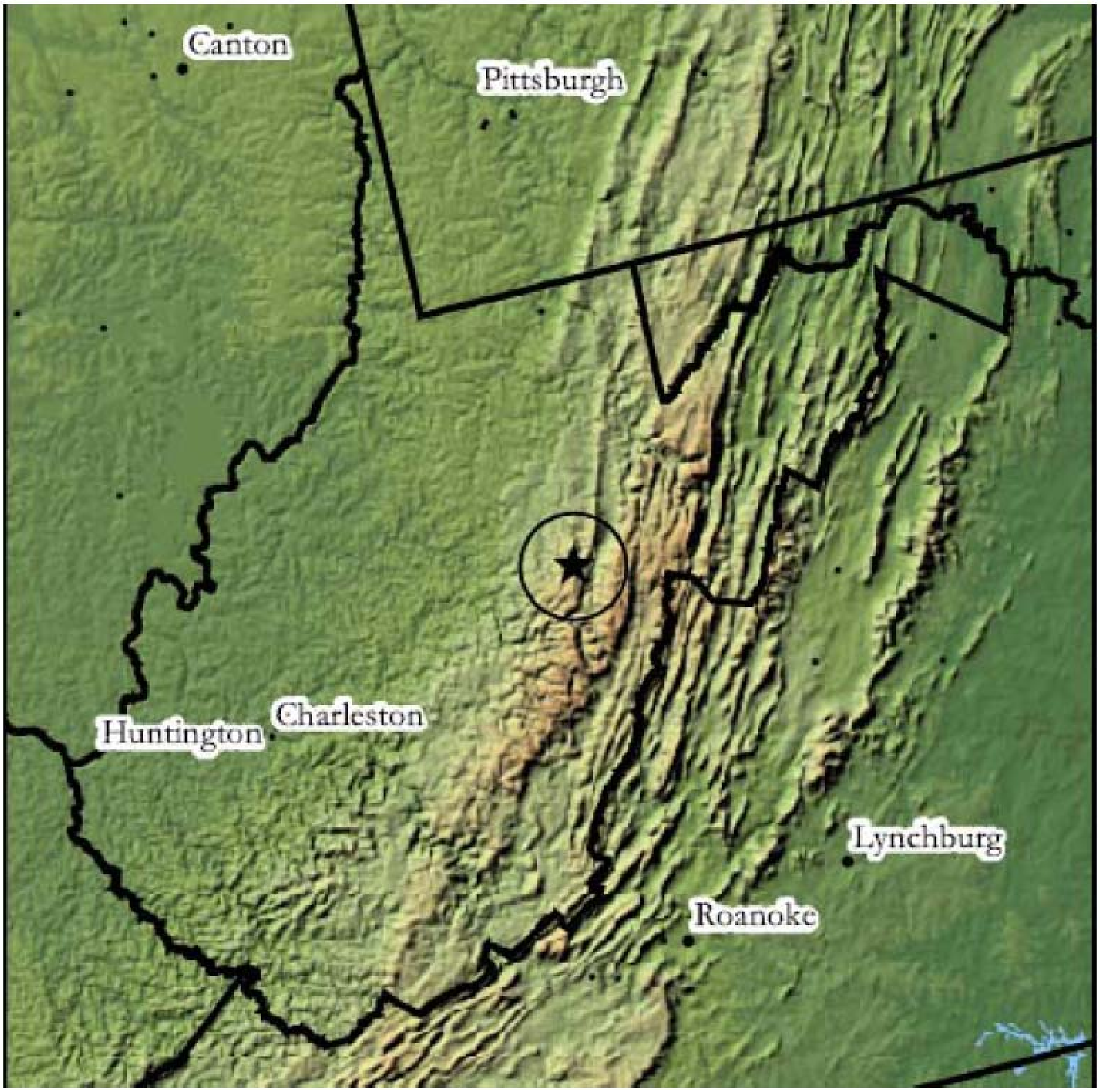

Figure 3. Location of the MWERF in Randolph County, WV.

(Created by Celeste Good)

Figure 4. Created pond with drift fence and trap array at Compartment 7 pond 3.

(Photo by: Amy Schneider)

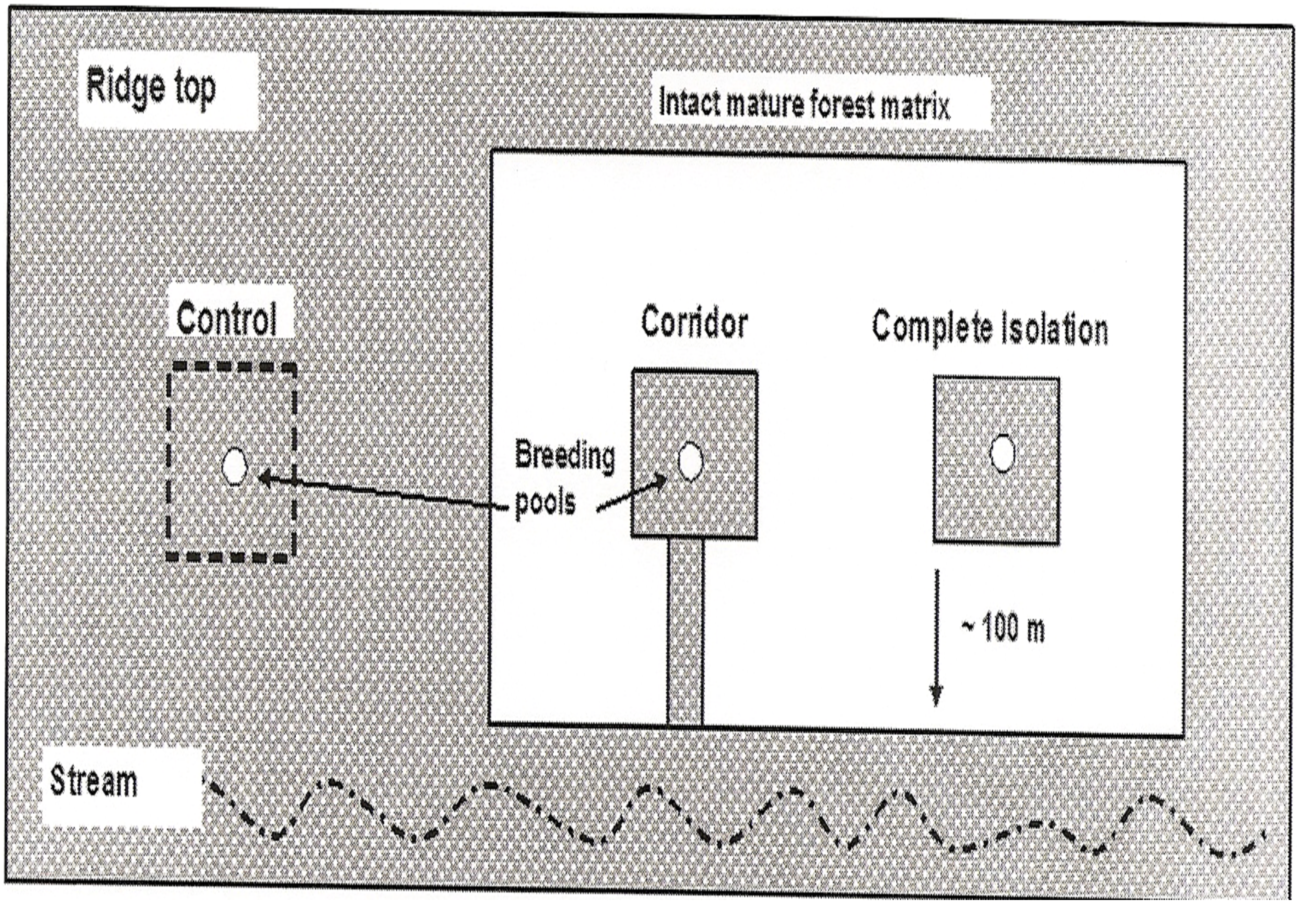

Figure 5. Schematic of the experimental treatments for the three constructed pond types.

(Created by: Celeste Good)

AMPHIBIAN MONITORING

In April 2004, amphibian eggs of *A. maculatum* and *R. sylvatica* were stocked in every pond at the Rocky Run site due to lack of adults naturally colonizing the ponds that first year (Figure 6). Eggs were collected from a natural pond on Birch Fork Road in the MWERF. One *R. sylvatica* and 8 *A. maculatum* masses were collected and staged (Gosner, 1960). Eggs and larvae were first distributed throughout the ponds at Compartment 9 on 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* eggs since it was retaining twice as much water as the other two. When these eggs hatched, *R. sylvatica* tadpoles were distributed throughout all the ponds in Compartment 7. Only ponds 1 and 2 received one *A. maculatum* egg mass as pond 3 did not have a sufficient water level at that time (Good, 2006). Ponds in Compartment 3 were not seeded because they were added to the original study in 2005 (Tables 1 and 2).

Ponds were monitored from March through October from 2004 to 2007. Traps were checked at least twice weekly until eggs hatched and tadpoles reached stage 30, as determined using the Gosner key to anuran development (Gosner, 1960) (Figures 7-11). This stage is discernible by limb bud growth to twice the diameter of its length. From May through August traps were checked at least 5 times per week to monitor for any *R. sylvatica* and *A. maculatum* juveniles that may egress (Good, 2006) (Figure 12).

Environmental information recorded included air temperature, water pH, dissolved oxygen levels, water temperature, soil temperature, and weather conditions (sunny, rain, etc). Air temperature was measured with I-buttons which are quarter sized data loggers created by Dallas Semiconductor, Incorporated. I-buttons were placed at each pond and at one forest edge and one clear cut area of each compartment. Water and soil temperature were taken with temperature probes every time 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 with a measuring tape. pH was taken with a Oakton Instruments Waterproof pHTestr 1 pH meter with probe near the pond surface. In the fall, dissolved oxygen was also measured twice a week with the YSI Model 55 handheld dissolved oxygen and temperature system. These were measured less often because it was believed that they would fluctuate less than the other environmental factors and, in the case of the dissolved oxygen reader, the equipment was unavailable for most of the field season. All ponds had their canopy cover measured by ocular estimation.

Amphibians trapped entering or leaving the ponds were measured with Swiss Precision Instruments 2000 calipers. Measurements included snout-to-vent length (SVL) and vent-to-tail length (VT) when appropriate. In 2004 and 2005, animals were given a visible implant elastomer (VIE) tags that was pond specific (Good, 2006). In 2004, each amphibian captured was given a pond specific batch mark, which consisted of one mark in one of the following: Pond one: right front axillary (RF), Pond two: left front axillary (LF), or Pond three: right rear inguinal (RR) on the ventral side of the animal. In 2005, adults captured were given a batch mark while juveniles received an individual specific tag. These consisted of 3 marks (yellow, red, green, or orange) per animal according to an assigned code. This permitted the possibility of population estimates and recapture information on initial size and location of capture (Good 2006).

In 2006 and 2007, adult *R. sylvatica* that were caught at the created ponds were fitted with an external transmitter that allowed for monitoring of movement via radio telemetry (Figure 13). Transmitters, created by Wildlife Materials, were fitted to the frog with an aluminum beaded chain as a belt (Rathburn and Murphy 1996). They were located using a Wildlife Materials receiver and a Yagi antenna everyday for the first 5 days to ensure the fit of the transmitter belt as well as to verify that the transmitter was functioning properly. After that, frogs were located every 2 days unless there was a rain event or a large movement was recorded. If either incident occurred, tracking commenced every day to guarantee the radio signal was not lost due to movement that would take the frog out of signal range. GPS coordinates were taken with a Garmin E-trex Legend unit every time a frog was located and that data was used to identify any trends in their movement. Movement patterns were compared to NOAA weather data (NOAA, 2008) to identify any correlation between movement and weather patterns and were mapped with ARCGIS software.

Nine natural ponds were identified and sampled for amphibians to study how similar the created ponds were to natural ponds (Figure 14). These ponds were found on topographic maps that were created for a study on Northern Green Frogs (*Rana clamitans melanota*) (Rogers 1999) and by driving around the MWERF visually searching for ponds. Once located, the natural pond's overall size and depth were measured with a measuring tape. Environmental measurements such as water temperature and pH as well as canopy cover were recorded. The edge of each pond was sampled by making approximately ten sweeps with a D-framed net for every 5 square meters (Heyer et al 1994). All amphibians caught were identified to species and their numbers were tallied.

STATISTICAL PROCEDURES

An ANOVA (F statistic = 4.88, degrees of freedom (DF) = 14, $p < 0.0001$) was performed using SAS to test for differences in capture numbers at different elevations and used an alpha value of < 0.05. Canonical Correspondence Analysis (CCA) was performed with the software PC-ORD 5 to investigate variation in amphibian abundance as they relate to microhabitat measurements at both the natural and created ponds. CCA is a type of ordination where there is no testing of any hypothesis. It is a multivariate regression that produces several orthogonal axes consisting of as much possible correlation between sites and species as possible (Palmer, 1993). An ANOVA (F statistic = 458.50, DF = 16, $p < 0.0001$) including a Tukey's Studentized Range Test was performed using SAS to test for temperature differences between elevation and silviculture treatments and used an alpha value of < 0.05. Descriptive statistics were used to quantify movement patterns of adult *Rana sylvatica*.

Table 1. Number of *Rana sylvatica* captures by site, pond, and year on the MeadWestvaco Ecological Research Forest. Superscript numbers indicate ponds that were stocked with eggs in the spring of 2004. Asterisks indicate what year the adult counts were from (*) and when the silvicuture treatments were implemented (**).

Year	**Site**	**Pond#**	**Silviculture Treatment****	**#Egg Masses**	**#Egressing Juveniles**	**#Adults***
2005	C9	1[1]	---	6-8	6	3
		2[2]	---	6-8	1	8
		3[1]	---	6-8	0	4
	C7	1[3]	---	6-8	5	6
		2[3]	---	6-8	27	6
		3	---	6-8	16	5
	C3	1	---	6-8	51	0
		2	---	6-8	1	5
		3	---	6-8	53	0
2006	C9	1	---	8-10	0	4
		2	---	8-10	0	36
		3	---	8-10	5	18
	C7	1	---	8-10	0	7
		2	---	8-10	0	14
		3	---	8-10	0	1
	C3	1	---	8-10	0	0
		2	---	8-10	0	3
		3	---	8-10	0	1
2007	C9	1	Corridor	1	1	---
		2	Clear cut	25-30	40	---
		3	Control	45-50	6	---
	C7	1	Control	10-15	0	---
		2	Corridor	10-17	1	---
		3	Clear cut	1-3	1	---
	C3	1	Clear cut	8-10	0	---
		2	Corridor	1	0	---
		3	Control	8-10	0	---

[1] ponds were stocked with 300 *R. sylvatica* egg masses in spring of 2004
[2] ponds were stocked with 600 *R. sylvatica* egg masses in spring of 2004
[3] ponds were stocked with *R. sylvatica* tadpoles from ponds in Compartment 9 in spring 2004
* counts were from the following spring
** silviculture treatments were implemented in the fall of 2006

Table 2. Number of *Ambystoma maculatum* captures by site, pond, and year on the MeadWestvaco Ecological Research Forest. Superscript numbers indicate ponds that were stocked with eggs in the spring of 2004. Asterisks indicate what year the adult counts were from (*) and when the silvicuture treatments were implemented (**).

Year	Site	Pond#	Silviculture Treatment**	#Egg Masses	#Egressing Juveniles	#Adults*
2005	C9	1[1]	---	0	0	0
		2[2]	---	0	0	0
		3[1]	---	0	0	0
	C7	1[3]	---	0	0	0
		2[3]	---	0	0	0
		3	---	0	0	8
	C3	1	---	0	0	0
		2	---	0	0	0
		3	---	0	0	0
2006	C9	1	---	0	0	0
		2	---	0	0	0
		3	---	0	0	0
	C7	1	---	0	0	1
		2	---	0	0	1
		3	---	1	0	1
	C3	1	---	0	0	0
		2	---	0	0	0
		3	---	0	0	0
2007	C9	1	Corridor	0	0	---
		2	Clear cut	0	0	---
		3	Control	0	0	---
	C7	1	Control	0	0	---
		2	Corridor	0	0	---
		3	Clear cut	7	0	---
	C3	1	Clear cut	0	0	---
		2	Corridor	0	0	---
		3	Control	0	0	---

[1] ponds were stocked with 300 *R. sylvatica* egg masses in spring of 2004
[2] ponds were stocked with 600 *R. sylvatica* egg masses in spring of 2004
[3] ponds were stocked with *R. sylvatica* tadpoles from ponds in Compartment 9 in spring 2004
* counts were from the following spring
** silviculture treatments were implemented in the fall of 2006

Figure 6. *Rana sylvatica* eggs and tadpoles.

(Photo by: Amy Schneider)

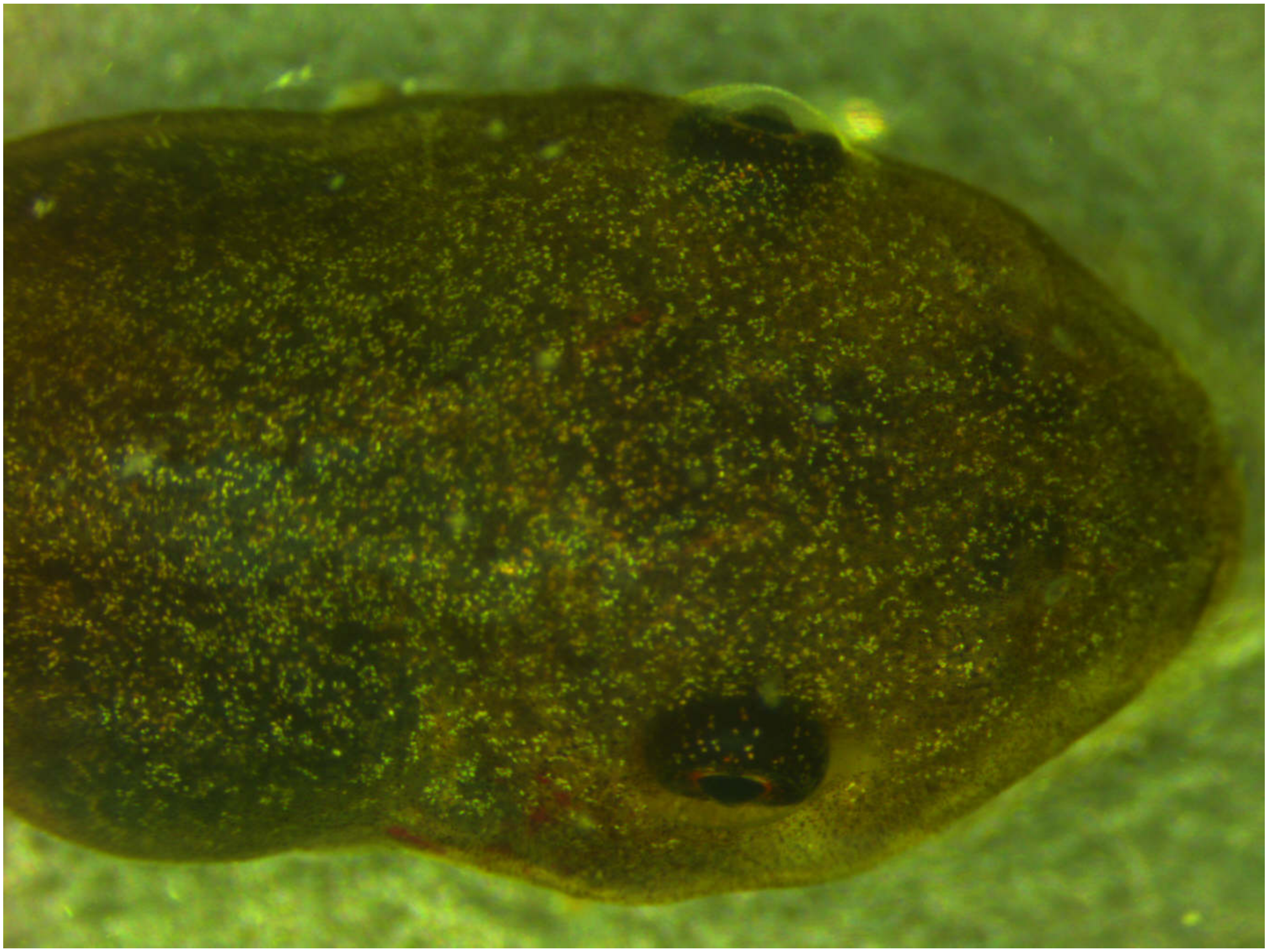

Figure 7. Dorsally positioned eyes of *Rana sylvatica* tadpole

(Photo by: Eric Diefenbacher)

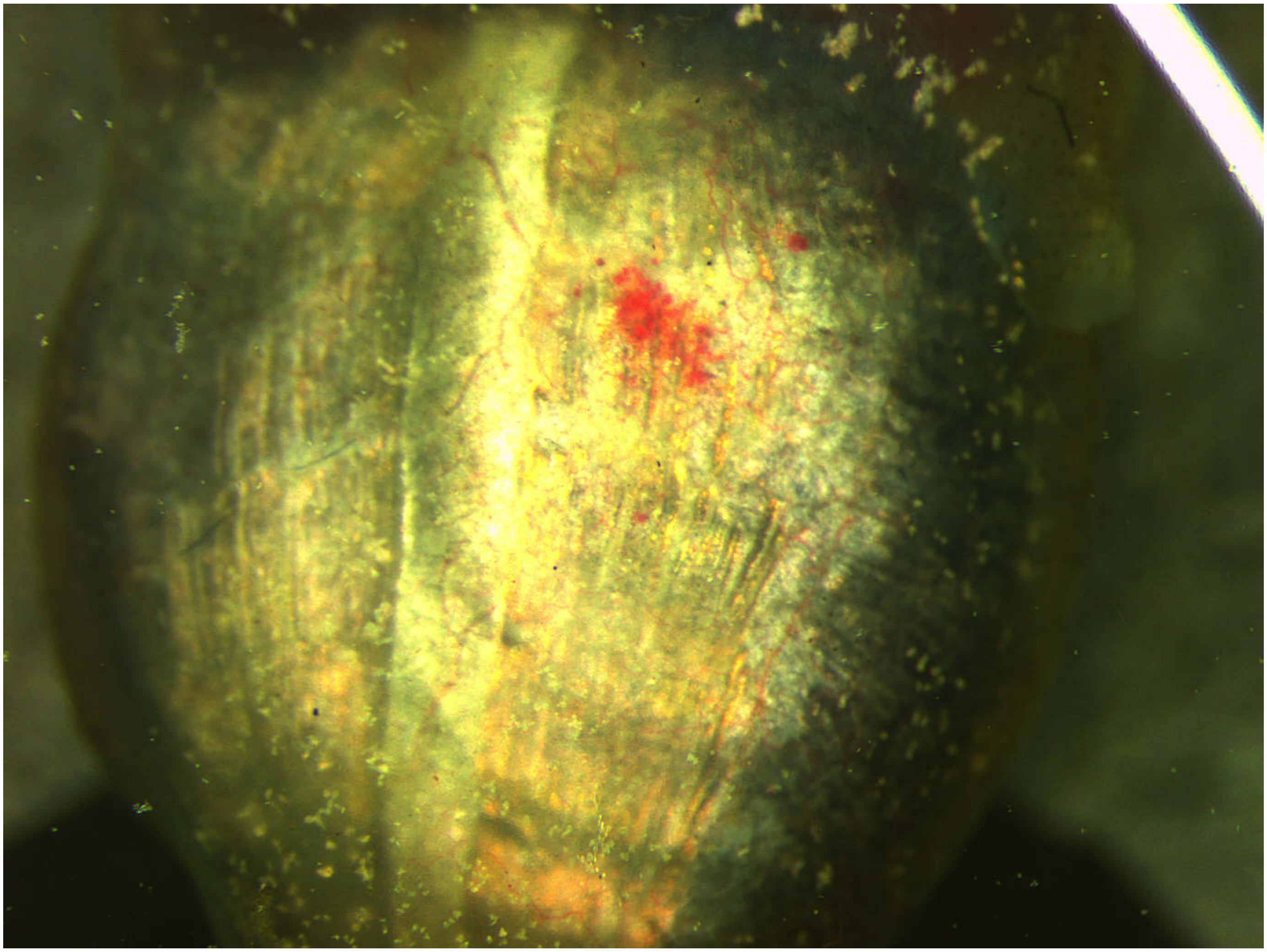

Figure 8. Visible intestinal coil of *Rana sylvatica* tadpole.

(Photo by: Eric Diefenbacher)

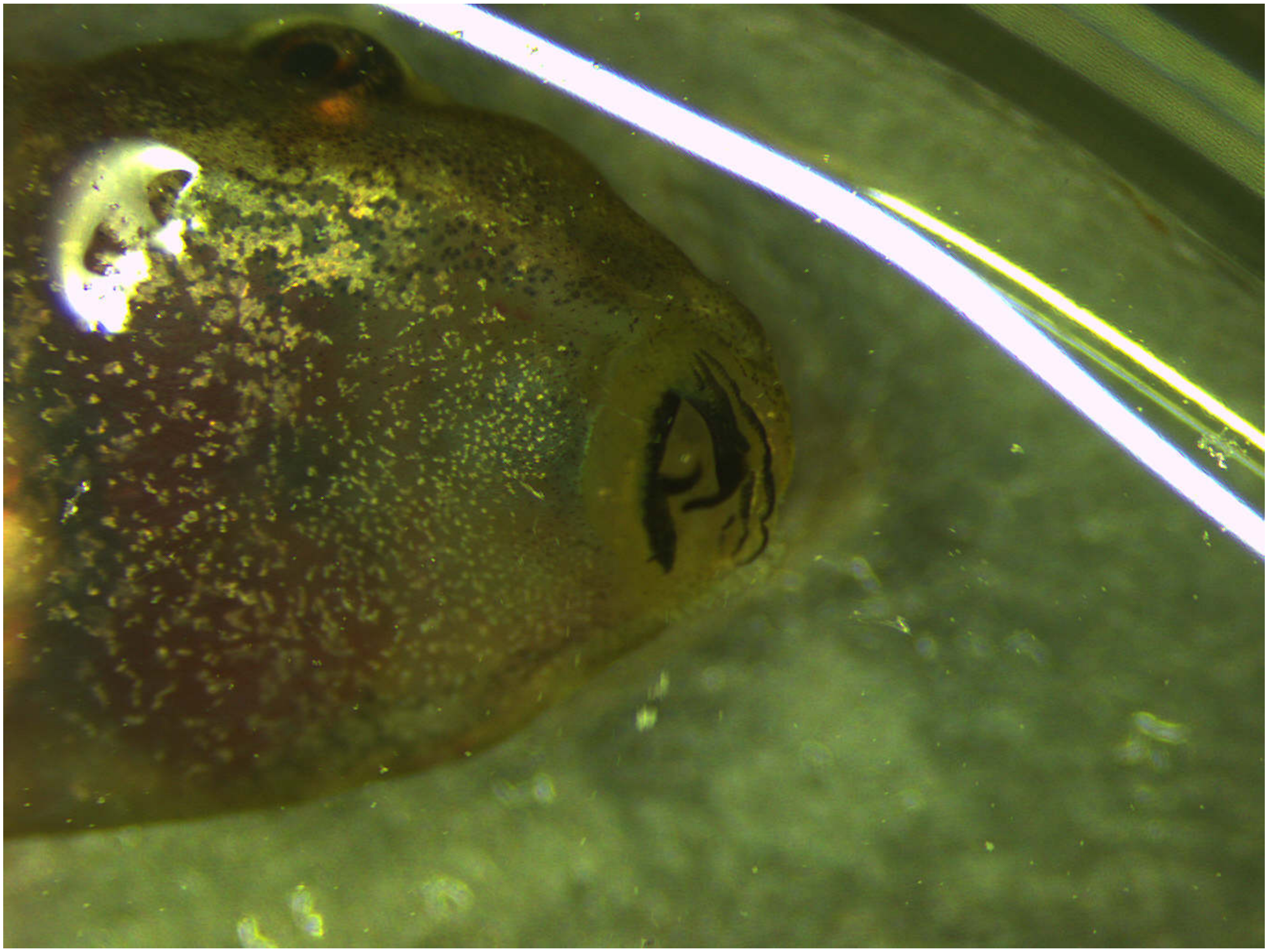

Figure 9. Three rows of top teeth of *Rana sylvatica* tadpole.

(Photo by: Eric Diefenbacher)

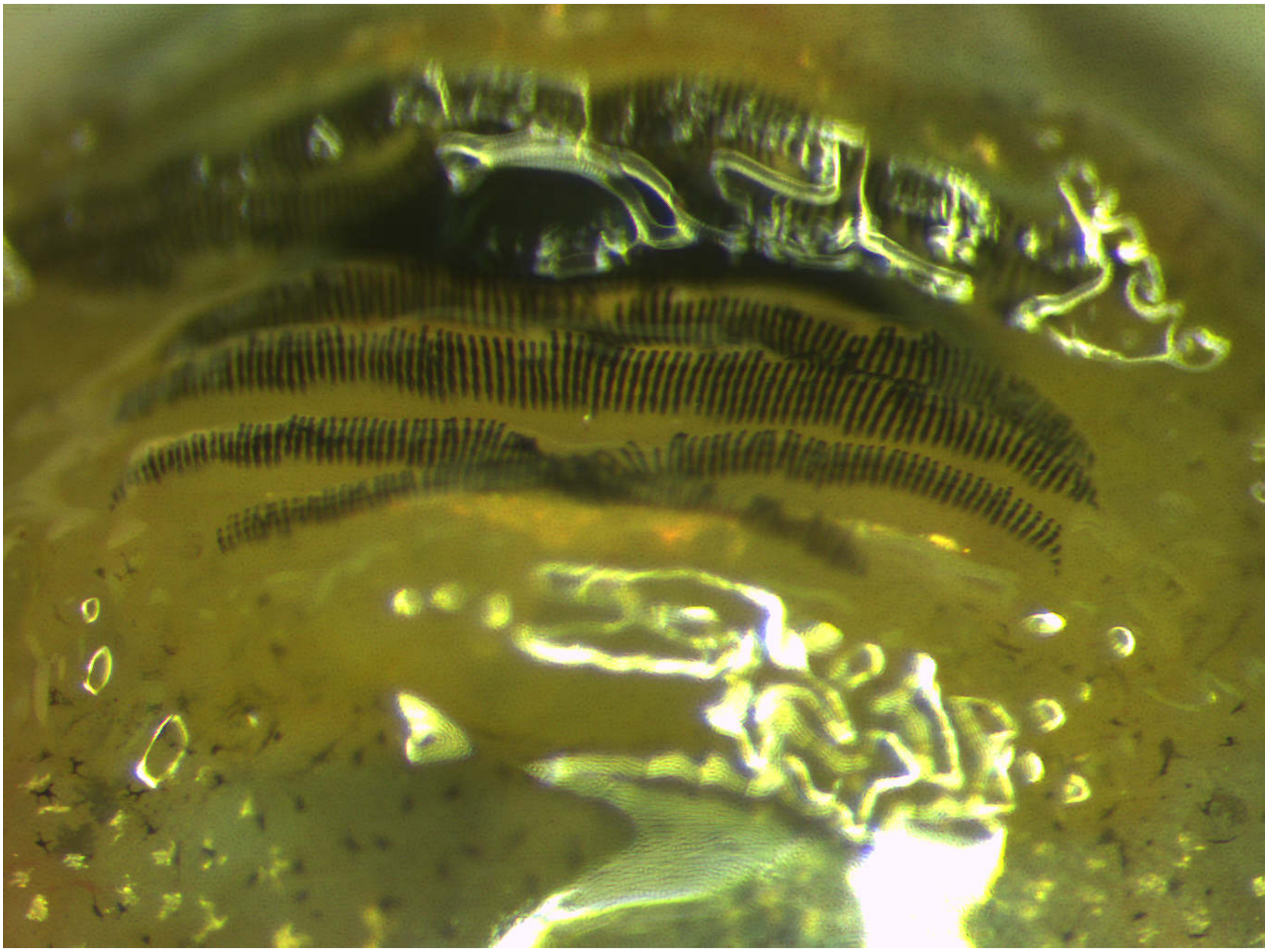

Figure 10. Four rows of bottom teeth of *Rana sylvatica* tadpole.

(Photo by: Eric Diefenbacher)

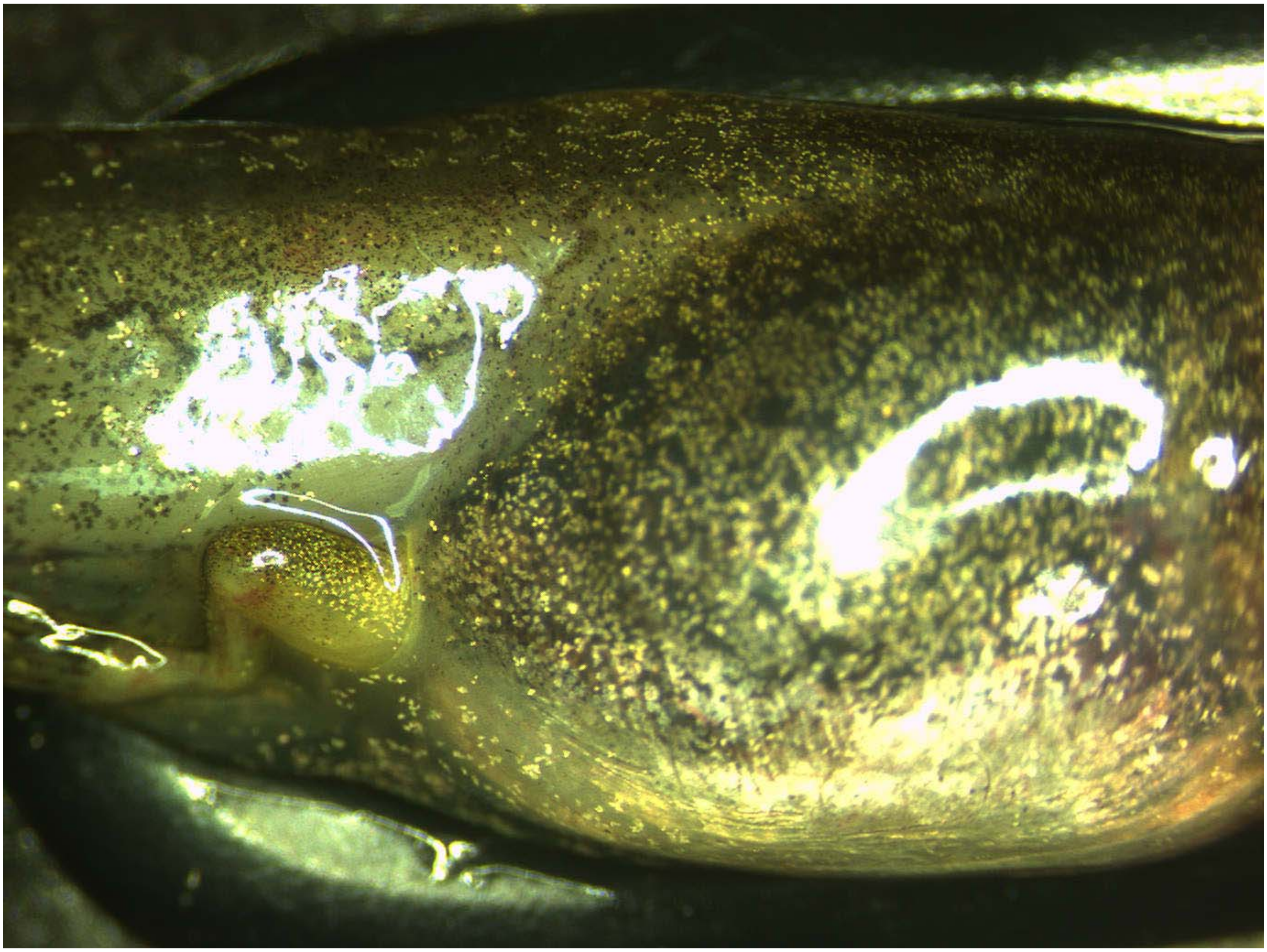

Figure 11. Tail-body interface of *Rana sylvatica* tadpole.

(Photo by: Eric Diefenbacher)

Figure 12. *Rana sylvatica* metamorph.

(Photo by: Amy Schneider)

Figure 13. Adult *Rana sylvatica* fitted with a radio transmitter.

(Photo by: Amy Schneider)

Figure 14. Natural Pond (P10) used to compare differences in species abundance and environmental factors between natural and constructed ponds.

(Photo by: Amy Schneider)

RESULTS

In 2004, no breeding amphibians were captured while in 2005 there were 17, 2006 35, and in 2007 86 adult *R. sylvatica* caught (Figures 15-19). Most were caught from the 28 March to 4 April in 2005, 28 March to the 1 April in 2006, 15 to 25 March in 2007. Eggs were laid mid to late March in all years with 6-8 egg masses per pond in 2005, 8-10 egg masses per pond in 2006 and anywhere from 1 to 50 egg masses per pond in 2007 (Table 1). Eggs hatched near the end of April in 2005 and early to mid April in 2006 and 2007. Tadpoles began metamorphosing between mid-June and early July in 2005 but did not start until the end of July in 2006 and 2007. Tadpoles remained at Compartment 9 in 2005 and 2007 through November while other sites had successful egression by mid-September for both years. There is limited data for juvenile metamorphosing from 2006 due to time and monetary constraints.

In 2004, 25 *R. sylvatica* juveniles from ponds 1 and 2 from both Compartment 7 and 9 and 4 *A. maculatum* juveniles from pond 2 in Compartment 7 and pond 1 in Compartment 9 were captured from stocked egg masses in late June-July. A pair of breeding *A. maculatum* was observed in March of 2004. In 2006, eight *A. maculatum* were captured and eggs were observed at one pond in Compartment 7. In 2007, three *A. maculatum* were captured and eggs were present at two ponds in Compartment 7.

Rana sylvatica juveniles were caught between July and October in 2005 (n=154), few were caught in late July in 2006 (n=5), and in 2007 were caught between late July and mid September (n=54). In 2005, most juvenile captures occurred in Compartment 3 while none occurred in 2006 and 2007. Most captures took place at Compartment 9 for both 2006 and 2007 with 5 captures occurring in Compartment 7 in 2007 and no captures occurring in 2006.

For all sites, snout-to-vent length (SVL) ranged from 48.1 mm to 69.3 mm (μ=55.8 mm) for adults and from 9 mm to 20 mm (μ=14.5 mm) for juvenile *R. sylvatica* in 2005. SVL ranged from 39.6 mm to 60.1 mm in adults (μ=51.9 mm) and from 17.1 mm to 18.6 mm in juveniles (μ=17.9 mm) for 2006. In 2007, adults SVL ranged from 47 mm to 79.8 mm (μ=60.6) and juveniles ranged from 12 mm to 21.5 mm (μ=14.2 mm).

Air temperature was statistically different between elevations. The mean temperature at the lowest elevation was 0.76 - 1.25 ºC higher than at the highest elevation and was 0.97 - 1.47 ºC (F statistic = 458.50, DF = 16, $p < 0.0001$) higher than at the middle elevation (Figure 20). There was no statistically significant difference between the temperatures at the middle and highest elevations. There were statistically significant differences in temperature for the silviculture treatments (Figure 21). The mean temperature in the clear cut was 0.06 - 0.80 ºC higher than at the edge and was 1.79 - 2.70 ºC (F statistic = 458.50, DF = 16, $p < 0.0001$) higher than all the ponds. The mean temperature at the forest edge was 1.36 - 2.27 ºC (F statistic = 458.50, DF = 16, $p < 0.0001$) higher than all the ponds. The silviculture treatments around the ponds created no statistically significant differences in air temperature at the ponds (Figure 22).

The telemetry study showed long distance movements for 7 adult frogs (median for 2006= 154 m and for 2007= 327 m) from late June through mid-July of 2006 and 2007 (Figure 23). In 2006, 4 adult *R. sylvatica* (3 males and 1 female) were tracked for ~ 1 month with movements averaging 31.4 m. In 2007, 2 adult *R. sylvatica* and 4 *R. c. melanota* (2 adults and 2 sub-adults) were tracked for ~ 1 month with movements averaging 34.5 m (37.1 m for *R. sylvatica*). *Rana c. melanota* were included in 2007 due to equipment maintenance which delayed the deployment of the transmitters past the breeding season of *R. sylvatica*. The 2 sub-adult *R. c. melanota* slipped their transmitters within 1 day to 2 ½ weeks after deployment. One adult *R. sylvatica* in 2006 and 1 adult *R. c. melanota* in 2007 were predated upon by unknown predators near the end of the telemetry study while one adult *R. sylvatica* disappeared with the transmitter in 2007.

Based on the CCA analysis of microhabitat measurements and species captures in 2007, 84% of the variation in relative abundance can be explained by all three axes (Figures 24-26). The first axis explained 66.6% (Eigenvalue = 0.87, Pearson Correlation, R = 0.989), the second axis explained 15.4% (Eigenvalue = 0.202, R = 0.83) and the third axis explained 2.1% (Eigenvalue = 0.27, R = 0.607). *Rana sylvatica* abundance appeared to be related to % canopy cover and low pH levels while *A. maculatum* abundance appeared to not be related to any of the environmental variables measured. Also, the different type of pools appeared to separate out across the axes.

Other species trapped for all years included *Ambystoma jeffersonianum* (Jefferson Salamander) (n=1), *Bufo a. americanus* (Eastern American Toad) (n=8), *Desmognathus ochrophaeus* (Allegheny Mountain Dusky Salamander) (n=6), *Hemidactylium scutatum* (Four-toed Salamander) (n=1), *Notophthalmus v. viridescens* (Red-spotted Newt) (n=75), *Plethodon cinereus* (Eastern Red-backed Salamander) (n=2), *Pseudacris c. crucifer* (Northern Spring Peeper) (n=18), *Rana clamitans melanota* (Northern Green Frog) (n=117), and *Thamnophis s. sirtalis* (Eastern Gartersnake) (n=1).

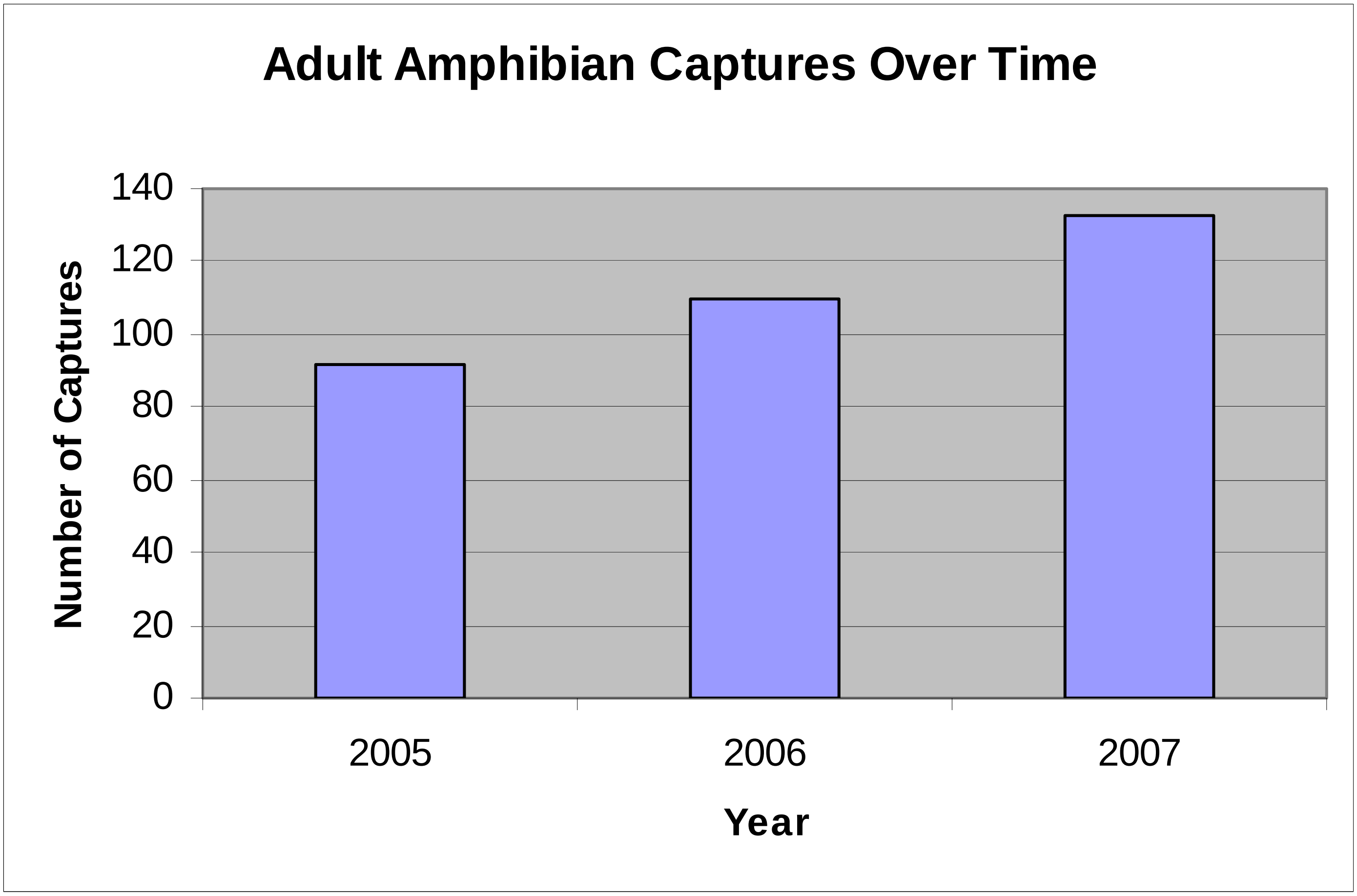

Figure 15. Adult amphibian captures for 2005-2007.

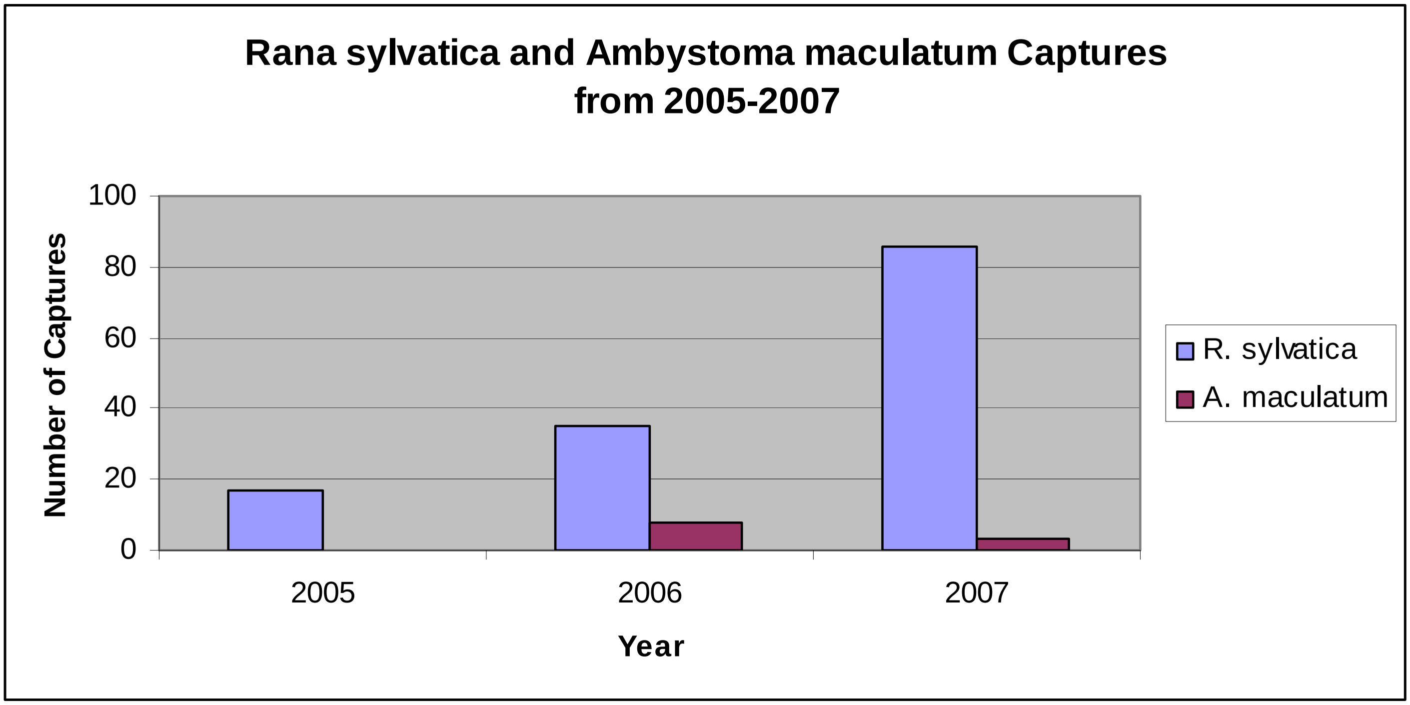

Figure 16. Adult *Rana sylvatica* and *Ambystoma maculatum* captures for 2005-2007.

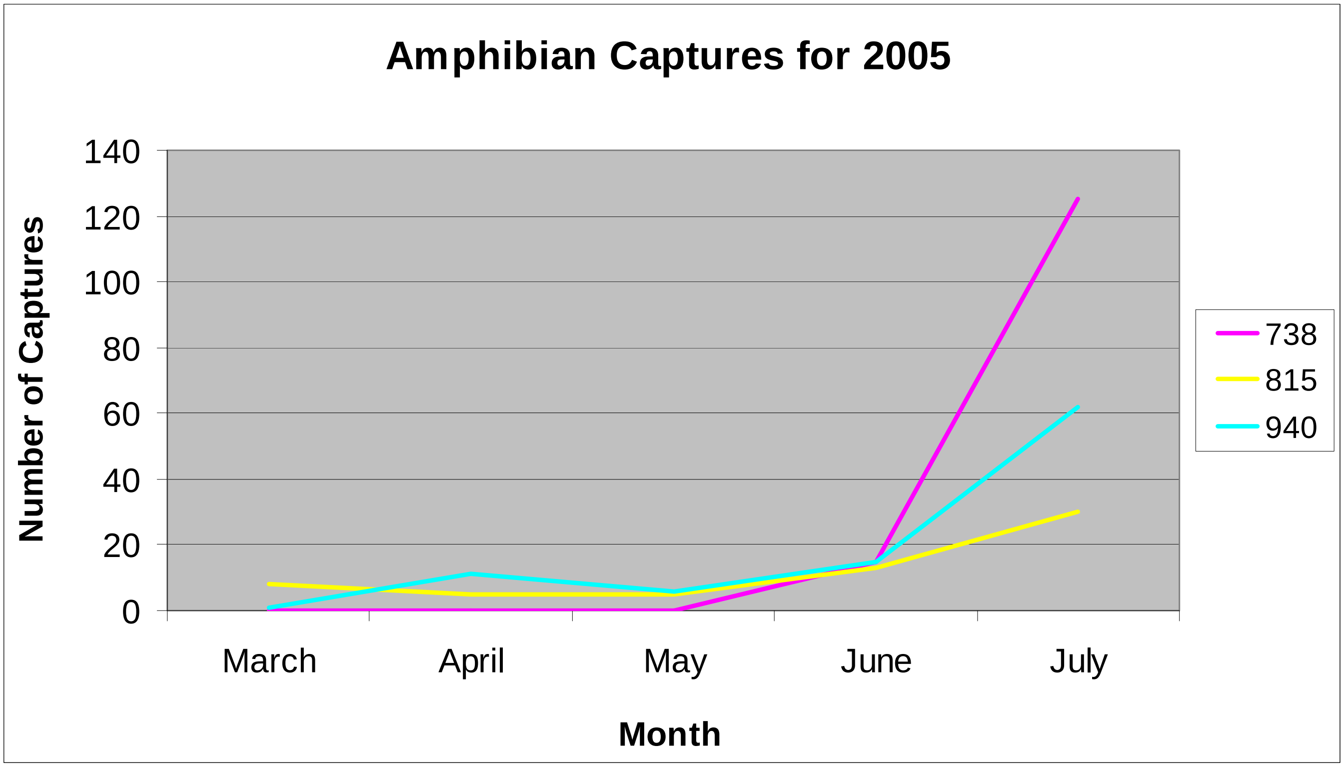

Figure 17. Total number of amphibian captures for 2005. A statistically significant difference was found between amphibian capture rates at different elevations using an ANOVA (F statistic = 4.88, DF = 14, $p < 0.0001$, $\alpha=0.05$).

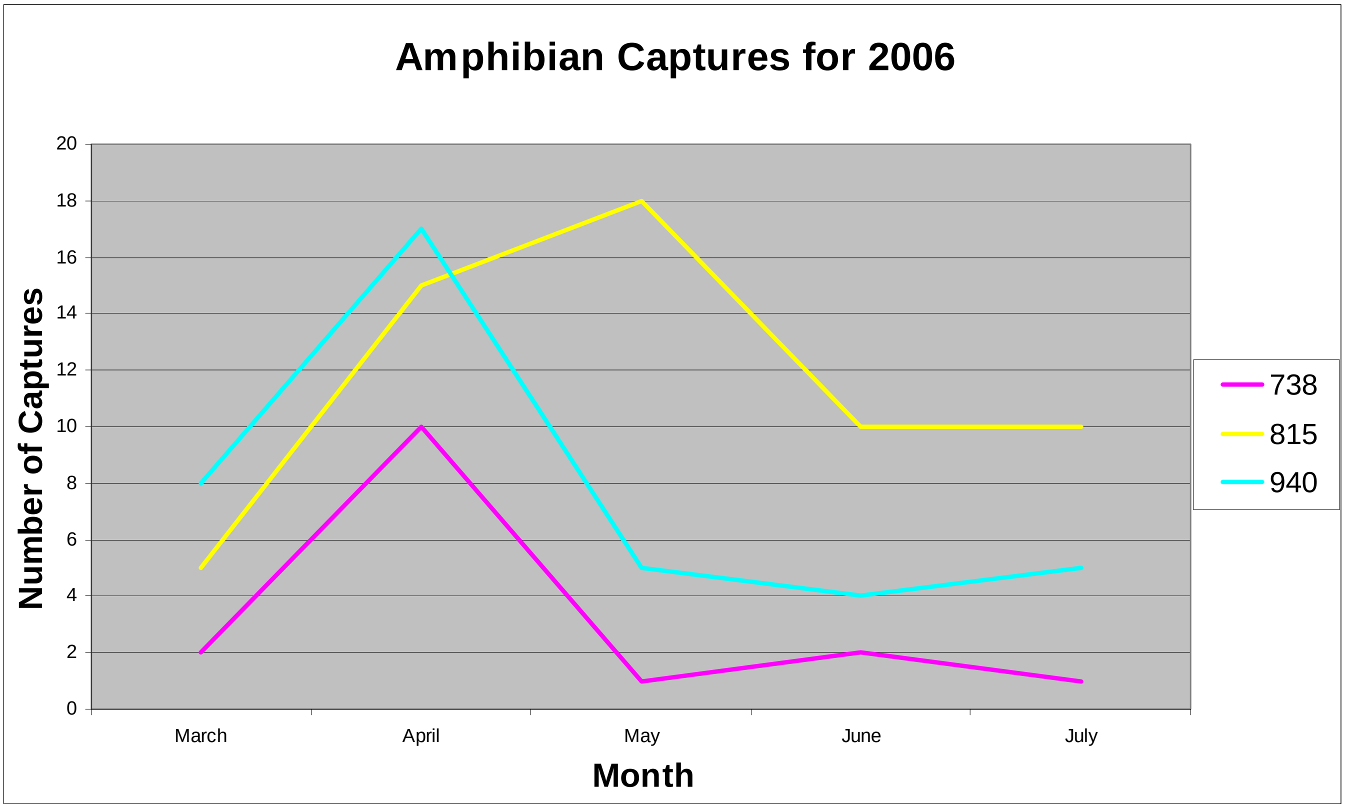

Figure 18. Total number of amphibian captures for 2006. A statistically significant difference was found between amphibian capture rates at different elevations using an ANOVA (F statistic = 4.88, DF = 14, $p < 0.0001$, $\alpha=0.05$).

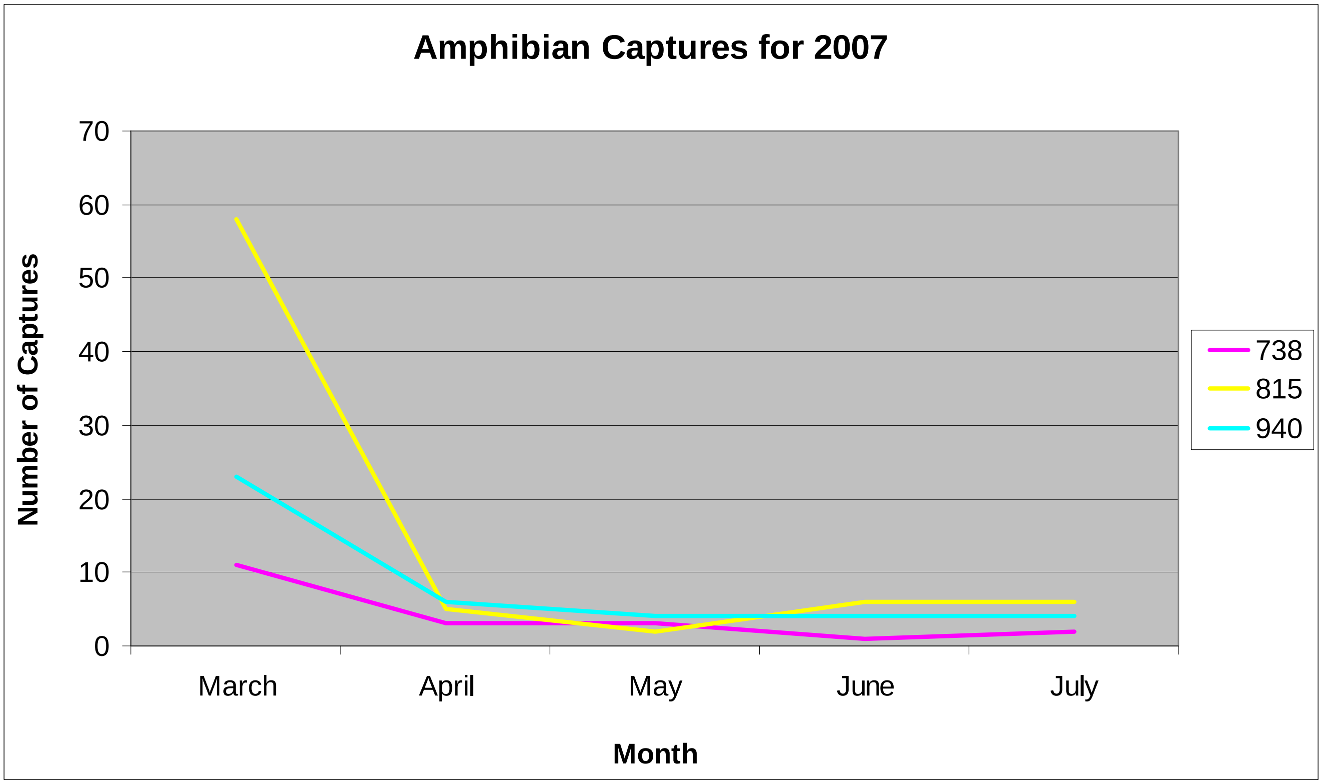

Figure 19. Total number of amphibian captures for 2007. A statistically significant difference was found between amphibian capture rates at different elevations using an ANOVA (F statistic = 4.88, DF = 14, $p < 0.0001$, $\alpha=0.05$).

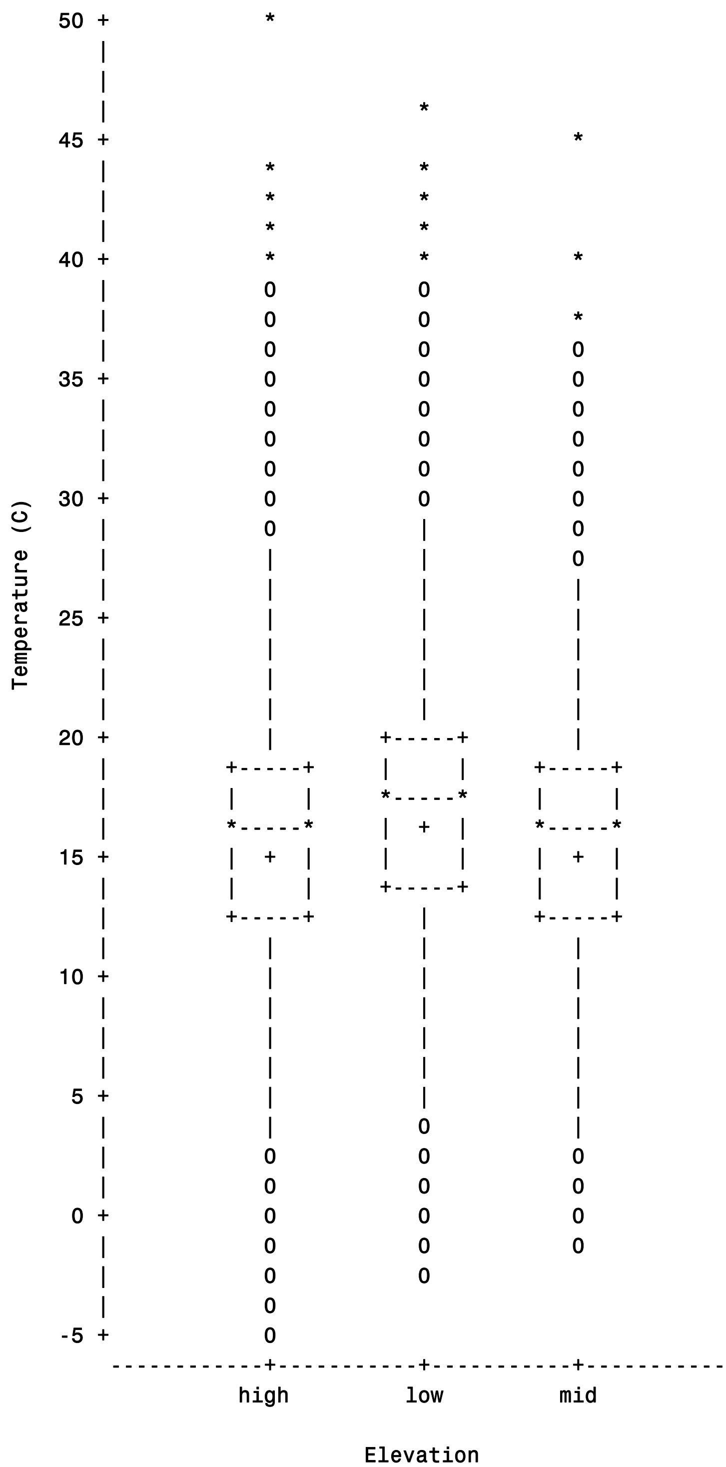

Figure 20. Box and whisker plot representing the average temperatures (ºC) for each elevation created in SAS. The lowest elevation was found to be statistically different from the middle and highest elevations (ANOVA, F statistic = 458.50, DF = 16, p < 0.0001).

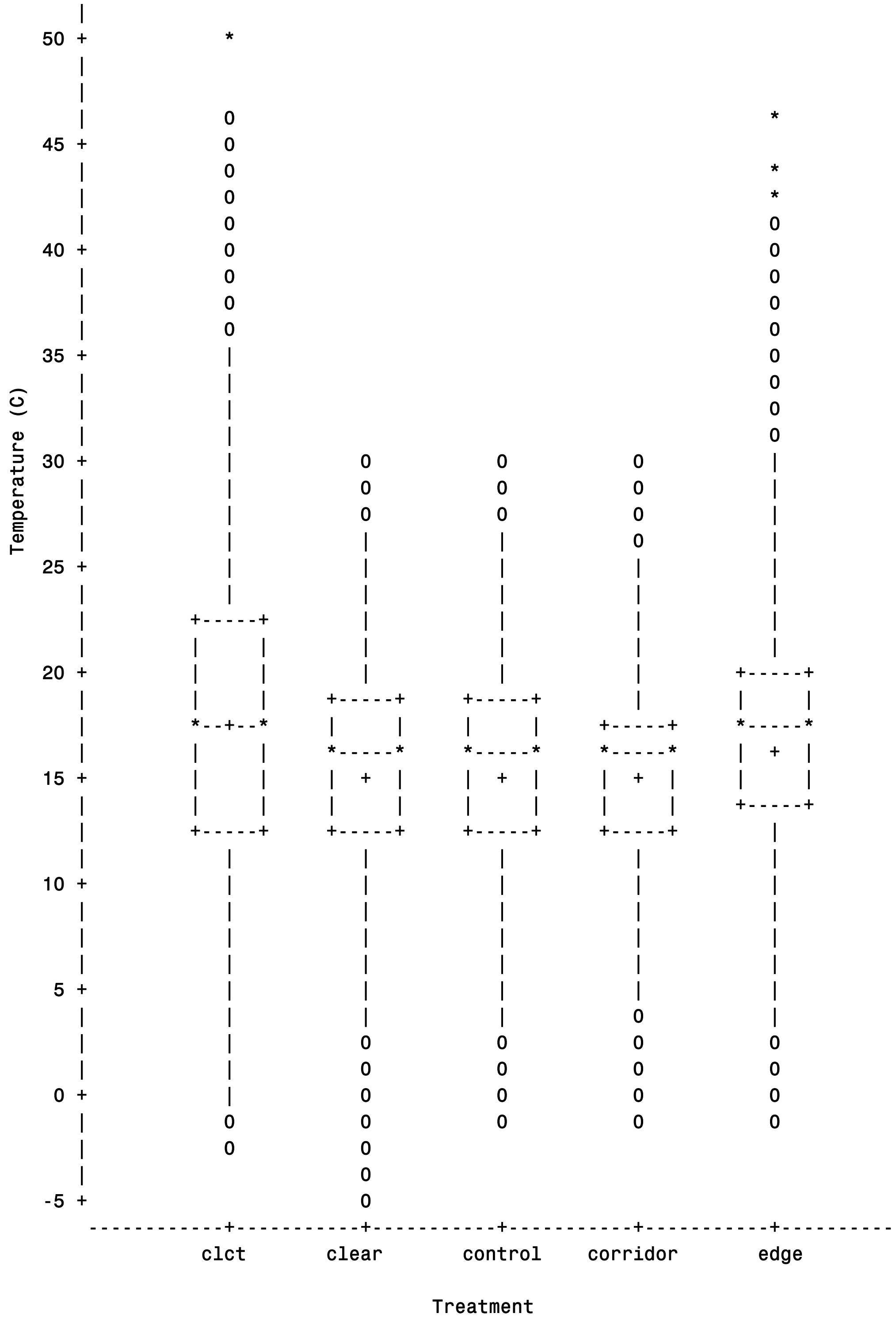

Figure 21. Box and whisker plot representing the average temperatures (ºC) for each silvicuture treatment plus a forested edge and a clear cut created in SAS. The clear cut (clct) and the forested edge (edge) temperatures were found to be statistically different from each other as well as from the other silviculture treatments (ANOVA, F statistic = 458.50, DF = 16, $p < 0.0001$).

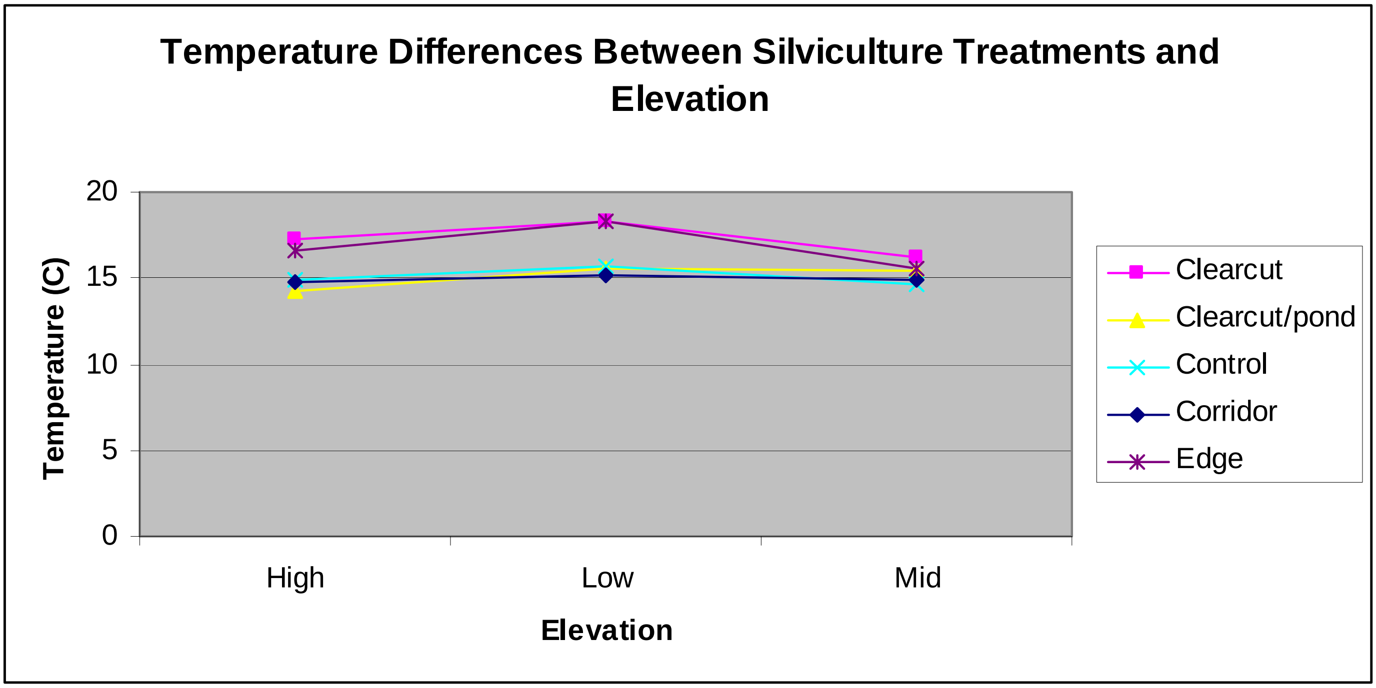

Figure 22. Comparison of mean temperature differences between silviculture treatments and elevation for 2007. A statistically significant difference was found between the clearcut temperatures and all other treatments as well as for the forest edge temperatures and all other treatments using an ANOVA with a Tukey Studentized test to test for differences between elevations and silviculture treatments

(F statistic = 458.50, DF = 16, $p < 0.0001$, α=0.05).

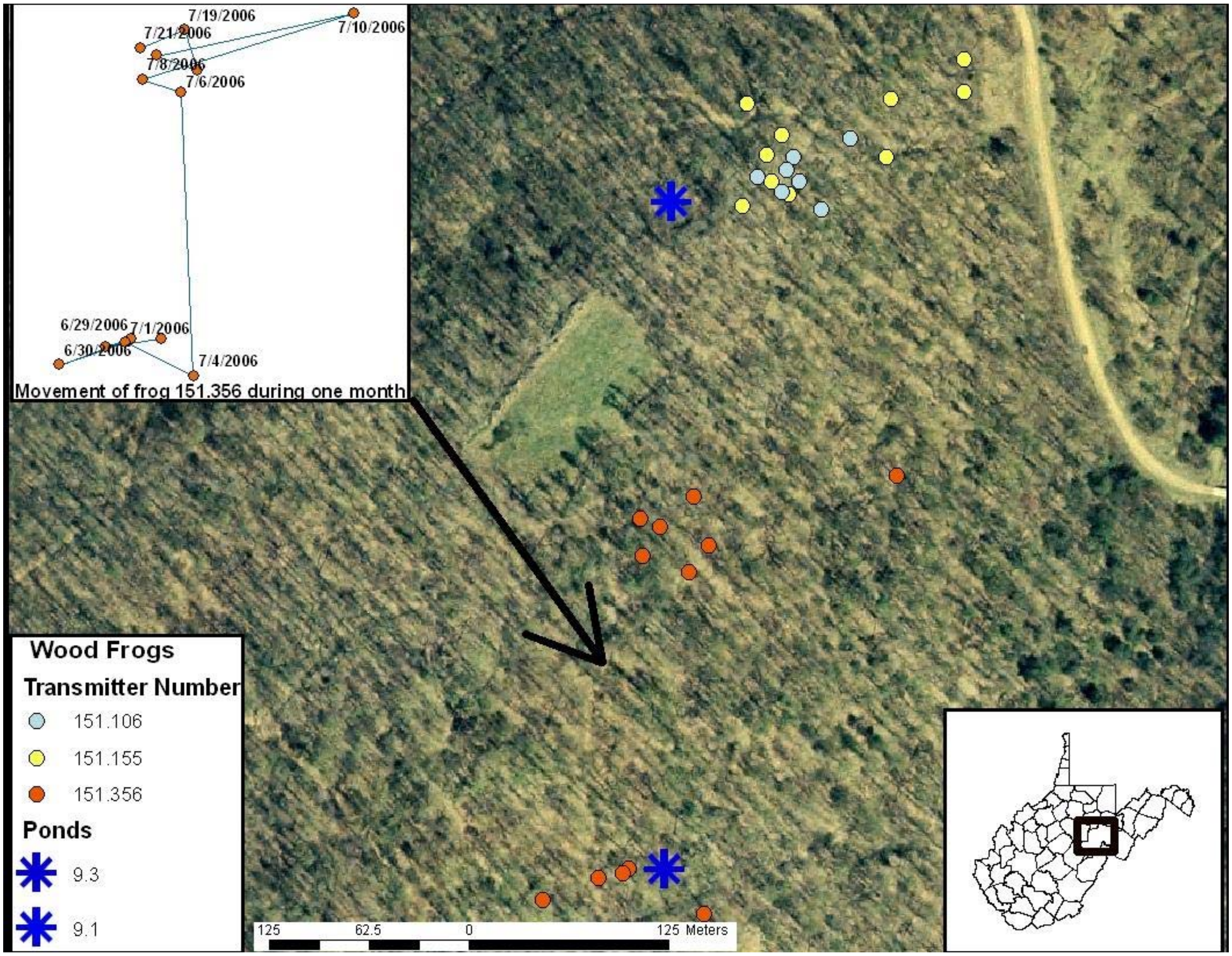

Figure 23. ArcMap recreation of *Rana sylvatica* movements for 2006. Each color represents a different frog. The picture in the upper left corner shows the actual route of one frog (151.356).

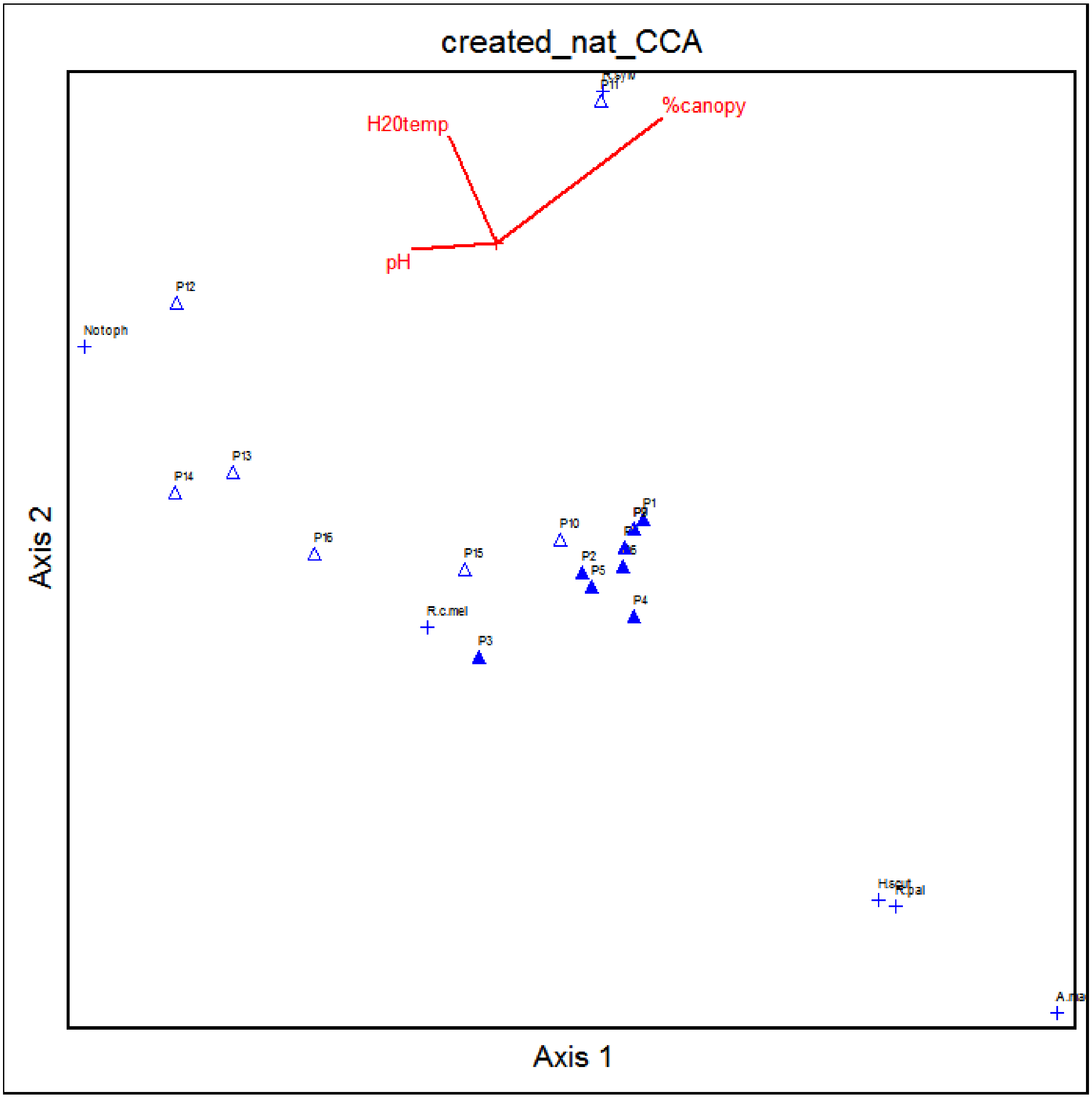

Figure 24. First and second Canonical Correspondence axes with species abundance and microhabitat variables for 2007. Created ponds are depicted by the solid triangles (numbers P1-P9), natural ponds are depicted by the open triangles (numbers P10-P16), and species captured are depicted by crosses.

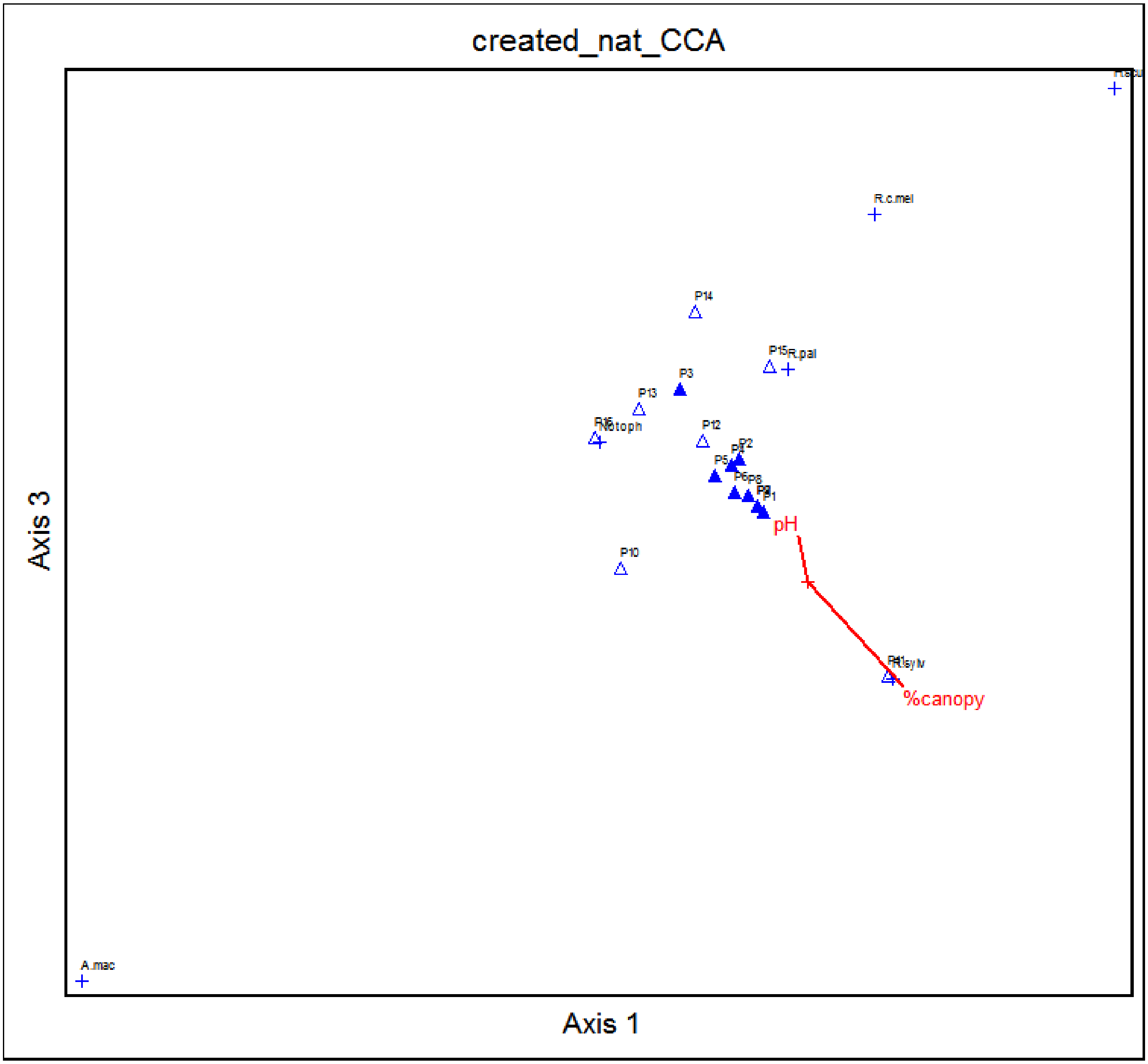

Figure 25. First and third Canonical Correspondence axes with species abundance and microhabitat variables for 2007. Created ponds are depicted by the solid triangles (numbers P1-P9); natural ponds are depicted by the open triangles (numbers P10-P16), and species captured are depicted by crosses.

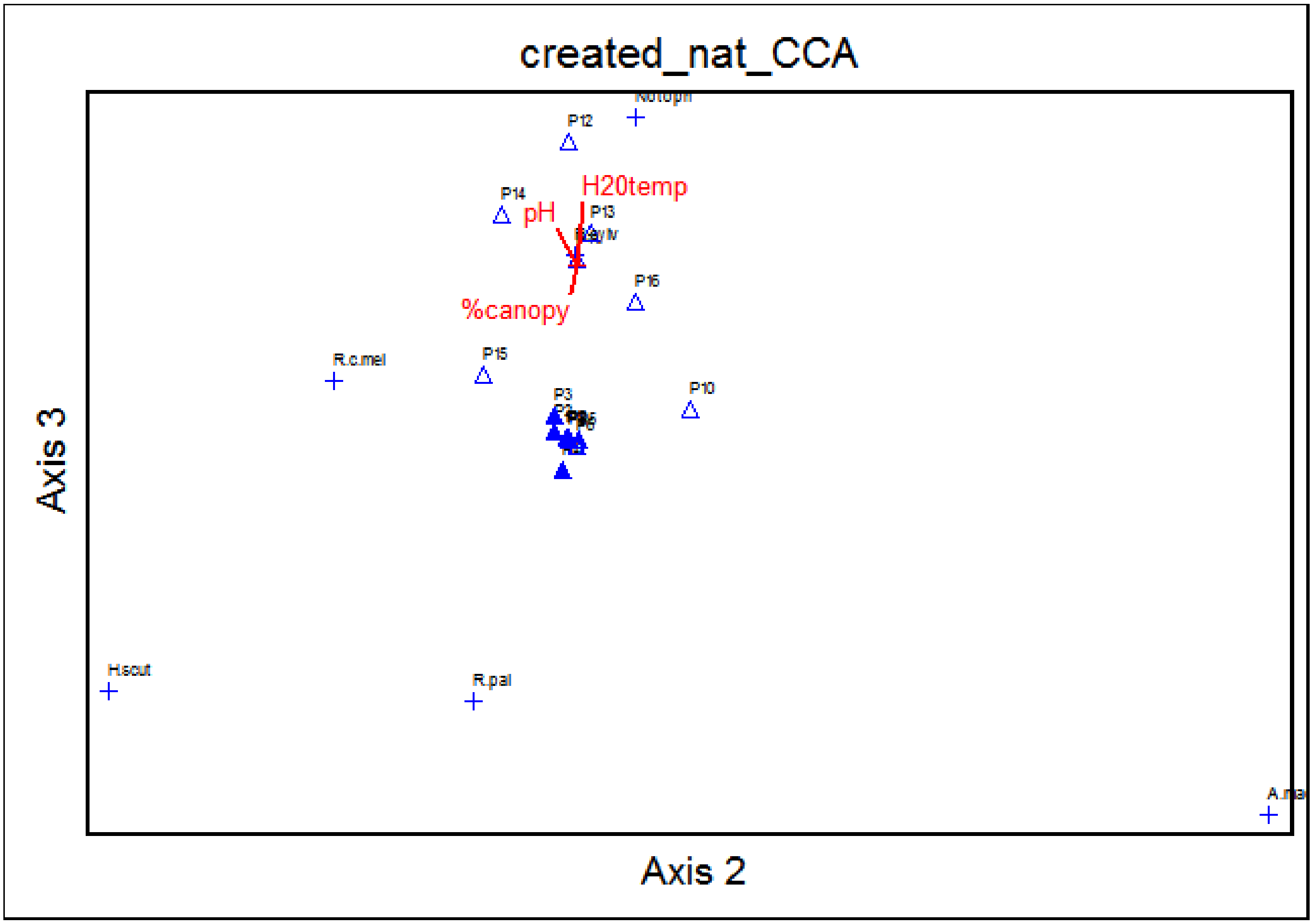

Figure 26. Second and third Canonical Correspondence axes with species abundance and microhabitat variables for 2007. Created ponds are depicted by the solid triangles (numbers P1-P9); natural ponds are depicted by the open triangles (numbers P10-P16), and species captured are depicted by crosses.

DISCUSSION

Monitoring created ponds for usage by breeding amphibians

It took 2 years for amphibians to utilize the created ponds for breeding purposes with usage increasing every year (Figure 15). There were a higher number of adults captured in Compartments 7 and 9 then in Compartment 3 for 2005. This could potentially be explained by the stocking of the ponds in Compartment 7 and 9. Since the ponds were seeded with egg masses in 2004, it is likely that some of the adults captured in subsequent years were from those eggs (Good, 2006). Studies have shown that most *R. sylvatica* reach sexual maturity within 1-2 years with females taking slightly longer than males (Berven, 1982).

Ambystoma maculatum did not use the created ponds until 2006 and egg masses were only present at two ponds throughout the study (Figure 16). The low number of *A. maculatum* caught in this study could be due to the difficulty of capturing semifossorial salamanders (Vasconcelos and Calhoun, 2004). Ambystomatid salamanders are highly philopatric and any movement to new ponds can probably be explained by chance encounters with other ponds while searching for their natal pond (Gamble et al., 2007). Also, *A. maculatum* have specific post-breeding habitat requirements that may restrict where they are found (Baldwin et al., 2006). This lag between pond construction and amphibian breeding usage is comparable to findings from other studies such as Pauley (2005) and Pechman et al (2001). In their study, Pechman et al. (2001) found that amphibians colonized constructed ponds over time with salamander species taking at least 2 years to use them while anurans used them within the same year they were constructed in some cases. Similarly, in Pauley's (2005) study, 4 anuran species colonized ponds the same year that they were created.

Another possible explanation for the delayed usage of these created ponds could be the high level of philopatry demonstrated by both study species (Berven and Grudzien, 1990; Vasconcelos and Calhoun, 2004). The location of other temporary pools of water next to natural pools may attract amphibians to less suitable habitats (Vanconcelos and Calhoun 2004, Baldwin et al. 2006). After the silviculture treatments were created, small puddles of water appeared and various breeding amphibians were located within them which could have kept them from finding the created ponds. The distance between natal ponds and these created ponds may also play a role in how many amphibians used the new ponds. While some amphibian species will travel great distances for foraging and breeding (Pauley, 2005), others may not be able to do so. Adult and juvenile *R. sylvatica* can travel in excess of 300 meters while *A. maculatum* can travel 153 meters from their source ponds (Vanconcelos and Calhoun, 2004). With no source ponds nearby any of the created ponds in this study, the distance may have been too great for *A. maculatum* to use some of these ponds.

There was a statistically significant difference in pond usage between the different elevations (Figures 17-19). There are many possible explanations for this difference some of which were discussed above including level of philopatry, movement ranges, and differences in environmental variables. One of the obvious environmental factors that elevation would affect would be temperature. Air temperature at the lowest elevation was found to be significantly higher than those at the middle and highest elevations (Figure 20, 22). Skelly et al. (2004) demonstrated that temperature plays an important role in amphibian embryo development. This difference in temperature could affect larval development which could ultimately affect the number of adults that return to use the ponds. Temperature can also play a role in the survival of amphibians by affecting their size at maturity. Sagor et al. (1998) found that larger females were able to hold more eggs and male frogs showed an affinity for larger mates.

Development, metamorphosis, and egression of juvenile amphibians from created ponds

There was slow tadpole development in most of the ponds, with 6 of the 9 ponds in 2005 and 5 of the 9 ponds in 2007 having successful egression of *R. sylvatica* juveniles. Due to time and monetary constraints, the field season ended before juveniles began to egress in 2006. The timing of juvenile egression from the created ponds differed between years of this study. In 2005, *R. sylvatica* juveniles began to egress near the end of June while in 2006 the limited number of juveniles caught occurred near the end of the field season which was mid-July and in 2007 egression did not begin until the very end of July. In contrast to my findings, Timm et al. (2007) found that for some amphibian species, such as *R. sylvatica,* there was consistent timing for juvenile egression among ponds and years.

Several factors could have an affect on timing of juvenile egression. Several studies have cited low pH levels as a factor affecting amphibian larval development (Freda and Dunson 1985, Pierce 1993, and Sadinski and Dunson 1992). In 2005 and 2007, pH levels were around the lower range of tolerance for *R. sylvatica* (pH ~5) (Pierce, 1993). In a study comparing *R. sylvatica* larvae development between closed and open canopies, Skelly et al. (2002) found that open canopies had faster development than closed ones. They also noted that ponds with open canopies had higher water temperatures and dissolved oxygen levels. In a similar study, Werner and Glennemeier (1999) found that ponds with high canopy cover had low dissolved oxygen and pH levels both of which could reduce growth rates and survivorship of *R. sylvatica*. In comparison, the most productive ponds for juvenile development and egression in 2005 were those at the lowest elevation (Compartment 3) which had slightly higher water temperatures and less canopy cover (Good, 2006). In contrast, the most productive ponds in 2007 were those at the middle elevation (Compartment 9), which had more canopy cover but slightly higher water temperatures and dissolved oxygen levels.

There are many possible explanations for the contrasting findings for 2007. One possible justification may be related to the number of eggs found within each pond. Waldman (1982) found that *R. sylvatica* females deposited their egg masses in clumps which kept all eggs warmer than the surrounding water. In 2007, there were a higher number of egg masses per pond in Compartment 9 then there were in Compartment 3, which could suggest warmer conditions for the developing embryos. Also, the air temperature was statistically warmer at the lowest elevation (Compartment 3) which could influence the water temperatures in the ponds potentially creating too warm of an environment for the tadpoles to survive. In contrast, Berven (1990) found that increased egg numbers were associated with reduced survival, decreased size at metamorphosis, and a longer larval stage. This could explain why tadpoles were present through November at 2 of the ponds in Compartment 9.

Although pH levels were fairly constant at all ponds there was a difference in dissolved oxygen levels between the two elevations. Compartment 9 had an average of 1.99 ppm while Compartment 3 had an average of 1.51 ppm dissolved oxygen. Several studies have suggested that dissolved oxygen levels fluctuate throughout the pond, with higher levels generally found along the pond margin (Fairchild et al. 2003, Studinski and Grubbs 2007). Werner and Glennemeier's (1999) study suggested that low dissolved oxygen levels could reduce growth rates and survivorship of *R. sylvatica*. Nie et al. (1999) had similar findings with bullfrog (*R. catesbeiana*) tadpoles and suggested that even though they were bimodal breathers (obtaining oxygen from both the air and water) low dissolved oxygen was detrimental from a respiratory standpoint. Therefore, this small difference in dissolved oxygen between the two elevations could play a role in explaining the differences in *R. sylvatica* development.

Another possible explanation may involve hydroperiod. Eagan and Paton (2004) found that *R. sylvatica* were most abundant in ponds that held water for 8-9 months. Skelly et al. (1999) also found that *R. sylvatica* were more ubiquitous in ponds that dried yearly and were absent from permanent ponds. Baldwin et al. (2006) had mixed results concerning hydroperiod with *R. sylvatica* present in ponds with both short (<16 weeks) and intermediate (>16 weeks) hydroperiods and *A. maculatum* present only in intermediate hydroperiod ponds. In 2007, 2 of the 3 ponds at Compartment 9 drastically dropped in water levels while only 1 of the ponds in Compartment 3 had a considerable drop. This difference in water levels could have contributed to the differences in development. It should be mentioned that 2007 was a much drier year than 2005 and that could potentially account for some of the difference in hydroperiod between years. An interaction between hydroperiod and the number of tadpoles in each pond could create another factor possibly explaining the difference in juvenile survival. Pearman (1993) suggested that competition between *R. sylvatica* tadpoles could occur due to slow development and a loss of resources due to pond drying.

Predators could also have an affect on tadpole metamorphosis and survival. Semlitsch (1993) found that newts, odonates and fish had a negative affect on tadpole survival when present in the ponds. *Notophthalmus v. viridesences* (red-spotted newts) were prevalent in all of the created ponds and could have preyed upon a number of amphibian larvae.

Although *A. maculatum* eggs and larvae were present in one pond in 2006 and two ponds in 2007, no juveniles were caught egressing into the surrounding forest. This could be due in part to their need for a specific hydroperiod (Skelly et al. 1999). The ponds that they were found in (pond 2 and 3, Compartment 7) dropped drastically in water level for both years which probably made it difficult for them to survive. The difficulty in trapping semifossorial salamanders could have also played a role in the seemingly absent egression of Ambystomatid juveniles (Vasconcelos and Calhoun, 2004). If the larvae did survive, they could have burrowed underneath the drift fence therefore avoiding the traps during their egression.

The results of my study seem to coincide with that of a few of the studies mentioned above. I believe that the number of egg masses per pond as well as the presence of certain predators were the major causes of delayed egression in these created ponds. Also, I believe more research into the levels of dissolved oxygen within these ponds and how they may affect amphibian larvae is needed to better understand what role it might be playing in this situation.

Effects of habitat fragmentation on adult migration and juvenile egression

One year after silviculture treatments created edges throughout parts of the forest surrounding the ponds, it is difficult to conclude that fragmentation has had an affect on amphibian movements. Amphibian usage at all of the ponds increased after the silviculture treatments, which could suggest that there was no affect (Figure 14). A study done by Regosin et al. (2003) focused on the wintering activities of adult *R. sylvatica* and found that they over-wintered in upland forests within 65 m of the breeding pond. However, these adult frogs did not use this area for foraging or shelter during the active season after breeding. They also found that the number of over-wintering frogs decreased as the distance from the pond increased and that there were more males than females closer to the ponds. The researchers also suggested that because *R. sylvatica* adults in their study have seasonal variations in habitat use it may render them more susceptible to habitat loss and fragmentation. The telemetry data from my study mirrored what Regosin et al. (2003) found with the adults spending most of their time away from the breeding ponds (Figure 22). Their study could also explain why the number of amphibians using the created ponds increased after the fragmentation of the surrounding habitat. If *R. sylvatica* over-winter close to the breeding ponds then it is not surprising that their numbers were not affected immediately after the silviculture treatments. The treatments were created in the fall, which was after the active season of most amphibians in the area, suggesting that they were already within the 1 ha buffer that remained around each pond. The results from Regosin et al. (2003) that mentioned that *R. sylvatica* foraged in different habitats then they over-wintered suggests that migrating adults or egressing juveniles from the 2007 breeding season would be some of the first to encounter this newly fragmented habitat. Further research could more accurately show how these silviculture treatments affect amphibian movements.

Several forest amphibians are associated with woody debris, litter depth and moisture, under-story vegetation, and canopy cover all of which are directly affected when timber harvesting creates edges (deMaynadier and Hunter, 1998). deMaynadier and Hunter's (1998) study found that *R. sylvatica* were more abundant in closed canopy forests and were considered a species sensitive to the effects of forest practices. They also found that management-sensitive species were negatively affected to distances 25-35 m from silviculture edges. This finding could suggest that the 1 hectare buffer that was left around the ponds in this study were enough to safeguard against edge effects at the breeding sites but left them a small amount of suitable habitat for foraging and migration. With the average movements of adult *R. sylvaticas* being 31.4 m in 2006 and 37.1 m in 2007, it would be likely that these frogs would encounter the created edges during their active season. If they attempt to avoid the edges, it could leave them with a small amount of suitable habitat to move within. *Ambystoma maculatum* require deep forest litter, rotting logs, and small mammal burrows for post-breeding habitat (Baldwin et al. 2006), which silviculture treatments can affect. Similarly, *R. sylvatica* require contiguous forests immediately surrounding the breeding pools as well as in the upland habitat (Baldwin et al. 2006), which are affected by silviculture practices.

Similar findings occurred in studies by Rothermel and Semlitsch (2002) and Rothermel (2004) where they looked at how *A. maculatum* and *Bufo a. americanus* metamorphs responded to forest edges. They mention that the smaller size of the metamorphs may cause these species to become intolerable of open areas because the chance of desiccation is much higher in those habitats. Movements for both species were 3-4 times higher in forest habitats than in field habitats which they contributed to how the different habitats filtered dispersing individuals. Migratory distance from the breeding pond to the surrounding forest was also found to affect the survival of juvenile amphibians (Rothermel, 2004). The greater the distance between the breeding pond and the surrounding forest, the less likely the egressing juveniles would survive. A study done by Saunders et al. (1999) found that small patches of forest exhibited a narrow and unstable edge influence for temperature. They also found that open areas had a higher depth of edge influence than did forested areas. Similarly, Gelhausen et al. (2000) found that canopy openness, air temperature, soil moisture, and relative humidity had as large as an 80 m depth of edge influence in 24 hectare forest patches. The findings of these studies are important because if the temperatures get too high or the soil moisture and humidity get too low it creates unsuitable habitat for many amphibians.

Brattstrom (1963) found that *R. sylvatica* had a mean thermal maximum temperature of 34.8º C. Putnam and Bennett (1981) had similar findings for other species in the *Rana* family in their study and concluded that some long term activities; such as migration, mating, and foraging; are temperature sensitive. That could be important because the air temperature was statistically different with temperatures at or above 35º C in both the clear cuts and the forest edges for most of the field season (Figure 20-21). Gibbs (1998) found that both *R. sylvatica* and *A. maculatum* had an intermediate sensitivity to forest fragmentation and were absent from forests where the forest cover was less than 30%. He also suggested that low population densities along with high dispersal rates and habitat specificity could predispose species, such as *R. sylvatica* and

A. maculatum, to fragmentation sensitivity. In another study, Homan et al. (2004) found critical threshold levels of habitat loss and fragmentation that fell between 10-30% forest cover for both species. They suggested that the site fidelity of these species may inhibit them from avoiding unsuitable habitat and that both species avoided crossing fields and clear cuts which could further limit their dispersal to suitable habitat.

In a study focused on how edges created by peat mining affected amphibians, Mazerolle (2001) found that all species' activity, movement patterns and size were influenced and that climatic variables were more inconsistent in the fragments. He made particular mention of *R. sylvatica* because their movement was more affected in the fragments and their movements were altered near fragmentation edges. During 2006 and 2007, adult *R. sylvatica* fitted with radio transmitters were never found in the clear cut or near the edges created in the silviculture experiment which could suggest possible avoidance of these areas. The frog movements were also compared to weather data to identify any correlation between the two but nothing significant was found. Other factors may confound amphibian sensitivity to habitat fragmentation. Gibbs (1998) mentioned that some ground-dwelling predators, including raccoons, skunks, shrews, and snakes, thrive in fragmented forests and have been found to eat amphibians; some of the snake species that feed on amphibians are only found in open field habitat (Rothermel and Semlitsch, 2002). A number of snake, mammal and bird species were mentioned as predators of anurans in a literature review (Toledo et al. 2007). One study found that *R. sylvatica* was avoided by shrews because of noxious skin secretions and mercy cries which scare off the predator (Formanowicz and Brodie, 1979), but no studies have looked at the response of other potential predators. Coyote, raccoon, bobcat, and bear tracks were present along the forest edges and also inside or near the ponds during the 2006-2007 seasons and all could be potential predators of migrating adult and juvenile amphibians. All of these findings propose that edges created by the silviculture treatments in this study could generate unsuitable habitat for most amphibians, specifically *R. sylvatica* and *A. maculatum*.

Environmental factors and species abundance in created and natural ponds

In a study by DiMauro and Hunter (2002), the reproductive success of *R. sylvatica* and different environmental factors were studied and compared in anthropogenic ponds and natural ponds occurring in the same forested area. They found that even though anthropogenic pools outnumbered natural pools in their study site, the anthropogenic pools were creating an ecological trap effect for *R. sylvatica.* They found that the two types of pools were different in respect to pool area and depth, sunlight, water temperature and canopy cover. They also found that different environmental factors affected the reproductive success of *R. sylvatica* in the different pool types. For the anthropogenic pools, pool area, water depth and canopy cover positively affected the frogs while water temperature negatively affected them. For the natural pools, there was a significant relationship between the number of wetlands in the area and pool pH. The findings in my study are similar to that of DiMauro and Hunter (2002). The CCA demonstrated that the created and natural ponds differed in % canopy cover, water temperature and pH levels (Figures 23-25). It also demonstrated that the created ponds (P1-P9) were similar enough to group together along the axes while the natural ponds (P10-P16) were more variable so that they were more spread out along the axes. One explanation for this difference in grouping could be the different habitats where these ponds were found. The created ponds were all created exactly the same way in exactly the same habitat while the natural ponds were found in many different areas. Only two of the natural ponds had canopy coverage over 90% while all of the created ponds had high canopy cover. Due possibly in part to the more open canopy, the natural ponds had slightly warmer water temperatures and higher pH levels than the created ponds as well. The graphs also illustrated the different preferences of different species. For example, they show that *R. c. melanota* prefers more open canopy and higher pH levels than *R. sylvatica*. Many amphibian species have environmental preferences that can determine their breeding habitat. For example, *R. sylvatica* prefer more canopy cover (deMaynadier and Hunter, 1998) which is illustrated in the CCA graphs (Figures 23-25). Species abundance can be related to specific environmental factors which should be taken into consideration when creating ponds for mitigation techniques.

FUTURE RESEARCH

Certain aspects of this study would benefit from future research. Further monitoring of the ponds following the silviculture treatments could more clearly show if and how the habitat fragmentation affects adult migration and juvenile emigration. Also, a second survey of the natural ponds could be warranted since some of the ponds dried up due to a dry summer season in 2007. Further research into why *R. sylvatica* tadpoles are prolonging their metamorphosis could also be necessary to pinpoint any possible environmental factors that could be affecting amphibian survival.

CONCLUSIONS

In conclusion, I believe that created ponds are an adequate method to provide vital breeding habitat for local amphibians when natal ponds have been removed due to timber practices. Ponds should be constructed in areas that are contiguous forest and monitored for activity to ensure that they are suitable habitat for adult breeding amphibians and so that they can encounter them during the non-breeding season. Seeding the ponds with eggs from amphibians found in the area could decrease the time it takes for them to colonize the new ponds. Placing several ponds close together would create a pond network that would relieve genetic drift and provide habitat for an adequate number of juveniles that would protect against mortality and pond drying. The elevation where these ponds are created should be considered since temperatures fluctuate enough between them to cause differences in successful juvenile metamorphosis. The ponds that are removed should be sampled before their decimation to ensure the newly created ponds mimic the environment lost for the amphibians in the surrounding area.

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