2011

Forest Succession and Amphibian Migrations: Implications for Landscape Connectivity

Viorel Dan Popescu

Recommended Citation

Popescu, Viorel Dan, "Forest Succession and Amphibian Migrations: Implications for Landscape Connectivity" (2011). *Electronic Theses and Dissertations*. 635.
http://digitalcommons.library.umaine.edu/etd/635

FOREST SUCCESSION AND AMPHIBIAN MIGRATIONS:
IMPLICATIONS FOR LANDSCAPE CONNECTIVITY

By

Viorel Dan Popescu

B.S. University of Bucharest, 2001

M.S. SUNY – Environmental Science and Forestry, 2007

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

(in Wildlife Ecology)

The Graduate School

The University of Maine

May, 2011

Advisory Committee:

Malcolm L. Hunter, Jr., Libra Professor of Conservation Biology and Professor of Wildlife Ecology, Advisor

Aram J. K. Calhoun, Associate Professor of Wetlands Ecology

James P. Gibbs, Professor of Conservation Biology and Wildlife Management

Robert S. Seymour, Professor of Forest Resources

Joseph D. Zydlewski, Assistant Unit Leader – U.S. Geological Survey, Maine Cooperative Fish and Wildlife Research Unit

THESIS

ACCEPTANCE STATEMENT

On behalf of the Graduate Committee for Viorel Dan Popescu, I affirm that this manuscript is the final and accepted thesis. Signatures of all committee members are on file with the Graduate School at the University of Maine, 42 Stodder Hall, Orono, Maine.

Dr. Malcolm L. Hunter, Jr.
Libra Professor of Conservation Biology
Professor of Wildlife Ecology

20 March, 2011

LIBRARY RIGHTS STATEMENT

In presenting this thesis in partial fulfillment of the requirements for an advanced degree at The University of Maine, I agree that the Library shall make it freely available for inspection. I further agree that permission for "fair use" copying of this thesis for scholarly purposes may be granted by the Librarian. It is understood that any copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Signature:

Date: 20 March, 2011

FOREST SUCCESSION AND AMPHIBIAN MIGRATIONS: IMPLICATIONS FOR LANDSCAPE CONNECTIVITY

By Viorel Dan Popescu

Thesis Advisor: Dr. Malcolm L. Hunter, Jr.

An Abstract of the Thesis Presented in
Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy
(in Wildlife Ecology)
May 2011

Conservation of forest-dependent amphibians is dependent on finding a balance between timber management and species' habitat requirements. Accurate predictions of the response of amphibian communities to disturbance rely on a good understanding of the scales at which ecological processes affect distribution and abundance through space and time. I investigated the response of 14 species to four different forestry treatments (partial harvest, clearcut with coarse woody debris [CWD] removed, clearcut with CWD retained, and uncut control) over a six-year period, using 2.1-ha experimental treatments. Forest amphibians showed a strong negative response to complete canopy removal at a broad spatial scale, but site-specific variation in the use of forestry treatments was the norm at a finer scale. Four forest-dependent species showed substantial declines in abundance beginning at 2 – 3 years post-disturbance. Avoidance of clearcuts by forest species and site-specific patterns of habitat use were maintained throughout the study.

Incipient vegetative succession and retaining CWD did not mitigate the effects of clearcutting; I found only a modest positive effect of succession on habitat use by emigrating juvenile wood frogs (*Lithobates sylvaticus*). I studied the permeability to juvenile wood frogs movements of four forestry treatments (recent clearcut, mature forest, 11-year-old conifers, and 20-year-old natural regeneration). I conducted experimental releases in 50 x 3 m terrestrial enclosures built in each treatment. Recent clearcuts and young coniferous stands were significant barriers to movements, and were three times less permeable to movement compared to the mature forest and 20-year-old regeneration. In addition, I found that juvenile wood frogs reared in semi-matural conditions did not show inherited directionality upon emergence, rely on proximate cues for orientation, and avoided forested wetland cues. Vegetative succession in young stands (5-6-year-old) mitigated the effects of clearcutting on microclimate, but juvenile wood frogs strongly avoided these stands. Thus, microclimate cannot be used as a sole parameter to predict potential habitat use by amphibians. Closed-canopy habitat was preferred by all terrestrial life stages of forest amphibians. A viable forest management strategy is to plan for spatially and temporally-structured harvests that retain canopy between high-quality breeding sites, and avoid clearcutting and conversion to conifer plantations.

ACKNOWLEDGEMENTS

Chapter 2 was published in the journal Ecological Applications (in press) as Maine Agricultural and Forest Experimental Station paper # 3147. I thank Malcolm L. Hunter for being co-author on this paper.

This research was primarily supported by the LEAP project (Land-use Effects on Amphibian Populations – NSF Grant No. 0239915) and the University of Maine. I received additional support from the Department of Wildlife Ecology, College of Natural Sciences, Forestry and Agriculture, Maine Agricultural and Forest Experiment Station, USGS Maine Cooperative Fish and Wildlife Research Unit, Maine Association of Wetland Scientists, and NSF-EPSCoR grant No. EPS-0904155. Experiments were conducted under University of Maine IACUC permits A2006–03–03 and A2009-04-04.

Foremost, I would like to thank my advisor, Mac Hunter, for his continuous support and encouragement, flexibility, and for being a great mentor. Mac, you are a great source of inspiration, and I have learned enormously from you about thinking ‘outside the box’ on conservation issues, and about science ethics. You always amazed me with your logic, lightning-fast feedback, and power to synthesize.

I would also like to thank greatly to my dissertation committee members, Aram Calhoun, Bob Seymour, Joe Zydlewski, and James Gibbs. They all contributed to my intellectual development and greatly improved this research through their thought-provoking comments, and willingness to participate in ad hoc discussions. I give special thanks to Dave Patrick who left behind some giant shoes that I had to fill. He guided me during the first stages of the project, provided data that I used in Chapter 1, and always

offered critical and insightful comments on the LEAP work. I also thank Catherine Burns and Betsie Rothermel for sharing their fieldwork expertise for Chapter 2. William Halteman provided helpful statistical advice on Chapters 2 and 4.

I thank many people that helped me in the field and in the lab: Ashley Hoskins, Andrea Long, Lindsey Munson, Kara Ray, and Brittany Wilson. I especially thank Scott Helmke for his hard work and perseverance with removing debris from the sites.

My graduate experience at the University of Maine was enhanced by a wonderful group of graduate students. I especially thank the other members of the Hunter-Calhoun labs for their input during this project. I give special thanks to Sean Blomquist, Steve Campbell, TR Morley for their friendship and their helpful insights into my research. Britt Cline, Kevin Ryan, Jessica Jansujwicz, Amanda Shearin, Erynn Call, and Dimitry Gorski were great colleagues and friends, and the 'coffee-train' kept me going while writing the dissertation. Emily Knurek and 'Team Bowling' kept my spirit high and provided much-needed distractions.

Finally, I would like to thank my family for their love and for supporting me in this endeavor. I especially thank my wife, Bekka Sue for her unconditional support, for putting up with my fieldwork schedule, and for giving me the best gift (as well as the greatest incentive to finish) – our son, Tavi. My Mom, Dad, and sister Veronica were a great source of advice and moral support, even from afar. Through their kindness and welcoming spirit, Stephen and Corina made the distance between Maine and Romania seem insignificant. Rexaroo worked hard in the field during those cold and wet breeding-season days. I dedicate this research to my family.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

PREDICTING THE RESPONSE OF AMPHIBIAN COMMUNITIES TO DISTURBANCE ACROSS MULTIPLE TEMPORAL AND SPATIAL SCALES

Abstract

Conservation of forest-dependent amphibians is dependent on finding a balance between timber management and species' habitat requirements. Accurate predictions of the response of amphibian communities to disturbance rely on a good understanding of the scales at which ecological processes affect distribution and abundance through space and time. To examine the role of spatial and temporal scales in shaping amphibian communities, I studied the response of 14 species to four different forestry treatments (partial harvest, clearcut with coarse woody debris [CWD] removed, clearcut with CWD retained, and uncut control) over a six-year period, using 2.1-ha experimental treatments in central Maine, USA. Forest amphibians showed a strong negative response to complete canopy removal at a broad spatial scale (across experimental sites), but site-specific variation in the use of forestry treatments was the norm at a finer scale. Four forest-dependent species showed substantial declines in abundance beginning at 2 – 3 years post-disturbance. Avoidance of clearcuts by forest species and site-specific patterns of habitat use were maintained throughout the study. Incipient vegetative succession and retaining CWD did not mitigate the effects of clearcutting; I found only a modest positive effect of succession on habitat use by emigrating juvenile wood frogs (*Lithobates sylvaticus*). Despite high yearly fluctuations in species richness and abundance, there was

a shift in species assemblage towards habitat generalists. In natural and semi-natural landscapes, implementing broad management prescriptions could be a viable strategy, while finer-scale variation has to be acknowledged when managers focus on a limited area especially in highly-modified landscapes.

Introduction

A key focus of applied ecology is predicting the long-term effects of habitat change on the spatial distribution and abundance of organisms. In order to make accurate predictions we need to understand the role that scale plays in determining patterns of habitat use, especially in fragmented landscapes (Fahrig 1992). Investigating ecological patterns at inappropriate spatial and temporal scales may limit our understanding of the responses of species to disturbance and lead to deficient conservation strategies (Doak et al. 1992, Ewers and Didham 2006). Contradictory results about the effects of disturbance, mainly stemming from investigations conducted across disparate spatial and temporal scales, have been recorded for a variety of taxa across multiple ecological systems (e.g., birds [Chalfoun et al. 2002, Stephens et al. 2004], birds and lepidopterans [Hill and Hamer 2004], habitat heterogeneity – species diversity relationship [Tews et al. 2004], dung beetles [Nichols et al. 2007]). Scale also influences the relative prevalence of deterministic versus stochastic processes in shaping the spatial and temporal dynamics of animal communities (May 1973), imposing additional constraints on our ability to generalize the predictions of species responses to habitat change.

Understanding the relative importance of scale in driving patterns of occurrence is particularly challenging for species with complex life cycles, where different life-history stages may occur in disjunct environments and function at different spatial scales. Many pond-breeding amphibian species are classic examples of such taxa, with population dynamics driven by environmental stochasticity and demographic fluctuations manifested at all life stages (Sjögren 1991). Within-pond factors, such as variable hydroperiod, larval density-dependence, and larval predation affect the aquatic stage (Vonesh and De la Cruz 2002, Altwegg 2003, Trenham and Shaffer 2005). These in turn affect the vital rates of the terrestrial stage through carryover effects (Chelgren et al. 2006). Terrestrial vital rates are further influenced by habitat quality, predation, and terrestrial density-dependence (Altwegg 2003, Harper and Semlitsch 2007). Aquatic and terrestrial factors act synergistically in space and time, generating multiple sources of variability that make species turnover common in amphibian communities (Werner et al. 2007). The net result of the interactions among the demographic rates of different life-history stages of amphibians is that predicting the effects of habitat change is extremely challenging. These difficulties are further exacerbated by the fact that the large degree of variation in life history traits of many amphibian species makes it hard to generalize about long-term population status and viability (Marsh 2001).

Due to their dual life-cycle, permeable skin, and generally low vagility, amphibians are often assumed to respond in a deterministic manner to changes in environmental conditions. Strong deterministic responses are likely to prevail when species are habitat specialists, with a direct link to a prominent habitat attribute (Bell 2001). Predicting such responses is critical to managers, who often rely on direct cause-effect relationships that can be generalized. The relative importance of such predictions may diminish when finer-scale variability inherent to ecological systems produces unexpected patterns (e.g., due to variation in individual behavior, species interactions, and landscape context [Campbell et al. 2010]). Although the abundance and distribution of organisms are shaped by processes at various spatial and temporal scales (Wiens 1989), management of natural resources often overlooks or ignores the finer-scale variability as background “noise” in a mostly predictable system. Given the known sensitivity of many amphibian species to habitat loss and alteration (Cushman 2006), it is important that we understand how predictable, or unpredictable, their responses to disturbance remain across a wide range of spatial and temporal scales.

In recent years, particular attention has been paid to understanding the effects of forestry practices on amphibian populations. This attention is likely a result of the known sensitivity of forest-dependent amphibian species to habitat change (Homan et al. 2004), and a move towards integrating timber management with the conservation of biodiversity (Lindenmayer 2009). Silvicultural practices such as clearcutting create extremely heterogeneous landscapes with profound differences in vertical and horizontal stand structure (Hunter and Schmiegelow 2010). In particular, clearcutting can have long-term negative effects on amphibian abundance, with some populations reaching pre-disturbance levels only two to seven decades post-harvesting (Ash 1997, Homyack and Haas 2009). Such wide estimates of time to population recovery suggest that the landscape context and the temporal scale of studies will determine whether or not we are able to predict the response of populations to disturbance. To investigate the predictability of amphibian response to disturbance created by forestry practices across multiple spatial and temporal scales, I selected a relatively simple system (i.e., a temperate forest amphibian community), and experimentally investigated the response of

14 amphibian species to canopy removal and vegetation re-growth for six years post-disturbance. I focused on two vernal pool-breeding species common in temperate and boreal North American forests: wood frog (*Lithobates sylvaticus*) and spotted salamander (*Ambystoma maculatum*), and gathered detailed information on habitat use during three distinct life stages: (1) breeding, (2) post-breeding, and (3) juvenile emigration. For non-vernal pool-breeders (all other species) I evaluated the general pattern of habitat use during post-breeding and juvenile emigration. The strongest deterministic response of amphibians to logging disturbance is likely to occur within the first 1 – 5 years due to harsh microclimatic conditions associated with canopy removal and associated edge effects (Keenan and Kimmins 1993).

Methods

Study sites

This research was part of the NSF project "Land-use Effects on Amphibian Populations" (LEAP), a collaborative investigation of amphibian community responses to forestry practices between the University of Maine, University of Missouri – Columbia, and University of Georgia (Semlitsch et al. 2009). This study uses a six-year dataset and data from years 1 – 3 was partly published in (Patrick et al. 2006, Patrick et al. 2008a, 2008b)).

Our study was conducted on the Penobscot and Dwight B. Demerrit Experimental Forests, Penobscot County, Maine. Four replications of four forestry treatments – partial cut (50% canopy removed), clearcut with coarse woody debris (CWD) retained, clearcut with CWD removed, and control (not harvested) – centered on a breeding pool, were created between November 2003 and April 2004. The treatments extended up to164 m from the pool, a distance assumed to include the life zone of 95% of the local salamander populations (Semlitsch 1998), and thus each quadrant covered approximately 2.1 ha (Figure 1.1). In the clearcuts, all marketable timber was removed, and the remaining standing trees were felled and left on site (in the CWD retained treatment) or removed (in the CWD removed treatment). The volume of coarse woody debris was highest in the CWD-retained treatment (45.6 ± 21.6 m^3/ha; Patrick et al. 2006). The orientation of treatments was randomly assigned among sites, and the forested treatments (partial harvest and control) were opposite of each other at each site. The pre-treatment vegetation of the experimental forests was mature mixed coniferous and deciduous forest (Patrick et al. 2006). The codominant tree species were balsam fir (*Abies balsamea*), eastern white pine (*Pinus strobus*), northern white-cedar (*Thuja occidentalis*), red maple (*Acer rubrum*), eastern hemlock (*Tsuga canadensis*), paper birch (*Betula papyrifera*), American beech (*Fagus grandifolia*), and bigtooth aspen (*Populus grandidentata*). Canopy cover amounted to 73.8 ± 22.7% in control and 53.0 ± 33.5% in the partial harvest (Patrick et al. 2006).

The experimental pools were encircled by silt fence (70 cm tall and 30 cm buried in the ground) and associated pitfall traps located every 5 m on both sides along the fence. Upland drift fences (10-m long) were also erected at 16.6, 50, 100, and 150 m (1, 3, 6, and 9 fences, respectively) from the experimental pool in each treatment, in a circular setup that allowed sampling approximately 38% of the circumference at each distance (Figure 1.1).

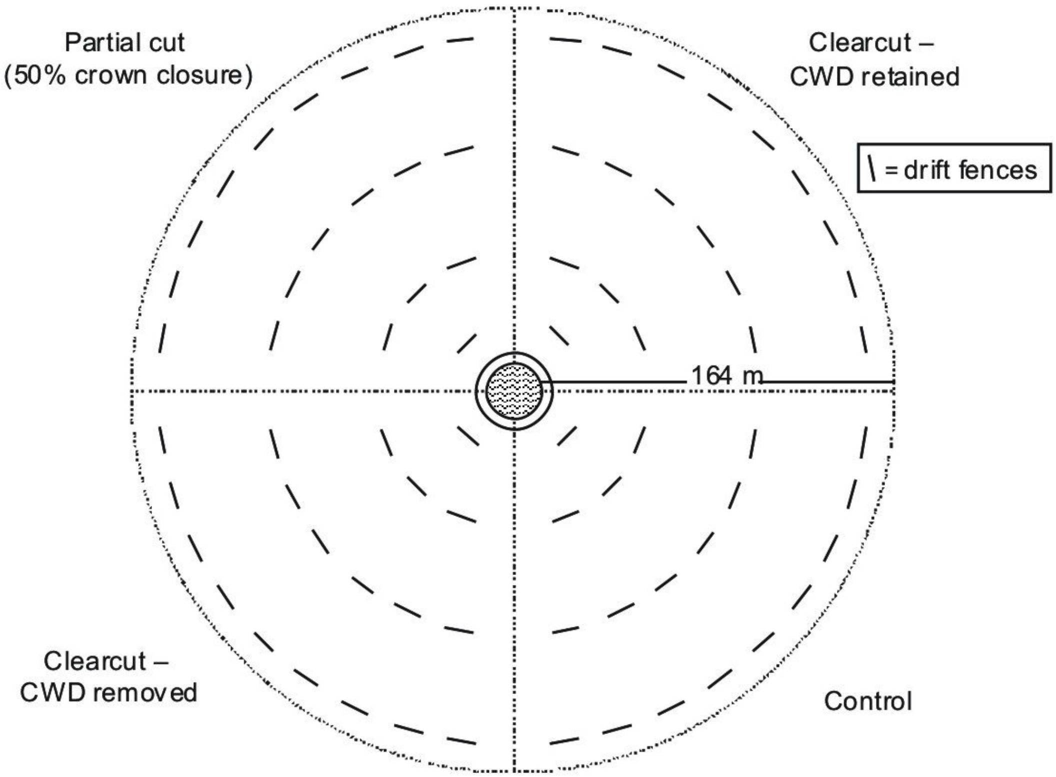


Figure 1.1. Experimental setup of the forest treatments centered on a breeding pool.

Study species

Fourteen species have been documented at our study sites (Patrick et al. 2006). These include two forest-dependent amphibians that successfully bred in the experimental pools– wood frog (*L. sylvaticus*) and spotted salamander (*A. maculatum*) – and were the most abundant at our experimental sites. Other members of the amphibian assemblage with sufficient captures to analyze the community dynamics included the red-backed salamander (*Plethodon cinereus*), eastern red-spotted newt (*Notophthalmus viridescens*), and four ranids: green frog (*L. clamitans*), bullfrog (*L. catesbeianus*), northern leopard frog (*L. pipiens*), and pickerel frog (*L. palustris*). Other amphibians either had very low captures: blue-spotted salamander (*A. laterale* complex), American toad (*Anaxyrus americanus*), mink frog (*L. septentrionalis*), and four-toed salamander

(*Hemidactylium scutatum*), or were not suitable for pitfall trap sampling: spring peeper (*Pseudacris crucifer*) and gray tree frog (*Hyla versicolor*), and were not included in the analysis.

Amphibian and habitat sampling

I sampled amphibians using a combination of drift fences and pitfall traps between 2004 and 2009, during two distinct periods: breeding season and non-breeding season. I captured adult *L. sylvaticus* and *A. maculatum* as they entered the four experimental pools to breed in early spring (13 April – 4 May 2005, 2 – 20 April 2006, 16 April – 3 May 2007, 12 – 30 April 2008, and 8 – 27 April 2009). During this period, I opened the traps associated with the pool fence, as well as the 16.6 and 50 m fences to capture the entire breeding population. Animals captured at the 16.6 and 50 m fences were released at the experimental pools. I did not attempt to capture breeding adults in 2004, as the experimental pools were stocked with egg masses from surrounding pools during the first year. I closed the traps at the end of the breeding season, re-opened the entire array at each site before the emergence season of juvenile *L. sylvaticus*, and continued sampling until the fall (1 July – 27 October 2004, 24 June – 17 September 2005, 30 June – 20 August 2006, 1 July – 12 September 2007, 30 June – 15 September 2008, and 30 June – 28 August 2009).

I checked the traps every other day during both the breeding and the non-breeding seasons. I released the animals on the opposite side of the fences so they could continue migrating or dispersing in their presumed direction of movement. Upon capture at the pool fence, juvenile *L. sylvaticus* emerging from the experimental pools were marked using a combination of Visible Implant Elastomer (VIE, Northwest Marine Technologies, Shaw Island, WA, USA) and toe clip (2004), or a single toe clip (2005 – 2009). Individuals recaptured at terrestrial fences were remarked using VIE (2005 – 2006), or Visible Implant Alpha Tags (VIAT, Northwest Marine Technologies) (2007 – 2009). Juvenile spotted salamanders were marked at the pool fence using VIE indicating the treatments they entered in 2004 – 2006 (limited or no recruitment occurred during 2007 – 2009).

I conducted repeated habitat sampling in August 2004, 2005, 2006, and 2008. Sampling was based on 96 9-m^2 permanent plots equally distributed among treatments and sites. The sampling plots were located approximately 25 m from equally-spaced selected drift fences (three 150-m fences, two 100-m fences and one 50-m fence in each treatment) in the direction of the pool. Each occasion, I sampled percent vegetation cover in four height classes (0 – 0.5, 0.5 – 1, 1 – 2, and >2 m) and dominant species composition, percent leaf litter cover and leaf litter depth, and percent canopy cover (using a Moosehorn densitometer, Moosehorn CoverScopes, Medford OR, USA).

Predicting the responses of amphibians to canopy removal

I assessed the effects of disturbance created by forestry practices on the spatial and temporal dynamics of the amphibian community using generalized linear mixed effects (GLME) models (Pinheiro and Bates 2000). I analyzed three groups of animals separately: (1) breeding adults *L. sylvaticus* and *A. maculatum*; (2) emigrating juveniles *L. sylvaticus* and *A. maculatum* emerging from the experimental pools (marked with VIE and VIAT); and (3) unmarked amphibians (all other species that used the experimental sites for foraging and migration across the six years of study, including *L. sylvaticus* and *A. maculatum* post-breeding adults, as well as juveniles from pools outside our experimental setup).

For model fitting and model selection I followed the procedure recommended by (Zuur et al. 2009). I started with a full fixed-effects model, fitted various random effects to find the optimal structure of the random component, and used AIC to compare among the models and select the optimal random structure. Fitted models had different fixed effects and the optimal random structure, and I used ANOVAs to identify the overall significance of the fixed effects, and likelihood ratio tests to compare between competing models.

I tested our ability to predict the use of forestry treatments by amphibians across spatial scales as well as changes in habitat use due to vegetative succession by fitting three models for each event, species, and life stage. The abundance and distribution of amphibians as a deterministic response to disturbance was investigated by examining: (1) the overall (broad-scale) effect of forestry treatments (Treatment only as fixed effect), (2) the between-sites (local scale) spatial variability (Treatment x Site interaction as fixed effect), and (3) the between-years (temporal) variability (Treatment x Year interaction as a fixed effect). For emigrating *L. sylvaticus* juveniles I also included the interaction term Treatment x Distance from natal pool (i.e., 16, 50, 100, and 150 m) as fixed effect to assess within-treatment (fine scale) variability in habitat use. For all models, the optimal random structure (Zuur et al. 2009) was a nested random intercept (separate intercepts for Year and Site within Year). I used Treatment contrasts using the forested control as the reference treatment to investigate differences among treatments. If differences between forestry treatments were found, I ran the same models using the CWD-retained as the reference treatment to identify the response of amphibians to retained microhabitat structures (CWD) in clearcuts. All analyses were conducted in R version 2.9.2 (R Development Core Team 2009).

Because the spatial and temporal extent of amphibian sampling varied between years, each life stage that I investigated using GLME's had a different response variable. For breeding adults I used the mean number of captures per trap as our dependent variable to account for the slightly different number of traps within each treatment and site. For emigrating juveniles, I assessed the initial habitat choice of newly metamorphosed *L. sylvaticus* and *A. maculatum* emerging from the experimental pools by using the mean number of captures per trap at the pool-encircling fence. I then used the raw number of animals recaptured at the upland terrestrial fences to investigate the response to forestry treatments during emigration movements. Only five juvenile *A. maculatum* were recaptured during 2007 – 2009 at all sites, and the 2004 – 2006 data was too sparse to fit a reliable model. For post-breeding *L. sylvaticus* and *A. maculatum* adults, juvenile *L. sylvaticus* and *A. maculatum* emerging from pools outside the experimental arrays, as well as for the other species composing the amphibian community that used the experimental sites for foraging or migration movements, I used the average number of captures per day as our predicted variable to account for the differences in trapping effort between seasons. I fitted different mixed effects models for adult and juvenile *L. sylvaticus*, adult and juvenile *A. maculatum*, juvenile *L. clamitans*, juvenile *L. catesbeianus*, and combined (juveniles and adults) *L. pipiens*, *L. palustris*, and *N. viridescens*. For all models assuming a Gaussian distribution, the response variable was transformed via [log(X + 1)] or [sqrt(X)] transformations to achieve normality (assessed using the Shapiro-Wilk test).

Results

Spatial and temporal variation in the response of migrating adult amphibians

I captured 1 278 adult *A. maculatum* and 1 176 adult *L. sylvaticus* during the five trapping seasons, and there were differences among sites and years with respect to the number of breeding adults (Appendix A). Adults of both species entered the experimental pools for breeding independent of forestry treatment (Tables 1.1, 1.2), but there was variability among sites with respect to the use of treatments (significant Treatment x Site interactions, Table 1.1). There were no differences among years in the use of treatments, suggesting no effects of forest succession on breeding migration (Table 1.1).

Spatial and temporal variation in the response of emigrating juveniles

I captured 14 066 juvenile *L. sylvaticus* and 1 521 juvenile *A. maculatum* emerging from the experimental pools between 2004 and 2009 (Appendix B). Newly metamorphosed animals did not show a strong preference for forested or clearcut treatments at the fences encircling the pools. For *L. sylvaticus*, forestry treatment was not a strong predictor of habitat use, with animals showing a slight preference towards the partial cut (Table 1.2). However, local spatial variability was manifested in the site-specific response to forestry treatments (significant Treatment x Site interaction, Table 1.1).

Table 1.1. Amphibian responses to disturbance reflected in the use of four forestry treatments up to 6 years post-harvesting yielded by generalized mixed effects models. Best models contained the interaction *Treatment x Site* as fixed effects and *Year* and *Site within Year* as random effects, unless otherwise noted. I am presenting p-values (bold emphasis for significance at α = 0.05) for each species and event; Treat = Treatment; A = adult, J = juvenile.

Event	**Species**	**Life stage**	**Fixed effects**			
			Treat*	**Treat** x **Site***	**Treat** x **Year ****	**Treat** x **Distance ****
Breeding migration	*L. sylvaticus*	A	**0.0005**	**0.0279**	0.5156	
	A. maculatum	A	0.2805	**0.0308**	0.7499	
Juvenile emergence	*L. sylvaticus*	J	**0.0320**	**0.0046**	0.1835	
	A. maculatum	J [a]	**0.0021**	0.1139	0.6877	
Juvenile dispersal	*L. sylvaticus*	J [b]	**<0.0001**	**0.0037**	0.7745	0.6829
	A. maculatum	J	- data not analyzed (too few captures)			
Use for foraging or migration	*L. sylvaticus*	J [b]	**<0.0001**	**0.0005**	0.4170	
		A	**<0.0001**	0.219	**0.0390**	
	A. maculatum	J [b]	**<0.0001**	**0.0019**	0.7509	
		A	- data not analyzed (too few captures)			
	L. clamitans	J	**<0.0001**	**<0.0001**	0.2471	
	L. catesbeianus	J [c]	0.0809	0.4345	0.3414	
	L. pipiens + *L. palustris*	J+A [b]	**0.0199**	**0.0003**	0.3748	
	P. cinereus	J+A [b]	**0.0004**	**0.0004**	0.2775	
	N. viridescens	J+A [b]	**0.0035**	**0.0122**	**0.0252**	

* from the best model

** from the model containing the interaction *Treatment x Year* or *Treatment x Distance* (for dispersing juveniles only) as fixed effects

[a] the best model contained *Treatment* as a fixed effect only

[b] model contained a variance function that allowed for modeling heteroscedastic variances specific to each *Site*

[c] model only contained *Year* as random effect, and a variance function that allowed for modeling heteroscedastic variances specific to each *Site*

Table 1.2. Forestry treatment use by amphibians up to 6 years post-harvesting. Coefficients and p-values (italics below coefficients, with bold emphasis for significance at $\alpha = 0.05$) are from the best mixed effects model for each event/species/life stage. For all models, I am comparing the mean *Control* value to all the other treatments (the coefficients show a higher (+) or lower (–) use and the p-values indicate departures from the mean *Control* value). No significant difference was found between the two clearcuts for any species and life stage (A = adults; J = juveniles).

Event	**Species**	**Life stage**	**Forestry treatments**			
			Control	**Partial Cut**	**CWD removed**	**CWD retained**
Breeding migration	*L. sylvaticus*	A	1.441	– 0.022 *0.9113*	– 0.127 *0.5377*	0.158 *0.4437*
	A. maculatum	A	- non-significant overall effect of Treatment			
Juvenile emergence	*L. sylvaticus*	J	3.283	0.445 ***0.0100***	– 0.014 *0.9310*	0.216 *0.1943*
	A. maculatum	J	1.759	– 0.101 *0.6559*	– 0.110 *0.6277*	– 0.010 *0.9640*
Juvenile dispersal	*L. sylvaticus*	J	3.727	0.526 *0.0646*	– 0.914 ***0.0023***	– 1.487 ***<0.0001***
	A. maculatum	J	- data not analyzed			
Use for foraging or migration	*L. sylvaticus*	J	1.525	– 0.390 ***0.0006***	– 0.662 ***<0.0001***	– 0.632 ***<0.0001***
		A	0.538	– 0.058 *0.3917*	– 0.287 ***0.0001***	– 0.268 ***0.0002***
	A. maculatum	J	0.794	– 0.303 *0.0959*	– 0.623 ***0.0010***	– 0.571 ***0.0023***
		A	- data not analyzed			
	L. clamitans	J	0.643	– 0.123 *0.2938*	– 0.236 ***0.0476***	– 0.139 *0.2371*
	L. catesbeianus	J	- non-significant overall effect of Treatment			
	L. pipiens + *L. palustris*	J + A	0.076	0.025 *0.3877*	0.028 *0.3326*	– 0.017 *0.5579*
	P. cinereus	J + A	0.340	– 0.145 ***0.0313***	– 0.190 ***0.0054***	– 0.212 ***0.0021***
	N. viridescens	J + A	0.108	– 0.017 *0.6991*	– 0.020 *0.6616*	– 0.046 *0.3205*

Juvenile *A. maculatum* showed no initial directionality, but the use of treatments did not vary across sites (Table 1.1). I found no effect of forest succession on the choice of the forestry treatment for both species upon exiting the natal pools. I recaptured 1 993 *L. sylvaticus* (14.2% of the total emerging from the experimental pools) and 87 *A. maculatum* (5.8% of the total) in the upland habitat. In the upland, the frequency of *L. sylvaticus* frog recaptures was strongly biased towards the forested treatments (Table 1.2), and did not vary temporally (non-significant Treatment x Year interaction, Table 1.1). Across all sites, there was no difference in use between the control and partial cut, and the animals showed a slight preference for the CWD-removed over the CWD-retained treatment. However, there was strong site-specific variability in the use of upland forestry treatments (significant Treatment x Site interaction, Table 1.1), which was maintained across seasons (Figure 1.2). Along with the strong preference for forested treatments, animals that entered each treatment maintained their direction of movement (non-significant Treatment x Distance interaction, Table 1.1). By examining the interaction plot between the mean proportion of recaptures (pooled across sites), treatment, and year (Figure 1.3), I found a slight trend toward an increasing proportion of individuals captured in clearcut treatments in later years. During the first year, the difference between the use of forests and clearcuts expressed as proportion of captures was approximately eight-fold (forest: clearcut = 0.89:0.11). This difference decreased to 3.3-fold by the third year (0.77:0.23), and it was maintained during the fifth year post harvesting.

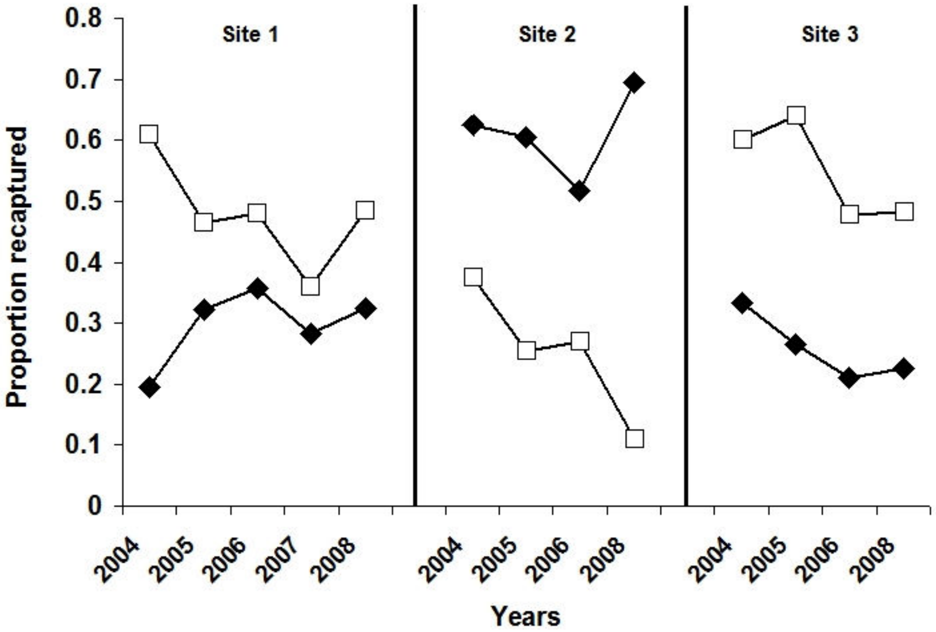


Figure 1.2. Site-specific difference in the use of the forested treatments (black diamond = *control*, empty square = *partial cut*) by emigrating juvenile *L. sylvaticus* emerging from experimental pools. Site 4 not graphed (only one year of data).

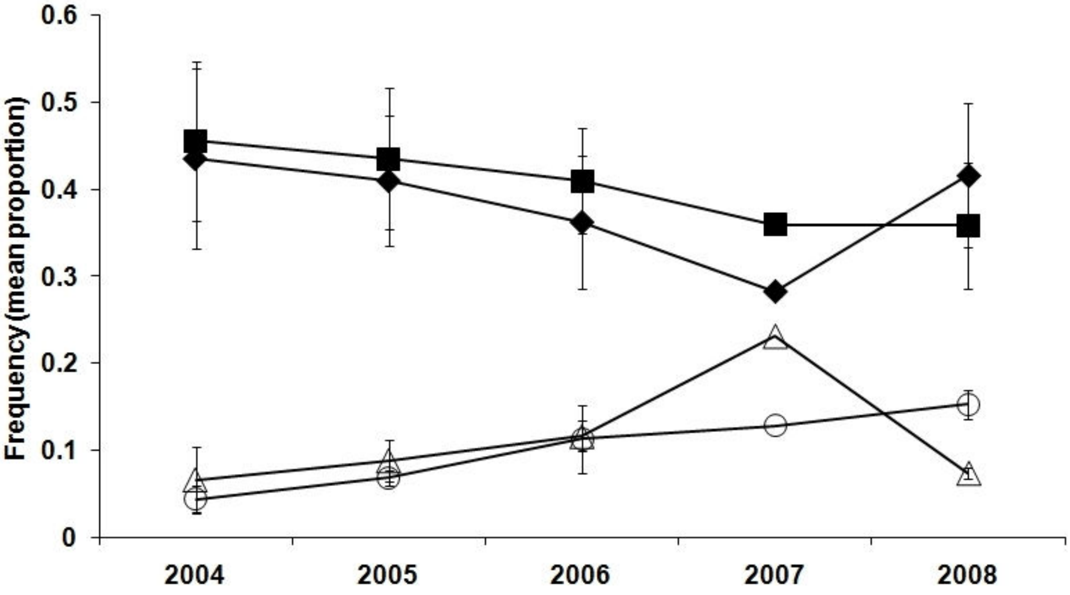


Figure 1.3. Use of upland habitat by juvenile wood frogs emerging from the experimental pools expressed as the mean proportion (± 1 SE) captured between 2004 and 2008; solid diamonds = *control*, solid squares = *partial cut*, empty circles = *CWD-retained*, empty triangles = *CWD-removed*. In 2007 only one site produced juveniles.

Unmarked adults and juveniles – shift in species assemblage

I captured 26 374 unmarked adults and juvenile amphibians (post-breeding adults, as well as juveniles emerging from pools outside the experimental forestry treatments) using the experimental arrays between 2004 and 2009. The number of species recorded varied between 14 in 2005 and five in 2009. Of these, 91% (24 015 individuals) were juveniles, and 54% of juveniles (12 884 individuals) were *L. sylvaticus* (Table 1.3). For most species, there were strong yearly and site-specific, demographic fluctuations, with juveniles of three forest-dependent species, *L. sylvaticus*, *A. maculatum*, and *P. cinereus* experiencing the greatest variation across the study period (Table 1.3). Despite the annual variation in the number of captures, the juveniles and adults of these three species exhibited a clear deterministic response to disturbance and vegetative succession, selecting for the uncut control throughout the study (Table 1.2, Figure 1.4). Overall, the number of captures for forest specialists (including *N. viridescens*) declined after the first 2-3 years of the study across all treatments, and the decline was related to time-since-harvest (significant effect of Year, p-value <0.0001). *P. cinereus* virtually disappeared from the clearcut treatments by the second year post-harvest and only five captures were recorded between 2006 and 2009 in these treatments.

Overall, the generalist *L. clamitans*, *L. catesbeianus*, *L. pipiens,* and *L. palustris* did not respond to disturbance or vegetative succession, but at the site scale there were differences in the use of treatments by most species (Tables 1.1, 1.2, Figure 1.4). The proportion of juvenile *L. clamitans* using the forested treatments increased post-harvesting, especially in the uncut control, which accounted for approximately 50% of the total captures in 2009 (Table 1.3, Figure 1.4). Juvenile *L. catesbeianus* and adult and

Table 1.3. Total number of unmarked amphibians captured between 2004 and 2009 (C = clearcuts; F = forested treatments).

	Adults											
Species	**2004**		**2005**		**2006**		**2007**		**2008**		**2009**	
	C	***F***	***C***	***F***	***C***	***F***	***C***	***F***	***C***	***F***	***C***	***F***
Salamanders												
A. laterale	6	7	-	2	-	3	2	2	2	-	-	-
A. maculatum [a]	3	5	5	20	4	6	4	8	1	3	-	-
H. scutatum	-	-	1	3	-	-	-	-	-	2	-	-
N. viridescens	4	1	1	10	3	3	6	9	3	5	1	5
P. cinereus	52	70	16	53	1	16	2	14	2	11	-	-
Anurans												
A. americanus	2	4	3	-	-	-	-	-	1	7	-	-
H. versicolor	-	-	-	-	-	-	-	-	-	-	-	-
L. catesbeianus	19	16	15	19	-	2	-	-	2	1	-	-
L. clamitans	113	68	61	92	10	13	6	17	2	7	8	12
L. palustris	2	6	6	1	2	-	13	11	-	4	-	-
L. pipiens	6	18	1	5	3	-	-	-	2	4	-	-
L. septentrionalis	-	-	1	6	-	-	-	-	-	1	-	-
L. sylvaticus	79	183	83	383	28	131	7	65	24	60	8	60
P. crucifer	-	2	-	-	-	1	-	1	-	3	1	-

	Juveniles											
	2004		**2005**		**2006**		**2007**		**2008**		**2009**	
	C	***F***	***C***	***F***	***C***	***F***	***C***	***F***	***C***	***F***	***C***	***F***
Salamanders												
A. laterale	2	8	8	13	11	17	-	-	-	2	-	-
A. maculatum [a]	13	199	130	741	184	404	2	32	3	23	5	55
H. scutatum	1	-	1	-	-	-	-	-	-	-	-	-
N. viridescens	17	58	13	39	6	9	9	14	4	18	2	3
P. cinereus	28	39	4	23	2	19	-	4	-	2	-	-
Anurans												
A. americanus	2	5	-	1	-	-	-	-	4	8	-	-
H. versicolor	1	-	-	1	-	-	-	1	-	-	-	-
L. catesbeianus	61	76	96	142	38	82	13	39	51	77	-	-
L. clamitans	236	346	464	696	474	1144	421	853	373	1215	36	164
L. palustris	4	29	11	13	25	19	17	41	11	20	-	-
L. pipiens	44	88	59	83	30	106	17	31	15	31	-	-
L. septentrionalis	-	-	-	-	9	22	-	-	-	-	-	-
L. sylvaticus	1211	2887	862	2172	1261	2618	200	448	280	749	48	149
P. crucifer	4	9	1	5	-	-	-	-	1	11	-	-

[a] In 2004, the bulk of *A. maculatum* adult and juvenile captures (approx. 83%) occurred late in the season (between 15 September and 27 October). For comparison purposes, I am presenting data truncated to 15 September to match the sampling period of the rest of the years.

juvenile *L. pipiens* and *L. palustris* fluctuated least during the study, and I did not record any of these species in 2009.

Heterogeneity of vegetative succession

Natural succession during the study period led to rapid changes in vegetation structure and composition, especially in the clearcut treatments. Due to specific regeneration processes of early-successional tree species (root suckers versus stump sprouts), micro-topography, existing dormant seed banks, and retained advance regeneration, the emerging spatial patterns were diverse and varied within and between experimental arrays. Overall, there was an increasing trend in percent cover in vegetation strata 1 – 2 m and >2 m in height (Figure 1.5), with the latter covering approximately 23% of the clearcuts five years post-disturbance. Low regeneration (up to 1 m) consisted mainly of pioneer species such as *Rubus* spp. (which in some cases formed continuous patches), and grey birch (*Betula populifolia*). Tall regeneration (which in some cases reached >4 m in 2008) was dominated by bigtooth aspen (*Populus grandidentata*) and red maple (*Acer rubrum*). Understory regeneration also increased following harvesting in the partial cut treatment, with the >2 m class reaching 19.4% (± 5.7) ground cover (Figure 1.5). Low woody understory cover was mostly composed of pioneer species, such as paper birch (*B. papyrifera*) and *Rubus* sp., while the taller class (>2 m) consisted of advance *A. balsamea* regeneration.

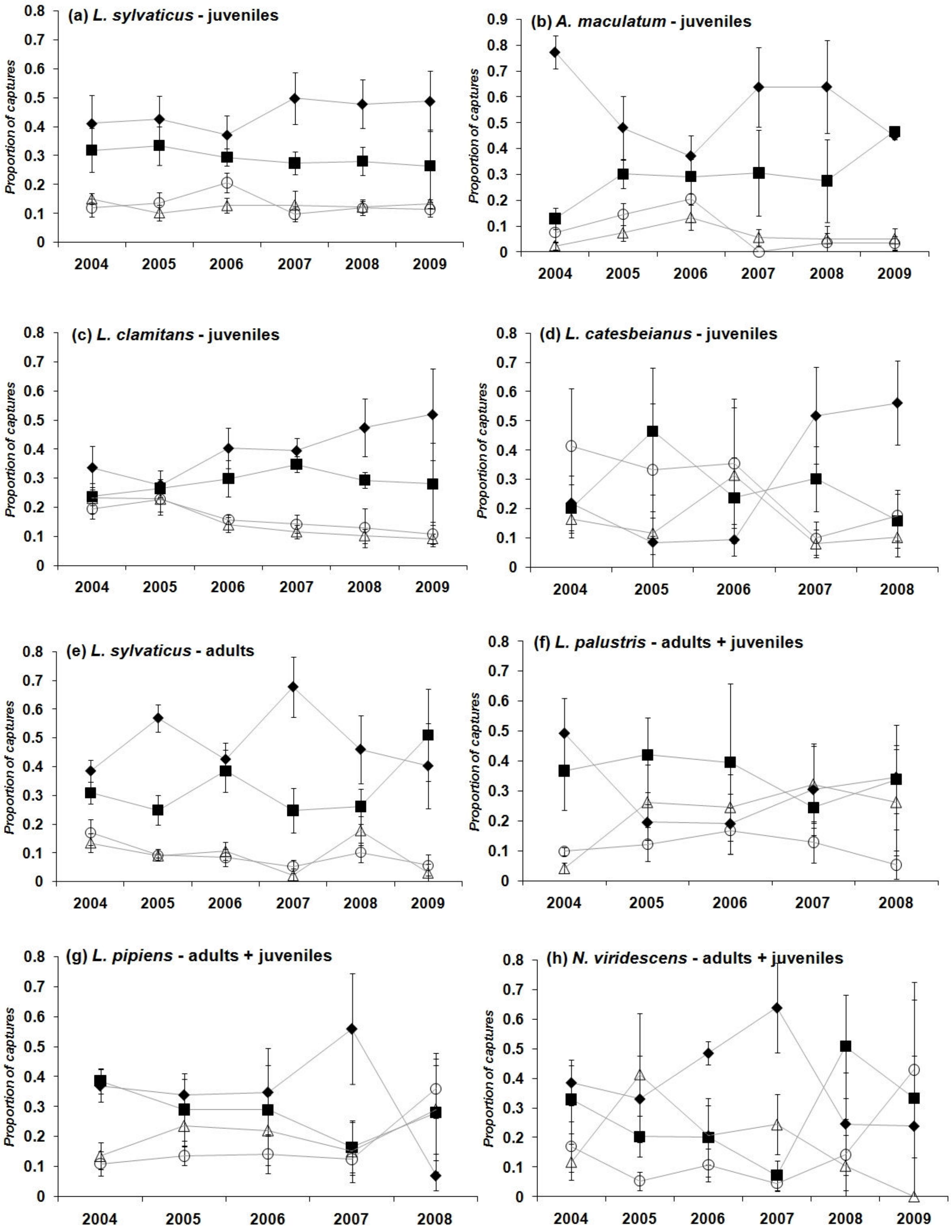


Figure 1.4. Mean proportions (± 1 SE) of unmarked amphibians captured between 2004 and 2009; solid symbols denote forested treatments (diamond = *control*, square = *partial cut*); empty symbols are clearcut treatments (triangle = *CWD-removed*, circle = *CWD-retained*).

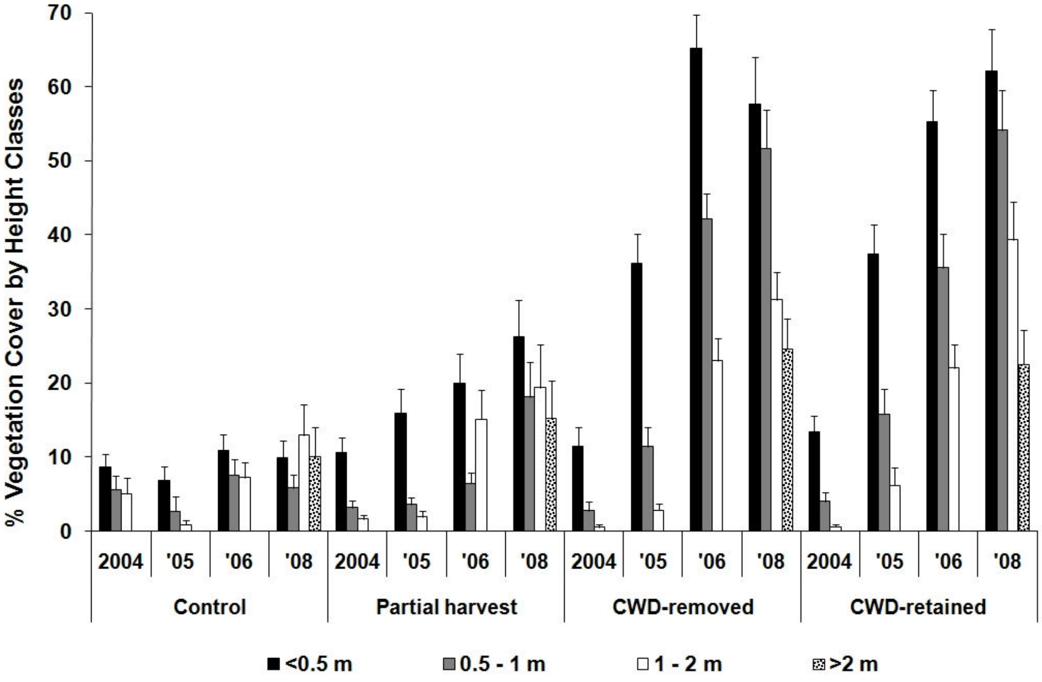


Figure 1.5. Changes in vegetation cover (by height classes) following canopy removal (black bars = *<0.5 m* in height, gray = *0.5 – 1 m*, white = *1 – 2 m*, black dots = *>2 m*).

Discussion

Anthropogenic habitat disturbance such as logging is known to affect forest amphibians, but predicting the long-term effects is often complicated by the underlying stochasticity characteristic of amphibian populations. Current approaches to management, however, are typically based on the premise that the responses of amphibians to management actions are predictable and remain relatively constant. Because of this, the spatial and temporal variability in habitat selection and fluctuations in demography seen both in our study and by previous researchers present a challenge for drafting management guidelines. Our study clearly showed interspecific and intraspecific differences in the extent to which forestry treatments can explain patterns of habitat use and community dynamics. Our ability to predict habitat use and abundance of amphibians related to the four forestry treatments was scale-dependent. Strong deterministic responses to clearcutting (i.e., avoidance, low abundance) were the norm for forest amphibians (Table 1.3, Figure 1.4). However, this overall broad-scale prediction was complicated by high site-specific variability in habitat use (e.g., local differences between forested treatments not apparent at the broader scale), likely linked to landscape context. Because of this, predicting the response of amphibians to habitat change at any one of our sites is subject to a high degree of uncertainty.

Temporal variability in abundance and distribution of amphibians was reflected in the year-to-year population fluctuations during both aquatic and terrestrial stages and in the marked decline of forest species after 2-3 years post-disturbance (Table 1.3). I found only a modest and non-significant positive effect of vegetative succession on habitat use by forest amphibians (Figure 1.3). On a temporal scale, the six years of the study reflect only very young, incipient succession (typically up to 15-20 years, [Oliver and Larson 1996]), but during this period observed changes should be more dramatic than changes that might occur later in succession. There was no year-to-year variation in habitat use by most species (Table 1.1). The less abundant members of the amphibian community, such as *A. americanus, L. septentrionalis*, and *H. scutatum* were also subject to natural fluctuations, as they appeared and disappeared from our study sites with no clear spatial or temporal pattern.

The different responses of species to canopy removal reflected their affinity to forest (forest specialists versus generalists), as well as their sensitivity to habitat alteration, both seasonally and during their ontogenetic development. For example, the spring breeding migrations of adult *L. sylvaticus* and *A. maculatum* were not influenced by clearcutting, while post-breeding adults and emigrating juveniles clearly preferred closed-canopy habitats over clearcuts throughout the study period, which corroborates findings of Todd et al. (2009) in a similar experimental setting. Contrary to our predictions, I did not detect a positive short-term effect of CWD on movements and habitat use by post-metamorphic or adult *A. maculatum* and *L. sylvaticus*. This suggests that CWD does not play a role in mitigating the effects of clearcutting for these species. The majority of migrating juveniles simply tend to avoid open habitat, irrespective of the existing microhabitat structures.

The role of spatial scales in predicting responses to disturbance

Deterministic processes were evident at a broad spatial scale (across all sites), and mainly arose from overall, relatively consistent differences in habitat use between forested and clearcut treatments. Clearcutting strongly affected the distribution and abundance of forest-dependent amphibians, while generalist species were less influenced by disturbance or showed an increase in abundance (Table 1.3). In contrast to the limited use of clearcuts by most forest amphibians, the partial harvest was characterized by high use, similar to the control treatment (Table 1.2, Figure 1.4).

The broader-scale landscape context also comes into play when considering the spatial population structure of vernal pool-breeding amphibians. Adult *L. sylvaticus* and *A. maculatum* did not show a preference for a particular forestry treatment during the breeding season (Tables 1.1, 1.2), but responded in a deterministic manner to the creation of the artificial experimental pools. Pool colonization occurred rapidly (within the first year) and breeding populations were maintained throughout the study period, indicating high plasticity in breeding site selection (Karraker and Gibbs 2009). This suggests that both species are abundant in the area of study, and that the degree of philopatry might be not as high as in other regions of the species' ranges (Patrick et al. 2008a). Evidence from molecular genetic analyses in other regions of these species' ranges revealed that *A. maculatum* might occur in metapopulations, with dispersal among population clusters occurring fairly frequently even in fragmented landscapes (Zamudio and Wieczorek 2007). High gene flow between populations separated by up to 20 km is also characteristic of *L. sylvaticus*, with frequent extinction-recolonization events (Newman and Squire 2001). This further questions the degree of philopatry in these species, and emphasizes the importance of the dispersing juvenile stage in maintaining connectivity of regional populations.

Spatial variability in terrestrial habitat use was manifested at the local scale through site-specific effects of the control and partial cut treatments on the distribution of forest-dependent amphibians. Notably, the observed patterns of habitat use of both adults and juveniles varied greatly between sites (significant Treatment x Site interactions for most species, life stages, Table 1.1). For example, juvenile *L. sylvaticus* emigrating from the experimental pools showed a pattern of forested treatment use that varied from site to site, but was maintained across years. At one site juveniles moved preferentially through the partial cut, another site was dominated by movements in the control, while at another site the number of captures was roughly equal between the two forested treatments (Figure 1.2). In contrast to the between-sites variation in habitat use and preferential use of closed-canopy upland habitat, emerging juveniles of both *L. sylvaticus* and *A. maculatum* exited the natal pools at random during the six years of the study. Given the narrow field of perception of juvenile amphibians and reliance on proximate cues for orientation (Rothermel 2004, Popescu and Hunter in press), we would expect random orientation when environmental cues are not very strong (i.e., our treatments converged towards the pool, masking the transition between forest ["good"] and no-canopy ["bad"] habitat), but non-random habitat use when prominent habitat features, such as sharp forest edges, are intercepting the movement paths. Moreover, the lack of the Treatment x Distance interaction in our study suggests that habitat choice in emerging juvenile *L. sylvaticus* animals occurs at distances <16.6 m from the pool edge.

Predictability of amphibian response to vegetative succession

Despite the modest mitigating effect of forest succession on habitat use by *L. sylvaticus* emigrating juveniles (Figure 1.3), the general preference for closed-canopy forested habitat by juvenile *L. sylvaticus* and *A. maculatum* was maintained throughout the six years of study, suggesting that the potential positive effects of vegetative succession (i.e., partial mitigation of microclimate and providing refugia) were overridden by other factors (i.e., lack of canopy cover). This is consistent with another study in our landscape in which movements of juvenile *L. sylvaticus* were affected by clearcutting for up to 10 – 20 years (Popescu and Hunter in press).

There was apparently an overall decrease in habitat quality for forest species at the site scale across the six years of the study, as evidenced by the continuous decline in abundance across the entire experimental sites, not just the clearcut portions (Table 1.3). Notably, the number of adult and juvenile *L. sylvaticus*, *A. maculatum*, *P. cinereus* and *N. viridescens* captured in the forested treatments decreased during the study period. Given the life-histories of these species, the causes for the decline in captures are likely to be different. The declining number of captures of juvenile and adult *L. sylvaticus* and *A. maculatum* across all treatments and sites might be due to overall avoidance of disturbed habitat (both clearcuts and the area of edge effect extending into the forested treatments). If we conservatively consider an edge effect depth of 30 m (deMaynadier and Hunter 1998), then almost half of the forested treatments fall outside what we might consider good quality habitat for forest amphibians. For example, migrating *L. sylvaticus* and *A. maculatum* juveniles show an active avoidance of abrupt edges, with fewer numbers entering and successfully migrating through habitats lacking canopy cover (Rothermel and Semlitsch 2002, Popescu and Hunter in press). *P. cinereus* is quite different from the other two declining forest species; it has the smallest home range, is less vagile, and does not migrate during summer (Jaeger et al. 1995). The rapid disappearance from clearcuts and the decrease in abundance in the forested treatments observed in our study corroborate other studies that found that plethodontid salamanders are very sensitive to canopy removal (Ash 1997), and have lower abundance in proximity to abrupt edges (deMaynadier and Hunter 1998). Despite not being affected by the forestry treatments, habitat use by the forest-dependent *N. viridescens*, which is known to be sensitive to fragmentation (Gibbs 1998) varied on a site-by-site and year-to-year basis, with juveniles showing the same site-wide decline after two years post harvesting (Table 1.3). In the absence of control sites with no logging at all we cannot exclude the possibility that the observed declines of forest specialists were due to a regional population reduction because of some other factor, such as disease. However, that seems unlikely given our observations of generalist species, which either showed an increase in abundance post-clearcutting (e.g., juvenile *L. clamitans*) or appeared to be unaffected by forestry treatments and vegetative succession (Tables 1.2 and 1.3). Despite annual fluctuations in the number of captures, the highest captures for a generalist species were for juvenile *L. clamitans*. Interestingly, the bulk of these captures occurred in the forested treatments, which was not expected for a habitat generalist that is known to use open habitat during upland movements (Birchfield and Deters 2005).

Accounting for spatial and temporal variability in terrestrial habitat use when managing amphibian populations

The effects of the local landscape context were evident in the abundance, distribution, and dynamics of habitat use of amphibians, which inevitably complicates managing populations and communities. Given that at the broad scale the responses to canopy removal were dominated by strong deterministic processes (e.g., avoidance of clearcuts by forest amphibians), conservation in forested landscapes that are relatively natural (either reserves or industrial forests that allow natural vegetative succession, without the threat of long-term deforestation) would likely be successful with just broad management prescriptions (e.g., avoid complete canopy removal, protect upland habitat in the proximity of breeding pools). However, because high variability in habitat use was prevalent at the site scale, conservation strategies for sensitive landscapes (i.e., under threat of intensive development) must account for this source of uncertainty. Thus, a multiple-scale approach to management of breeding aquatic habitat and upland habitat is most applicable to urbanizing landscapes (Baldwin and deMaynadier 2009).

Our research findings have specific implications when considering the conservation of the full complement of species in the amphibian community. The species in this study exhibit a variety of life history traits, representative for temperate amphibian assemblages (i.e., habitat generalists, forest specialists, pond-breeding, completely terrestrial), and I observed year-to-year and site-specific variability in habitat use. From a management perspective, instead of focusing on specific conditions to create optimal habitat for a particular species, general recommendations, such as maintenance of canopy cover and minimizing site disturbance during peak migration season would be more practical (Campbell et al. 2010). Similar guidelines for conservation strategies that meet both the habitat requirements of species breeding in or using small, isolated wetlands and forest management goals have been previously suggested by Calhoun and deMaynadier (2004). The findings of our study fully support their recommendations.

Specifically for the conservation of forest amphibians breeding in vernal pools, I found detrimental effects of clearcutting, while timber harvesting practices that retained at least 50% canopy cover (i.e., selection harvesting) still apparently provided suitable habitat at least for emigration. Generally, post-harvest silvicultural practices that lead to changes in tree species composition and stand structure negatively affect biodiversity (Lindenmayer et al. 2006), especially where natural forests are converted into single-species plantation forests (Lindenmayer and Hobbs 2004). Silvicultural alternatives to clearcutting that retain a certain proportion of the canopy and increase habitat heterogeneity appear to have a more benign effect on maintaining biodiversity (Rosenvald and Lõhmus 2008). This suggests that a forest management strategy for pool-breeding amphibians should combine: (1) the retention of a minimum protective buffer around breeding pools (sensu Calhoun et al. 2005) and (2) harvesting operations that are spatially and temporally structured to retain canopy both between highly productive pools and between pool and high-quality terrestrial habitat (Baldwin et al. 2006).

In conclusion, local (spatial) and year-to-year (temporal) variability in habitat use plays an important role in amphibian community dynamics. Deterministic processes are certainly going to continue to provide the most straightforward information for drafting management plans, but stochasticity should not be perceived just as noise inherent to the system. Rather, acknowledging that variability in habitat use and stochasticity occur at a finer scale, would result in more flexible conservation strategies, thus allowing for wider margins of error during the implementation process. Acknowledging fine-scale variation in the responses of amphibians to habitat change may be particularly important for managers focusing on a single site or limited set of sites.

Chapter 2

CLEAR-CUTTING AFFECTS HABITAT CONNECTIVITY FOR A FOREST AMPHIBIAN BY DECREASING PERMEABILITY TO JUVENILE MOVEMENTS

Abstract

Conservation of forest amphibians is dependent on finding the right balance between management for timber production and meeting species' habitat requirements. For many pond-breeding amphibians, successful dispersal of the juvenile stage is essential for long-term population persistence. I investigated the influence of timber harvesting practices on the movements of juvenile wood frogs (*Lithobates sylvaticus*). I used a chronosequence of stands produced by clearcutting to evaluate how stand age affects habitat permeability to movements. I conducted experimental releases of juveniles in 2008 (n = 350) and 2009 (n = 528) in unidirectional runways in four treatments: mature forest, recent clearcut, 11-year-old, and 20-year-old regeneration. The runways were 50 x 2.5-m enclosures extending into each treatment, perpendicular to a distinct edge, with four tracking stations at 10, 20, 30, and 40 m from the edge. I recorded the number of animals reaching each tracking station, and the proportion of animals changing their direction of movement at each distance. I found that the mature forest was 3.1 and 3.7 times more permeable than the 11-year-old regeneration and the recent clearcut, respectively. Animals actively avoided open-canopy habitats and sharp edges; significantly more animals returned towards the closed-canopy forest at 0 m and 10 m in the less permeable treatments. There were no significant differences in habitat

permeability between the mature forest and the 20-year-old regeneration. Our study is the first to directly assess habitat permeability to juvenile amphibian movement in relation to various forestry practices. I argue that habitat permeability at this scale is largely driven by the behavior of animals in relation to habitat disturbance and that caution needs to be used when using spatial modeling and expert-derived permeability values to assess connectivity of amphibian populations. The effects of clearcutting on the migratory success of juvenile *L. sylvaticus* are long-lasting. Forestry practices that involve canopy removal and conversion of natural forest to conifer plantations may affect regional population viability by hindering successful dispersal.

Introduction

Animals move across the landscape to gain access to various resources and thus understanding animal movements is often critical to conservation strategies (Semlitsch 2002). The conversion of natural land cover types to other uses disrupts movements of organisms, affecting habitat selection, and causing local declines in abundance or species richness (Cushman 2006). Because of their biphasic life cycles and generally low vagility and high sensitivity to disturbance (Semlitsch 2000), movement behavior and migratory success of amphibians are likely to be significantly altered by land-use conversion. For pond-breeding amphibians, one mechanism behind observed declines relates to decreased connectivity between breeding ponds, and between breeding and foraging or overwintering habitats (Laan and Verboom 1990, Sjögren-Gulve 1994). Changes in land cover induced by forestry practices impact forest amphibians and the effects can be long-lasting (deMaynadier and Hunter 1995). In particular, clearcutting and conversion of natural forests to single-species plantations are known to have detrimental effects on amphibian occupancy due to changes in habitat quality and permeability to movements (Parris and Lindenmayer 2004, Semlitsch et al. 2009). Permeability can be quantified in terms of both the costs imposed by a habitat to movement (e.g., physiological stress, risk of predation) (Joly et al. 2003), and behavior (i.e., willingness to move). Thus, research aimed at investigating habitat permeability has to account for the individuals' propensity to move through a particular habitat and the effects of habitat alteration on behavior (Russell et al. 2003, Semlitsch et al. 2008). Juvenile amphibians pose a particularly interesting challenge for quantifying movements and habitat permeability because of their small size and cryptic lifestyles. Moreover, juveniles are typically the dispersing life stage in many amphibian species, dispersal being defined here as the one-way trip from a natal pond to a different pond for breeding (Semlitsch 2008). Dispersers promote gene flow that maintains genetic variation among populations (Slatkin 1987) and contribute to metapopulation processes through recolonization following local extinction events (Marsh and Trenham 2001).

For juvenile forest-dwelling amphibians, permeability is assumed to decrease following timber harvesting, but empirical data on the magnitude of the decrease, as well as on how habitat alteration influences movement behavior is lacking (a notable exception is Rothermel and Semlitsch [2002]). Hence, most investigations of how landscape permeability affects amphibian movements, spatial population structure, and gene flow have been based on computer models that relied on expert opinions of the permeability of various land cover types (Ray et al. 2002, Compton et al. 2007, Zellmer and Knowles 2009). Despite the lack of empirical data on habitat permeability, this field of research is likely to become even more popular with the advent of circuit theory-based software that includes "isolation by resistance" parameters (McRae 2006).

In this study, I used an experimental approach to quantifying habitat permeability for juvenile wood frogs (*Lithobates sylvaticus*) in a forested landscape. *L. sylvaticus* are a vernal pool-breeding species, widely distributed in North American temperate and boreal forests, and the juveniles represent the only source of dispersing individuals (Berven and Grudzien 1990), critical to the persistence of regional populations (Zellmer and Knowles 2009). *L. sylvaticus* are highly sensitive to forest removal, and their local abundance is strongly affected by proximity to abrupt forest edges (deMaynadier and Hunter 1998). Habitat selection of post-metamorphic *L. sylvaticus* differs between a dispersing and a settling phase, with animals responding to coarse variation in habitat during the dispersing stage (Patrick et al. 2008).

I studied the movements of juvenile *L. sylvaticus* in a forest management context in central Maine, USA, using a chronosequence of stands resulting from even-aged silviculture (i.e., clearcutting) as a time-series proxy to evaluate how stand age affects permeability. The specific objectives of this research are: (1) to quantify the dispersal success of juveniles in various-aged stands resulting from clearcutting; (2) to identify mechanisms that drive the movement behavior of juveniles; and (3) to evaluate the influence of forest edges on the movement patterns of juvenile amphibians.

Materials and Methods

Study sites and experimental design

I conducted the research in Penobscot County, Maine, USA, on the University of Maine Dwight B. Demeritt Experimental Forest and Henderson Forest, and on a tract in Milford, managed by American Forest Management, Inc. Central Maine has a long history of forest management, and as a result is a mosaic of various-aged mixed wood stands, part of the Acadian Forest region, which covers most of northeastern U.S. and the Canadian Maritime Provinces (Saunders and Wagner 2008). I selected four forestry conditions that are common in the region: control *mature forest*, *recent clearcut* (2-3 years), *11-year-old regeneration* treated with herbicides, and *20-year-old natural regeneration*. I first identified a linear edge between a closed-canopy forest and each of the regeneration treatments then randomly selected the location of the experimental setup along the edge. The recent clearcut (hereafter clearcut) completely lacked canopy cover, as well as any tree regeneration, containing mostly herbaceous and low shrub (< 50 cm) vegetation layers. The 11-year-old regeneration stand (11-yr stand) was treated with herbicide 5-6 years post-clearcutting, a silvicultural treatment known as “conifer release”. The resulting stand resembled a dense conifer plantation, composed of white pine (*Pinus strobus*), red spruce (*Picea rubens*), and eastern hemlock (*Tsuga canadensis*), and the average tree height was 3.8 ± 0.4 m. The 20- year-old regeneration stand (*20-yr stand*) was left untreated post-clearcutting, and resulted in a dense, mixed stand dominated by white birch (*Betula papyrifera*) and grey birch (*B. populifolia*), with sparse red maple (*Acer rubrum*), white pine, balsam fir (*Abies balsamea*), red spruce, and quaking aspen (*Populus tremuloides*), averaging 9.2 ± 0.8 m in height. The mature forest was a 70-80 year-old closed-canopy mixed stand composed of hemlock, red spruce, red maple, quaking aspen, and American beech (*Fagus grandifolia*) >15 m in height, with patchy balsam fir regeneration, and was not harvested in the past 20 years.

Experimental runways

I tested the permeability of four forestry treatments to juvenile *L. sylvaticus* movements by building terrestrial *runways* using a design modified from Rothermel and Semlitsch (2002). Runways consisted of 50 m x 2.5 m silt fence enclosures (60 cm tall and buried 30 cm into the ground) oriented perpendicular to the forest edge, starting at approximately 3 m from the edge and extending into each treatment. The runways in the clearcut, 11-yr stand, and 20-yr stand were adjacent to closed-canopy mature stands. The runways in the mature forest were adjacent to a clearcut. I decided to place our runways adjacent to edges because: (1) newly metamorphosed *L. sylvaticus* tend to settle in good quality habitat and placing runways in interior closed-canopy conditions might not elicit movement behavior, the main focus of our experiment (Patrick et al. 2008), and (2) I wanted to investigate behavior in relation to harsh edges, which were found to alter amphibian movement (deMaynadier and Hunter 1998, Rothermel and Semlitsch 2002). I only selected one stand for each treatment because I could not identify other suitable stands that were close enough to allow us to implement the experiment, given our labor-intensive and time-sensitive approach. In 2008, I built six runways in mature forest, clearcut, and 11-yr stand (two per treatment). In 2009, I built another six runways: three in the 20-yr stand, and one for each of the other treatments, for a total of 12 runways. For each treatment, the runways were spaced 30 – 50 m apart, parallel to each other.

In each runway, I built four tracking stations (at 10, 20, 30, and 40 m from the start of the runway) to evaluate the number of animals reaching each distance (Figure 2.1). The tracking stations were constructed by cutting entrance and exit openings into large plastic containers with lids (45 x 65 x 20 cm). A silt fence funnel extending from the edge towards the middle of the runway directed the experimental animals into the tracking stations. Inside the trays, I placed a mix of orange fluorescent powder (DayGlo Color Corp., Cleveland OH) and mineral oil (RiteAid®) on waterproof paper (Rite-in-the-Rain, J.L. Darling Corporation, Tacoma WA), next to a white sheet of plain paper. Thus, individuals passing through the fluorescent powder mixture self-recorded their tracks on the white paper. The oil allowed us to accurately count tracks even after heavy rain events. The mixture is harmless to amphibians and was used in other studies of amphibian movements (Eggert 2002). The white paper was changed daily, and was recoated with powder and oil as needed.

I used pitfall traps to estimate the number of animals reaching the end of the runways, or returning towards the forest edge. Each runway contained 12 pitfall traps: two at the start and two at the end of the runway, and two at 10, 20, 30, and 40 m, in the sharp angle formed by the silt fence funnel and the runway wall (Figure 2.1). Animals that changed their direction of movement at a particular distance would be captured in these traps, thus indicating a change in behavior related to the conditions in the respective treatments.

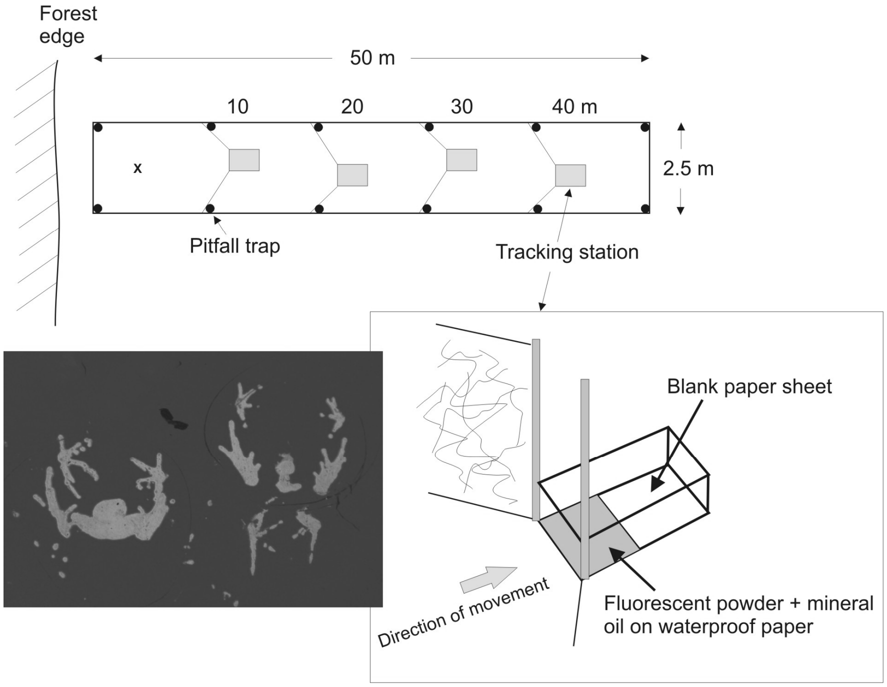


Figure 2.1. Experimental setup for evaluating the permeability of four forestry treatments to juvenile *L. sylvaticus* movements; **x** = release location; drawing not to scale. The inset on the right provides the tracking station design. Picture on left: fluorescent powder tracks from two different individuals.

Amphibian releases

I collected egg masses in mid April 2008 and 2009 from the University of Maine's Penobscot Experimental Forest, Maine, USA, and transported them to a site near campus where I hatched them in plastic wading pools (1 m diameter). Upon emergence, larvae at Gosner stages 21-23 (Gosner 1960) were added to 1500-liter cattle tanks. Two weeks prior to larval stocking, I added plankton collected from natural vernal pools and leaf litter to the cattle tanks to mimic a semi-natural rearing environment. I maintained a density of 70 larvae per tank. The cattle tanks were located under closed canopy cover, and were covered by screen lids to prevent colonization by other organisms. During the emergence season (July 1 – August 10 in Maine), the tanks were checked daily for metamorphosed *L. sylvaticus* (stage 42 and higher). Upon capture, I added the metamorphs to large plastic bins (200 liters) with moist leaf litter for up to three days until they reached the final stage of their metamorphosis. At this stage, they were measured, marked, and randomly assigned to the experimental treatments.

I released 350 juvenile *L. sylvaticus* in four batches in 2008, and 528 individuals in five batches in 2009. The number of animals per runway released in each batch ranged between 13 – 19 in 2008, and 8 – 10 in 2009, depending on availability, and it was consistent across runways within the same batch. The experimental animals were removed from the plastic bins during the day of release, measured (snout-vent length), marked with a single toe clip batch mark, and placed in plastic containers (one per runway). A subsequent batch was released only after no new tracks were recorded from the previous batch for at least 1 day. I released the animals 1 – 2 hours after sunset in the center of the runway, approximately 3 m from the starting point (i.e., approximately 6 m from the forest edge). I checked the runways daily between 7:00 and 11:00 AM during 9 – 24 July 2008, and 11 July – 6 August 2009.

Orientation arena

Due to landscape setting constraints, it was not possible to build runways with different cardinal directions in each treatment. However, I consider this not to be a limitation to our study because: (1) similar studies (Rothermel and Semlitsch 2002) did not find differences in movement patterns of juvenile amphibians among different cardinal directions, and (2) the animals used in this experiment were collected as eggs from skidder ruts and ditches along forest roads in an area (Penobscot Experimental Forest, Penobscot County, Maine) where metamorphosing *L. sylvaticus* orient randomly upon exiting the natal pools (Popescu V.D., unpublished data). However, to eliminate any doubt about their initial orientation biasing the outcome of the study, I tested a subsample of the experimental animals by releasing them in a circular arena (3 m diameter) in closed-canopy conditions. The circular arena was located >50 m from the forest edge, to minimize any potential edge effects that could potentially influence juvenile *L. sylvaticus* directionality (deMaynadier and Hunter 1998). Prior to release in the runways, I randomly selected three containers containing experimental animals, and released them in the center of the circular arena. After placing the tray in the arena, I waited for two minutes to overcome the effect of handling (Diego-Rasilla and Luengo 2002) then gently lifted the lid to release them. Using a pair of night-vision binoculars (Rigel Optics, DeWitt, IA), I recorded the bearing for each individual when it reached the edge of the arena.

Microclimate and habitat variables

Because microclimate might affect both habitat selection and survival of juvenile *L. sylvaticus*, I recorded hourly temperatures, relative humidity, and daily precipitation at each treatment location. I used 27 iButton data loggers (Maxim, Dallas Semiconductor, Dallas TX) to record hourly air, ground-level, and refuge (i.e., 5 cm below coarse woody debris or root channels) temperatures, as well as ground-level relative humidity, at two of the three runways in each treatment each year. Precipitation was measured daily using a rain gauge.

I characterized the vegetation cover in each 10 x 2.5-m compartment of the runways in July 2008 and 2009. I collected data on percent cover for each vegetation layer (herbaceous, shrub, canopy trees), percent leaf litter, ground cover, canopy closure (using a moosehorn densitometer, Moosehorn CoverScopes, Medford OR), canopy tree height (using a Recta DP10 professional prismatic clinometer), and tree density.

Statistical analyses

The runways yielded three indices that characterized amphibian movements and relative habitat permeability: (1) number of tracks at each tracking station distance, (2) proportion of individuals captured in pitfall traps, and (3) movement rate. The first two indices directly estimated the overall migratory success of juveniles, as well as their propensity to move through each forestry treatment. Besides the number of animals returning towards the edge, as well as reaching the end of the runways, which is a direct effect of habitat permeability and weather conditions, I was also interested in the number of individuals that changed their initial direction of movement to return towards the edge, as reflected in captures at 10, 20, and 30 m. The third index, rate of movement (m/day), is indicative of how quickly the animals moved through each forest type.

I used generalized linear mixed effects models (GLME) to investigate whether treatment, release, and individual runways had an effect on the number of tracks recorded at 10 – 40 m from the forest edge. GLME's have fewer assumptions than traditional regression (Pinheiro and Bates 2000) and provide a more flexible approach to analyzing non-normal data when random effects are present (Bolker et al. 2009). In GLME, fixed effects are factors that describe experimental treatments or are sources of systematic variance; random effects are associated with particular experimental units that are selected at random from the population of interest (Pinheiro and Bates 2000). I used block random effects that apply equally to all the individuals within a group, leading to a single level of correlation within groups (Bolker et al. 2009). Because the dependent variable was the proportion of animals that reached each tracking station following a release (inferred from the track count), I used a binomial mixed effects regression implemented in R version 2.8.1 (R Development Core Team, 2008; (package *lme4*; Bates and Maechler, 2009). I only used data from batches 2 – 4 in 2008 and batches 2 – 5 in 2009, because the first release in each year was regarded as trial and did not contain equal number of animals in each treatment and runway. I ran models for each tracking station distance (10, 20, 30, and 40 m) to avoid the autocorrelation emerging from counting the same individuals in successive tracking stations. For each model, I used variables *treatment* (the four forestry treatments) and *runway* (individual runway) as fixed effects and variable *batch* as both fixed and random effects. Due to the relatively small size of our dataset, and difficulty in interpreting regression coefficients for complex models, I only ran simple models followed by the first-order interactions of *treatment* x *runway* as fixed effects. I used quantile-quantile plots, residual plots, and plots of fitted versus observed values to assess the performance of each model, and likelihood ratio tests to assess the overall effect of the fixed effects. Finally, I used the Akaike Information Criterion (AIC) to select the model that had the greatest support for each of the four tracking distances (Burnham and Anderson 2002). For all four distances, the models that had the greatest support contained *treatment* as fixed effect and *batch* as random effect. The interaction term *treatment* x *runway* was not significant for any of the four distances, suggesting that there are no differences in the number of tracks among runways within treatment, and that the microhabitat heterogeneity observed among runways was overridden by other factors.

For the second index, I investigated the differences in the proportion of animals recaptured among treatments for each distance (0 – 50 m) and year separately. Specifically, I evaluated what fraction of animals that reached 10, 20, and 30 m (i.e., as deduced from the total number of tracks recorded at each distance) were captured in pitfall traps at that particular distance (i.e., total number of animals captured at 10, 20, and 30 m). I pooled the number of captures at 40 and 50 m in each runway because all four traps were located in the same runway 'compartment' (Figure 2.1). Thus, for both 0 m and pooled 40 – 50 m traps, I considered the fraction of animals captured out of the total number of animals released, as a measure of complete avoidance and dispersal success, respectively. I first assessed whether or not the frequency of captures and distance are associated with each other, and are independent of treatment using a three-way contingency table. If non-independence was found, I used pairwise tests for proportions to quantify differences in proportion of captures between treatments at each distance.

I investigated whether or not there were differences in the rate of movement (m/day) between the four treatments using a Kruskal-Wallis non-parametric test implemented in R (package *coin*; Torsten et al., 2008). If the general test was significant, I performed pairwise comparisons without adjusting the significance level. For quantifying movement rates, I only used the data on individuals that were captured past the first tracking station (89 individuals in 2008, and 91 in 2009).

I tested whether or not our experimental animals exhibited some directionality that might potentially bias the outcome of the permeability experiment using omnibus tests for circular uniformity in R (package *circular*; Lund and Agostinelli, 2007). Omnibus tests, such as Kuiper's and Watson's tests, are more powerful when there is little knowledge concerning the alternative hypothesis (Fisher 1993). For all statistical tests I used a significance level of 0.05.

Results

The three indices that I used to assess habitat permeability – number of track counts, proportion of captures, and rates of movement – differed significantly among treatments. The average size of the released juvenile *L. sylvaticus* was 17.19 ± 1.24 mm, and did not differ among treatments (ANOVA; $F_{3, 407} = 1.23$, p-value = 0.298).

Proportion of animals reaching tracking stations

There were differences in the number of tracks recorded among the four treatments for all distances. As a general rule, the proportion of released individuals decreased with increasing distance from the edge in all treatments, but was consistently higher in the mature forest and 20-yr stand compared to the two younger stands (Figure 2.2a, Appendix C). More specifically, a lower proportion of animals reached the 10-m tracking station in the clearcut compared to the 11-yr stand. A higher proportion of the released animals reached the 10-m tracking station in both the mature forest and 20-yr stand compared to the other two treatments. This pattern changed at 20 m from the forest edge, and remained consistent at 30 and 40 m from the edge. At these distances, there were no differences between the clearcut and the 11-yr stand, while a significantly larger proportion of animals migrated through the mature forest and the 20-yr stand (Figure 2.2b, c, Appendix C). The only difference between the mature forest and the 20-yr stand occurred at the 40-m tracking station, with a moderately lower proportion of animals moving through the 20-yr stand (Figure 2.2d, Appendix C). Considering the differences in the proportions of juvenile *L. sylvaticus* reaching 40 m, the mature forest was 3.1 - 3.7 times more permeable than the clearcut and 11-yr stand, and 1.5 times more permeable than the 20-yr stand (although the latter is a consequence of behavior, rather than absolute permeability; see Discussion).

Proportion of recaptures

The pattern of pitfall captures was complementary to that of the number of tracks. I recaptured 179 individuals in 2008 (51% of the total released) and 240 individuals in 2009 (45%). For both years, the proportion of captures at each distance was dependent on forestry treatment (2008: $\chi^2_{10} = 53.7$, p-value <0.001; 2009: $\chi^2_{15} = 116.8$, p-value <0.001). Overall, in the clearcut and *11-yr* stand most of the captures were recorded at 0 m, presumably reflecting animals trying to return towards the forest (30% and 48% in clearcut and 25% and 44% in 11-yr stand for 2008 and 2009, respectively; Table 2.1). Animals released in the mature forest were significantly less likely to return towards the edge, with only 19% and 18% captured at 0 m in 2008 and 2009, respectively. Individuals released in the 20-yr stand behaved similarly to those in the mature forest, with 17% captured at 0 m. In contrast, the proportion of animals traveling the entire length of the runways was significantly higher in the mature forest than in all the other

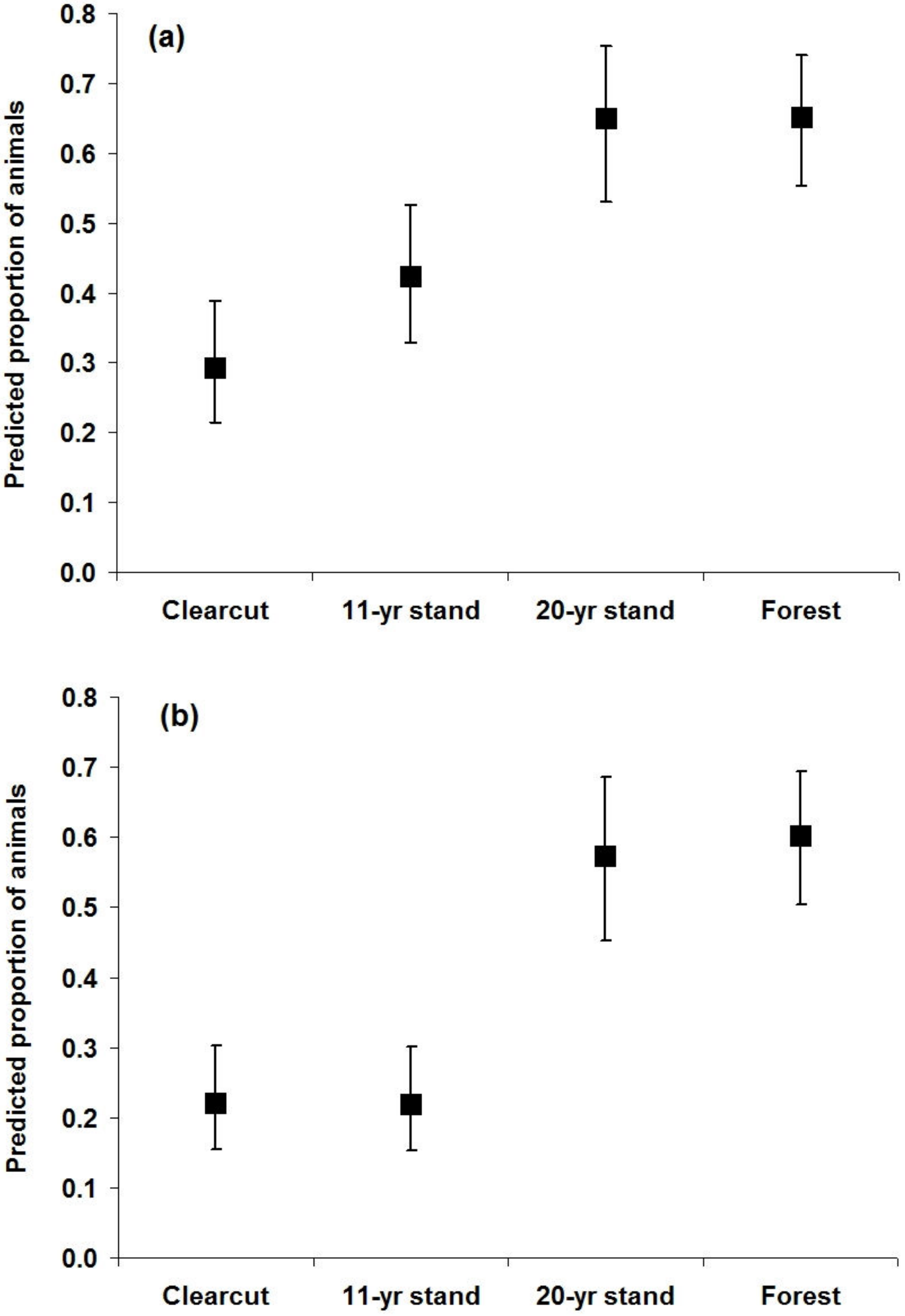


Figure 2.2. Predicted 95% confidence intervals around the mean proportions of juvenile *L. sylvaticus* reaching: (a) 10 m and (b) 20 m tracking stations (values are predicted proportions obtained by inverse logit-transforming the coefficients of the best binomial mixed effects model fitted for each distance).

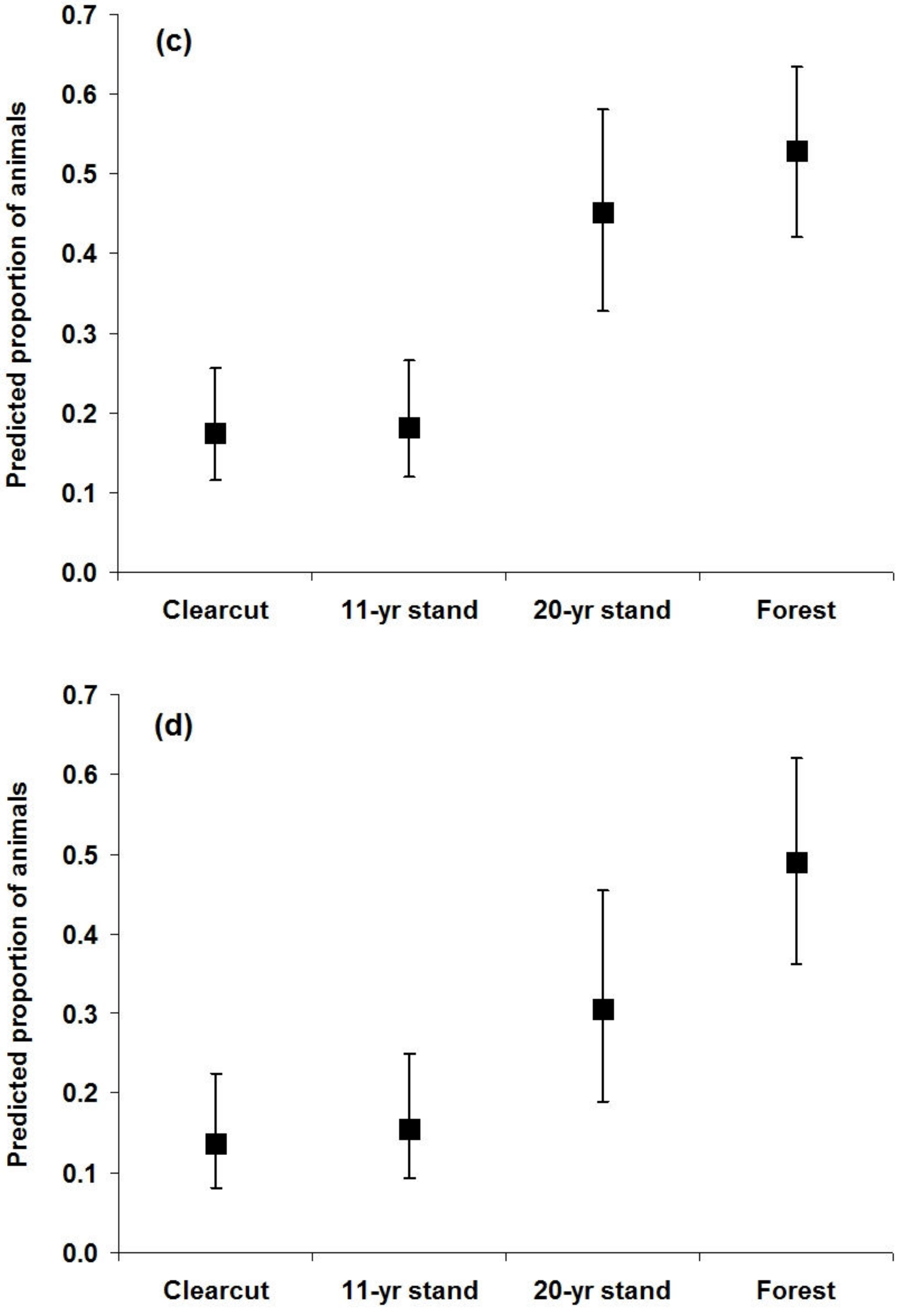


Figure 2.2 (continued). Predicted 95% confidence intervals around the mean proportions of juvenile *L. sylvaticus* reaching: (c) 30 m and (d) 40 m tracking stations (values are predicted proportions obtained by inverse logit-transforming the coefficients of the best binomial mixed effects model fitted for each distance).

treatments, with 29% and 31% captured at 40 and 50 m in 2008 and 2009, respectively (Table 2.1).

The proportion of captures at 10 m deserves special attention. In 2008, a significantly higher proportion of animals that passed through the first tracking station were captured in the clearcut (23%) and 11-yr stand (20%) than in the mature forest (5%) (Table 2.1). In 2009, the same pattern was observed (24% captures in clearcut, 18% in 11-yr stand and 11% in mature forest), but the pairwise tests for proportions did not yield statistically significant results (Table 2.1). The 20-yr stand had significantly lower captures at 10 m than all the other treatments (2%). This result suggests that juveniles starting to travel in the clearcut and 11-yr stand are able to assess potentially inhospitable habitat, and take the decision to return towards the forest. The number of captures at 20 and 30 m was very low (<5) for all treatments and years and not suitable for testing differences among treatments. Thus, once juveniles started to move away from the release point, and did not turn around to be captured at 10 m, they traveled the entire length of the runways regardless of forestry treatment.

The majority of captures occurred during the first three days post-release (96% in 2008 and 86% in 2009), suggesting that juveniles try to depart and move through all treatments relatively quickly. Only 3 and 11 animals spent >4 days in the runways in 2008 and 2009, respectively.

Movement rates and timing of movements

There were differences among the four treatments with respect to the rates of movement, as well as timing of movements post-release. In all treatments, the movement rates ranged between 5 and 50 m/day, but the omnibus Kruskal-Wallis test yielded significant differences among the four treatments (χ^2_1 = 10.471, p-value = 0.0135). Further, the pairwise comparisons revealed that: (1) there was no difference in the movement rate between the clearcut (median = 20 m/day) and 11-yr stand (median = 15.5 m/day) (χ^2_1 = 2.238, p-value = 0.138); (2) there was no difference between clearcut and mature forest (median = 17 m/day) (χ^2_1 = 0.829, p-value = 0.36), and (3) animals moved faster through mature forest and clearcut than through the 20-yr stand (median = 10 m/day) (χ^2_1 = 6.689, p-value = 0.008 and χ^2_1 = 4.248, p-value = 0.038, respectively) (result of juvenile behavior, rather than habitat permeability; see Discussion).

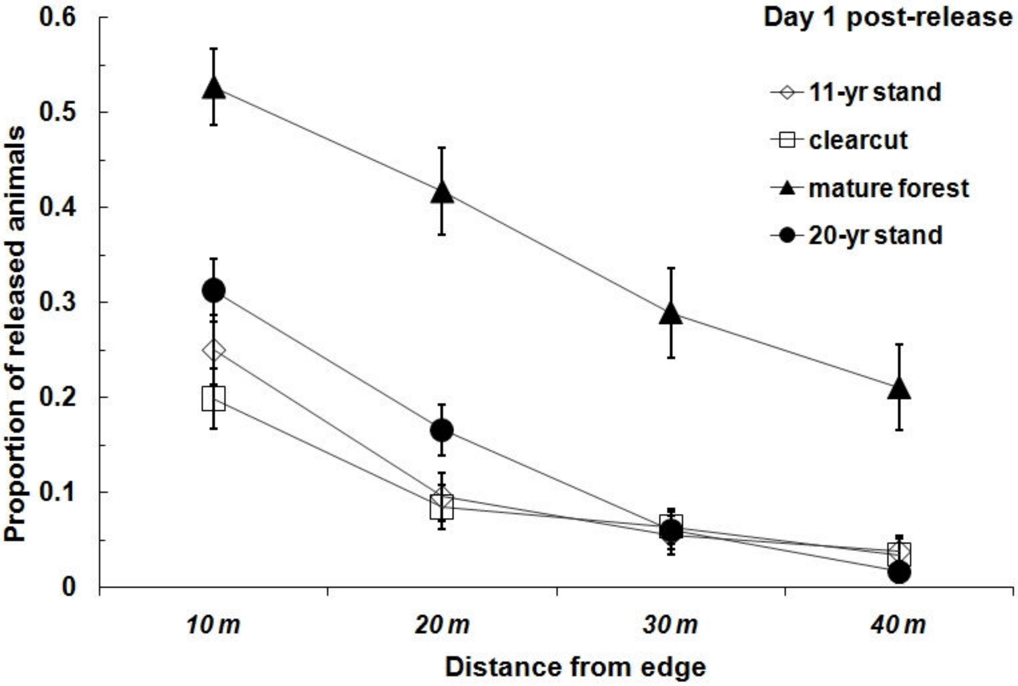
Table 2.1. Percentage of juvenile *L. sylvaticus* recaptured in the runways in 2008 and 2009. The superscript letters denote similarity or dissimilarity among treatments for each distance and year separately (along each row) resulting from pairwise tests for proportions. The number of captures at 20 and 30 m was very low in all treatments and data were not analyzed. Values at 10, 20, and 30 m represent percentage recaptured relative to the number of animals that reached that particular distance.

	Distance from edge (m)	**Clearcut**	**11-yr stand**	**20-yr stand**	**Mature forest**
2008					
Total # released		112	105	-	133
% of individuals recaptured	0	30^{B}	25^{AB}	-	19^{A}
	10	23^{B}	20^{B}	-	5^{A}
	20	13	3	-	0
	30	10	8	-	1
	40 and 50	11^{B}	7^{B}	-	29^{A}
2009					
Total # released		118	116	115	117
% of individuals recaptured	0	48^{B}	44^{B}	17^{A}	18^{A}
	10	24^{A}	18^{A}	3^{B}	11^{A}
	20	5	0	2	0
	30	8	2	0	6
	40 and 50	7^{B}	5^{B}	9^{B}	31^{A}

The timing of movements post-release reflected the choices of individuals to travel through the four treatments, and helps understanding how juvenile *L. sylvaticus* perceive differences in habitat quality. Most of the movement occurred within the first two days post-release (Figure 2.3a, b). In the mature forest, over 40% of the released individuals moved quickly past the second tracking station (20 m) during the first day, and 50% reached the end of the runway after four days. In the clearcut and 11-yr stand the bulk of movement also occurred during the first day, but only past the 10-m tracking station, and was notably lower than in the mature forest (20% and 25%, respectively, Figure 2.3a). Very few animals moved through these two treatments during the third and fourth days post-release, and only 13% and 16% of the total released actually reached 40 m after four days. Animals released in the 20-yr stand exhibited a different, more constant movement pattern. Although relatively few animals reached 10 m during the first day (30%), similar to the two younger treatments, substantial movements were recorded at 20 m during the second day and at 30 and 40 m during the third day (Figure 2.3b, c). On average, 40% of the animals reached 30 m during the four days post-release in this treatment.

Microhabitat and microclimate

The differences in habitat permeability among the four forestry treatments could be explained by differences in vegetation, ground cover, and microclimate. The mature forest and 20-yr stand had high canopy cover (90% and 78%, respectively), as well as extensive leaf litter ground cover (>90%, Table 2.2). Bare ground and moss together accounted for approximately 40% of ground cover in the clearcut and 11-yr stand. The



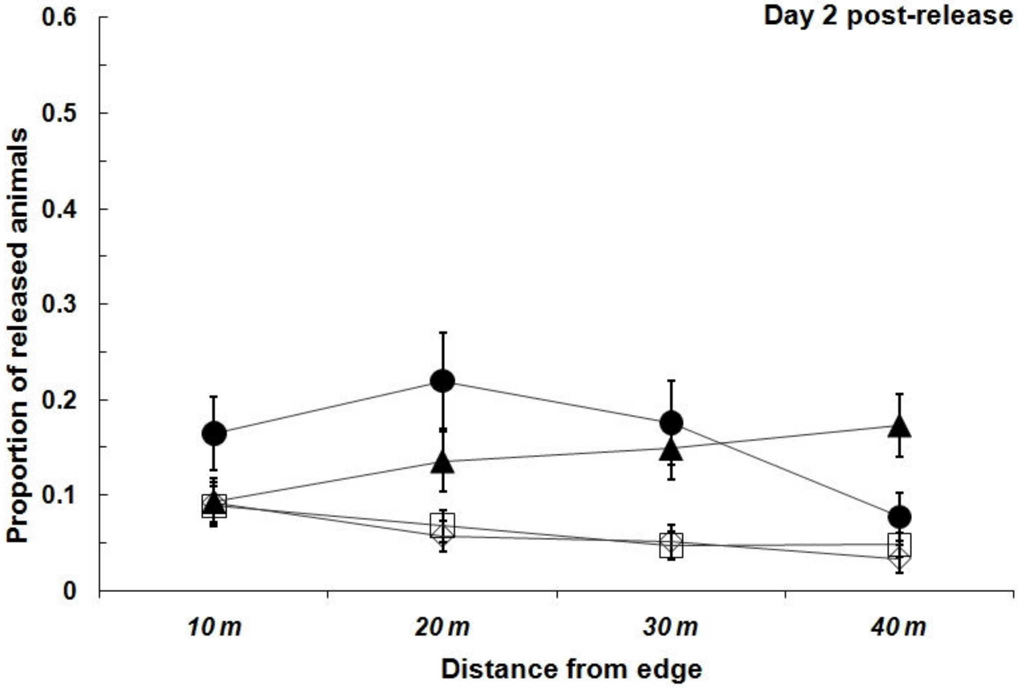
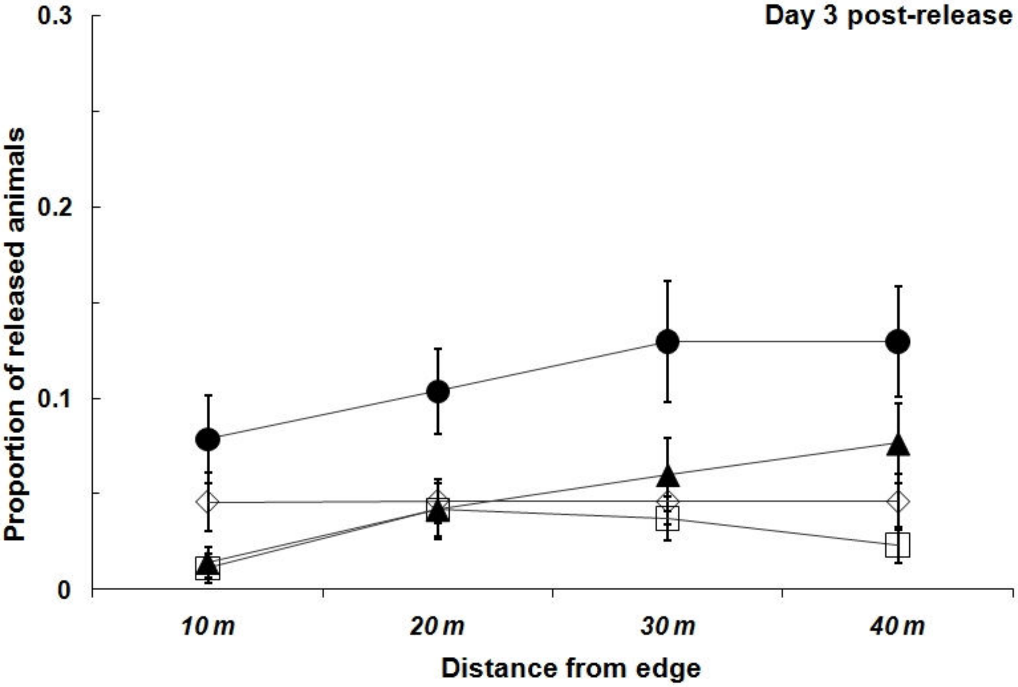


Figure 2.3. Timing of movements of juvenile *L. sylvaticus* released in four forestry treatments in 2008 and 2009. Values on the Y-axis are proportions of released individuals moving through the tracking stations averaged across runways and batches (error bars are 1 SE). I am showing the first 4 days post-release only, because these days accounted for most movement.



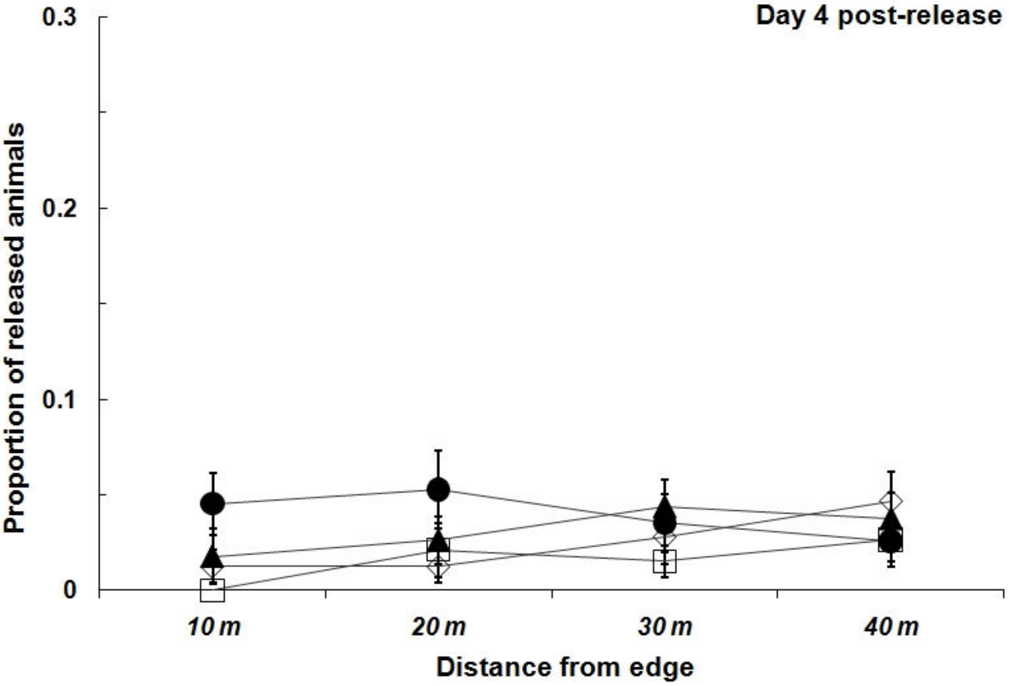


Figure 2.3 (continued).

herbaceous and low shrubs layers dominated the clearcut (65-70%), and to a lesser extent the 11-yr stand (≈25%, Table 2.2). Tree height varied from >15 m in the mature forest to 9.2 m in the 20-yr stand and 3.8 m in the 11-yr stand.

These differences in habitat structure were reflected in the microclimate of the four treatments. As I expected, the clearcut was warmer than the other treatments in both years (Table 2.3). The average daily maximum temperatures at ground level were higher (7.34 – 8.57°C in 2008, and 4.46 – 5.38°C in 2009) than in the older stands. In both years, the differences in average maximum temperatures between the mature forest and the 11 and 20-yr stands were minimal (0.9 – 1.2°C), suggesting that the shade provided by regeneration alleviates the microclimate near ground. The highest temperature at ground level was 39.1°C and was recorded on 16 July 2008 at 3:00 PM in the recent clearcut.

Table 2.2. Habitat characteristics of the four forestry treatments (mean ± 1SE).

Treatment	**Ground cover (%)**			**Cover of vegetation layers (%)**			**Stand density (stems /ha)**	**Tree height (m)**
	Leaf litter [a]	**Moss**	**Bare ground**	**Herba-ceous**	**Shrubs**	**Tree canopy cover** [b]		
Clearcut	0	14.3 ± 3	25.6 ± 4	55.0 ± 4	11 ± 3	0	0	0
11-yr stand	37.3 ± 6	30.5 ± 3	10 ± 2	14.0 ± 7	9.5 ± 3	69.5	5440	3.8
20-yr stand	92.6 ± 1	6.3 ± 1	0.3 ± 0	17.6 ± 5	0	77.7	9947	9.2
Mature forest	90.0 ± 2	10.0 ± 2	0	0	0	90.0	1120 [c]	>15

[a] for the *11-yr stand* I measured the percent ground covered with coniferous duff

[b] for the *11-yr stand* I measured the percent cover of conifer regeneration similar to the herbaceous and shrub layers rather than the canopy cover

[c] canopy trees only (in addition, the Mature forest contained 3573 stems/ha of balsam fir (*Abies balsamea*) seedlings and saplings in the understory)

Table 2.3. Microclimate of four forestry treatments in central Maine during experimental amphibian releases (July 9 – July 24 2008, and July 11 – August 6 2009). Relative humidity was recorded at ground level only (average ± 1SE).

	Average daily maximum temperature (°C)				**Relative humidity (%)**	
Treatment	**2008**		**2009**		**2008**	**2009**
	Ground level	**Refugia**	**Ground level**	**Refugia**	**Ground level**	
Clearcut	31.59	23.36	26.26	22.08	62.91 ± 6.3	86.33 ± 1.8
11-yr stand	24.26	19.79	21.79	17.81	76.14 ± 5.4	90.77 ± 1.7
20-yr stand			21.22	17.87		96.03 ± 1.1
Mature forest	23.02	18.65	20.88	17.02	78.70 ± 4.5	96.77 ± 0.9

The recent clearcut was also drier than the other treatments; daily minimum relative humidity varied between 27.23 and 100% (2008) and between 62.91 and 100% (2009). The quantity of precipitation that fell during the experimental releases was high (66 mm in 2008 and 76 mm in 2009), which places both study years above the long-term normal by 20% and 64%, respectively (*www.ncdc.noaa.gov*). However, in 2008, one rain event accounted for two thirds of the total rainfall (45 mm on 21 July).

Orientation of juveniles pre-release

I used a total of 87 juvenile *L. sylvaticus* to test the possibility that innate directionality might influence their propensity to move through the runways. I did not detect any departure from circular uniformity (Kuiper $V = 1.338$, p-value >0.15; Watson $U^2 = 0.073$, p-value >0.10), suggesting that the juveniles oriented randomly when released at night under closed canopy at >50 m from a forest edge (Figure 2.4). Hence, the direction of movement in the runways was apparently influenced by the juveniles' perception of experimental treatments only.

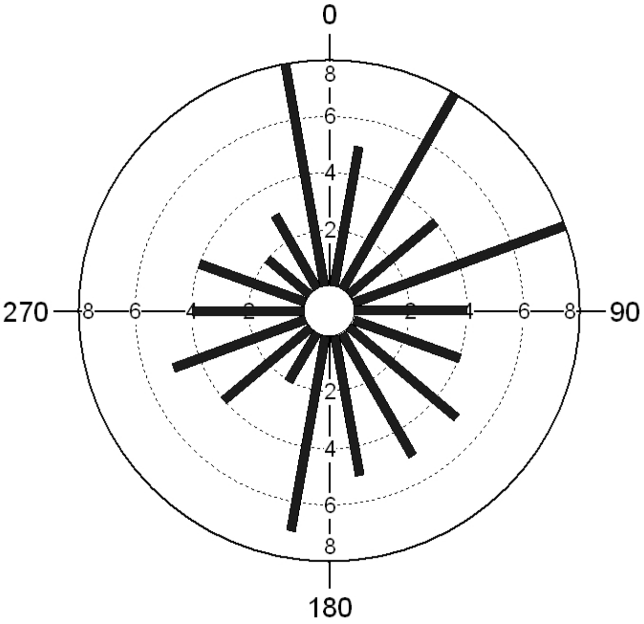


Figure 2.4. Orientation of juvenile *L. sylvaticus* (n = 89) prior to release in experimental dispersal runways in 2009. Bars represent number of animals reaching the edge of the circular arena. Scale is given by numbered concentric dotted circles.

Discussion

In this study I investigated the permeability to amphibian movements of a chronosequence of stands generated by even-aged silviculture. The greatest challenge in studying the movements of small organisms is being able to successfully track and detect them, while interfering as little as possible with their movements. For example, mark-recapture studies employing drift fences and pitfall traps are generally used for quantifying movements, but trapping interrupts animal movements and does not provide information on fine-scale behaviors. Fluorescent powder tracking is useful for small amphibians, but its effectiveness is highly dependent on weather conditions and substrate (Roe and Grayson 2008). In this respect, I consider that our “self-tracking” approach that combines the two techniques is preferable to other methods, because it allowed an in-depth exploration of juvenile amphibian movement ecology with minimal disturbance to their fine-scale movements.

Our study suggests that clearcuts are significant barriers, altering the movements of emigrating juveniles, and that the effects are long-lasting. The clearcut and 11-yr stand had lower permeability to movement than the older treatments. In the absence of subsequent silvicultural intervention (e.g., thinning, use of herbicides), the 20-yr stand was mature enough to provide good migratory or settling habitat for juvenile *L. sylvaticus*. While the low permeability of clearcuts or other open-canopy habitats to juvenile amphibian movement has been previously demonstrated (Rothermel and Semlitsch 2002, Rothermel 2004), our findings for the 11-yr stand deserve further discussion.

During both years of the study, the 11-yr stand had the same low permeability to movements as the clearcut (approximately 3.1 – 3.7 times less permeable than mature forest). Overall, a larger proportion of animals reached the 10-m tracking station compared to the clearcut, indicating that the 11-yr stand facilitated initial movements. However, the proportion of animals reaching >20 m from the forest edge dropped to a level similar to that of the clearcut. This is surprising given this treatment's microclimatic similarities to the mature forest and 20-yr stand. Despite microclimate similarities, there were striking differences in vegetation cover between this and the two older habitats, pertaining mainly to the presence of canopy cover and percentage leaf litter (<30% versus >90% in older habitats, Table 2.2). In southern Quebec, Canada, Aubin et al. (2008) characterized young coniferous plantation stands (of similar age and structure to our 11-yr stand) as open-canopy habitats, with understory physical and biological attributes radically different from natural regeneration stands. Although vegetative succession mitigates for microclimate, our results suggest that other physical factors, such as canopy cover (and the correlated percent leaf litter) exert a larger influence on the movement behavior of juveniles than favorable microclimate. Hence, actions aimed at microclimate mitigation in harvested stands (i.e., by providing refugia such as burrows and coarse woody debris) might not be successful if juveniles actively avoid such sites based on the lack of canopy cover (Patrick et al. 2006, Popescu V.D., unpublished data).

The forestry conditions studied here resulted from even-aged management. The trajectory of stand development for even-aged stands (i.e., spatial pattern, tree species mixing, and tree size differentiation) is quite predictable and yields comparable stand structures across the Acadian Forest region (Saunders and Wagner 2008). Our study sites were in stand initiation (clearcut), stem exclusion (11-yr and 20-yr stands), or understory reinitiation (mature forest) stages (sensu Oliver and Larson 1996). While there is natural site-to-site variation, these stand-level processes and disturbance regimes lead to a clear differentiation between naturally-regenerated stands treated and not treated with herbicides, and conifer plantations (Newton et al. 1992, Aubin et al. 2008). Studies quantifying differences in physical and biological attributes of stands resulting from even-aged management found vegetation structure comparable to our 11-yr and 20-yr stands throughout the Acadian Forest region (Newton et al. 1992, Ross-Davis and Frego 2002, Ramovs and Roberts 2003). Additionally, because I studied silvicultural practices that are common throughout North America (i.e., clearcutting) our results may be applicable elsewhere within the species' range, outside the Acadian Forest.

Abrupt changes in habitat resulting from even-aged silviculture negatively influence the abundance of amphibians, and the negative impacts of altered microclimate and microhabitat extend well into the unharvested forest (deMaynadier and Hunter 1998). The rates of movements and proportion of captures were strongly influenced by both habitat quality and proximity to edge. In the clearcut and 11-yr stand, the juveniles that chose to move away from the forest edge moved quickly, presumably in response to being exposed to poor-quality habitat, consistent with the evacuation hypothesis (Semlitsch et al. 2008). Furthermore, the difference in timing of movements between the 20-yr stand and mature forest might be due to adjacency to different quality habitats: (1) animals in the mature forest moved quickly away from the mature forest/clearcut edge, showing an active avoidance of clearcuts and edges (deMaynadier and Hunter 1998, Gibbs 1998), and (2) animals in the 20-yr stand moved away from the 20-yr stand/mature forest edge relatively slowly and tended to settle (Patrick et al. 2008). Thus, the differences in the proportion of animals captured at 40-50 m (i.e., successful dispersers) between these two treatments are probably not due to permeability *per se*, but to edge-related movement decisions. Settling behavior was also observed in the mature forest (lower number of individuals passing through the 40 m compared to the 20 and 30 m tracking stations). No settling behavior was observed in the clearcut and 11-yr stand. Released animals that were not recaptured either settled in the mature forest and the 20-yr stand or died due to predation or desiccation. Predation is likely to be higher in the open-canopy habitats, and I recorded garter snakes (*Thamnophis sirtalis*) in runways in both the clearcut and 11-yr stand. Notably, some juvenile *L. sylvaticus* traveled 50 m per night through all four treatments, sometimes in no-rain conditions, suggesting that the vegetation and substrate of our experimental treatments did not represent physical resistance to locomotion.

Permeability of clearcuts to amphibian movements has been found to be highly dependent on climate parameters such as rainfall or high temperatures (Chan-McLeod 2003, Graeter et al. 2008, Veysey et al. 2009). During our study, the maximum temperatures at ground level did not reach the critical thermal maximum for *L. sylvaticus* of 34.8°C (Brattstrom 1963) in any treatment, and the existing refugia provided a cool, moist microclimate throughout the study period (Table 2.3). However, I found that the study clearcut and 11-year-old coniferous regeneration have limited permeability to juvenile *L. sylvaticus* even in wet conditions. In both years, I released animals in both "wet" (rain during the first two days post release) and "dry" conditions, but the rainfall did not have a large impact on the movement patterns. For example, in 2009, the third release (22 July) coincided with two heavy rain events (≥30 mm on 22 July and 25 July), but juveniles in the clearcut and 11-yr stand did not show a higher propensity to move in these treatments compared to the other releases. Only two animals (7%) reached 40 m in the 11-yr stand and three (10%) in the clearcut during this release, which was similar to batches that completely lacked rain: four (13%) and two (7%) animals, respectively, during the fifth release (2 August).

Dispersal plays a critical role in the ecology and biology of many species of amphibians. Dispersers are able to colonize new breeding habitats, recolonize pools following extinction, and affect gene flow. For *L. sylvaticus*, contemporary metapopulation processes drive population dynamics and maintain high genetic diversity in fragmented landscapes (Zellmer and Knowles 2009). Predicting the process of animal movement and its implications for population or metapopulation dynamics cannot rely solely on assessing habitat cover and landscape configuration (Winfree et al. 2005), although this overly simplified approach has been applied in previous studies of amphibians (see for example, Stevens et al. 2005). Dispersal in heterogeneous landscapes involves an interaction between habitat structure and configuration (such as various aged clearcuts in an industrial forest landscape), and behavioral responses of individuals to these structures (Ricketts 2001). Our results suggest that decision behavior (in our case, willingness to travel and avoidance of clearcuts, which dispersers perceive as inhospitable habitat), is more important than the effect of the physical structure on locomotion.

Pond-breeding amphibians are able to recognize and preferentially use suitable habitat, and avoid less optimal habitat at all life-stages. In large-scale experiments on the effects of forestry practices on amphibian communities, adult ambystomatid salamanders (*Ambystoma maculatum, A. opacum*), frogs (*Lithobates* spp., *Pseudacris ornata, Scaphiopus holbrooki*), and southern toads (*Anaxyrus terrestris*) preferentially used closed-canopy habitats (uncut forest and light partial cuts) during their post-breeding migrations (Patrick et al. 2006, Todd et al. 2009, Semlitsch et al. 2009). Similarly, adult European common frogs (*Rana temporaria*) actively avoided agricultural lands and moved preferentially through hedgerows and meadows (Vos et al. 2007). Juveniles of spotted salamanders (*A. maculatum*) and American toads (*Anaxyrus americanus*), are able to recognize and preferentially use suitable habitat, and avoid less optimal old-field habitat (Rothermel and Semlitsch 2002). Our observations on the behavioral response of juvenile *L. sylvaticus* add to this body of evidence. Almost 30% of the juveniles released in the clearcut and 11-yr stand in 2008, and 50% in 2009 actively avoided these habitats by immediately returning towards the adjacent forest. Moreover, of the individuals that decided to move away from the forest in these two treatments, a large proportion was captured at 10 m from the release point (20 – 25%). However, this pattern was not observed at 20 and 30 m from the release point, suggesting that juveniles that decided to move traveled the entire length of the runway. Juvenile amphibians are likely to use proximate orientation cues, such as olfactory or visual cues, and presumably have a limited range of perception given the interaction between understory vegetation and weather conditions (i.e., rainy nights) that usually characterize amphibian migrations (Semlitsch 1985). Support for this idea comes from the study of Rothermel (2004) who found that juvenile salamanders and anurans failed to orient towards the nearest forest when placed 50 m from the forest edge in an open field.

The concept of habitat permeability offers a practical way to predict population dynamics and set conservation priorities across broad spatial scales using Geographic Information Systems (Compton et al. 2007, Baldwin and deMaynadier 2009). In a recent study, Janin et al. (2009) introduced a new method for assessing landscape permeability for the natterjack toad (*Bufo calamita*) using a calibration/validation method that yielded improved permeability measures that did not require much biological data (compared to the expert-based values). While such exercises have the potential to offer valuable results for conservation planning, our simple forestry-oriented experiment suggests a cautious approach to assigning expert-based permeability (resistance) values to various habitat types. Differing silvicultural management practices lead to different outcomes in terms of vegetative succession, which may affect habitat permeability in the long term. This poses problems for modeling exercises that rely on land cover or habitat maps derived from aerial or satellite imagery. First, the identification of forest successional stages using satellite imagery is difficult (Liu et al. 2008). Second, given the dynamic nature of forest succession, it is necessary to minimize the time lag between acquisition of spatial data and its analysis (Popescu and Gibbs 2009). Moreover, the type of management subsequent to harvesting cannot be extracted from land cover or habitat maps. Without such information, the permeability values assigned to forests are likely to be overestimated.

Our research was limited by the fact that I had no stands with natural regeneration of intermediate age (the 11-yr stand had been sprayed with herbicides to favor conifers and resembled a coniferous plantation). I do have evidence that the avoidance behavior observed in the clearcut and 11-yr stand persisted up to six years post-clearcutting, even when natural vegetative succession was allowed (Popescu V.D., unpublished data). The second limitation is the scale at which I conducted the study. Given that animals reached the end of the runways in one night, I underestimated the true dispersal abilities of juvenile *L. sylvaticus* by constraining their maximum movements to 50 m. Also, permeability of clearcuts might be slightly overestimated due to the shade provided by the enclosure walls (Patrick et al. 2008).

Our finding of active avoidance of open-canopy habitat, as well as habitats where the successional vegetation mitigates for microclimate effects, but lacks canopy closure, suggest that silvicultural practices that retain greater canopy cover (i.e., partial harvests) are less likely to inhibit juvenile dispersal than clearcutting. Evidence from large-scale experiments on the effects of various forestry practices on amphibian movements, replicated across three ecoregions support this finding, and partial harvests that retain at least 50% of canopy cover are as permeable to migrating amphibians as uncut forests (Semlitsch et al. 2009). Coniferous plantations have been found to negatively affect amphibian abundance and species richness (Pough et al. 1987, Waldick et al. 1999, Parris and Lindenmayer 2004) and the strong avoidance of the 11-yr coniferous stand during two successive years in our study corroborates these results. Whether or not amphibians exhibit metapopulation dynamics (Smith and Green 2005), extinction and recolonization are landscape-level processes common to many amphibians. Because recolonization of a breeding site is in part the realization of dispersal processes, highly fragmented habitats that have low permeability to movements may hinder recolonization success (Richter-Boix et al. 2007).

The lack of directionality and the preference for closed-canopy habitats observed in this study suggest that in heavily forested landscapes activities that lead to habitat alteration or loss (e.g., timber harvesting, development) need to pay attention to the spatial arrangement of potential breeding sites. Thus, movements of dispersing amphibians might be directed by retaining a certain level of canopy cover between identified high-quality breeding sites (Baldwin and deMaynadier 2009). This management strategy would also be beneficial for the local populations that generally require high-quality habitat for foraging and overwintering in the immediate vicinity of the breeding pool.

Movement ecology of amphibians is taxon-specific owing to differences in vagility, vulnerability to desiccation, and habitat preferences. Thus, empirical investigations of habitat permeability for other forest-associated species are warranted.

Focusing on the dispersal life stage of various species is likely to give a better understanding of regional population or metapopulation dynamics (Smith and Green 2005). Investigations on the amount of canopy cover retained during partial cuts that will allow for successful amphibian dispersal could also be fruitful. Because I only used recently metamorphosed animals for our experiments, I am uncertain how movement behavior might change during ontogenetic development, and testing habitat permeability for older/larger individuals should be a priority. Furthermore, a better grasp on the cues that dispersing amphibians rely on for orienting in natural forested landscapes would aid our understanding of movement behavior in fragmented urbanizing landscapes and better inform land-use planning.

Chapter 3

USE OF OLFACTORY CUES BY NEWLY METAMORPHOSED WOOD FROGS (*LITHOBATES SYLVATICUS*) DURING EMIGRATION

Abstract

Juvenile amphibians are capable of long-distance upland movements, yet cues used for orientation during upland movements are poorly understood. We used newly metamorphosed wood frogs (*Lithobates sylvaticus*) to investigate: (1) the existence of innate (i.e., inherited) directionality, and (2) the use of chemical olfactory cues, specifically forested wetland and natal pond cues during emigration. In a circular arena experiment, animals with assumed innate directionality did not show a departure from randomness when deprived of visual and olfactory cues, suggesting that juveniles from two different landscape settings in Maine (USA) most likely rely on proximate cues for orientation. Juvenile wood frogs reared in semi-natural conditions (1500-l cattle tanks) showed a strong avoidance of forested wetland cues in two different experimental settings, although they had not been previously exposed to such cues. This finding is contrary to known habitat use by adult wood frogs during summer. Juvenile wood frogs were indifferent to the chemical signature of natal pond (cattle tank) water. Our findings suggest that management strategies for forest amphibians should consider key habitat features that potentially influence the orientation of juveniles during emigration movements, as well as adult behavior.

Introduction

Understanding the movements of migrants and dispersers and identifying habitats selected during the movements to and from breeding ponds is critical for identifying conservation strategies for pond-breeding amphibians Semlitsch (2008). Orientation and navigation play an important role in guiding amphibian movements in both the aquatic and terrestrial environments (see reviews by Sinsch 1991, 2006, Rozhok 2008). Amphibians rely on a multisensory orientation system (Ferguson 1971) that uses a wide variety of mechanisms, including path integration, beaconing, pilotage, compass orientation, and true navigation (Papi 2006). Orientation during the transition between the aquatic and terrestrial stages (i.e., immediately post-metamorphosis) raises particularly interesting questions because of the change in locomotion, the availability of cues, as well as the potential carryover of sources of information between the larval and juvenile life stages (Hepper and Waldman 1992). For example, orientation information acquired during the larval stage was found to influence movements during the onset of emigration in the terrestrial environment (Goodyear and Altig 1971).

Migratory movements of adult amphibians towards breeding ponds and overwintering sites are well understood and have been shown to rely on a multitude of cues that are used in a hierarchical manner depending on the relative availability of cues (Sinsch 1990, Dall'antonia and Sinsch 2001). Olfaction plays an important part in adult orientation, with odors being used as directional cues for both short-distance orientation towards mates and long-distance homeward orientation (Tracy and Dole 1969, McGregor and Teska 1989). Adult breeding migrations were found to be guided by the chemical signature of the breeding ponds in both anurans (Grubb 1973, Forester and Wisnieski

1991, Sanuy and Joly 2009) and urodeles (Hershey and Forester 1980, Joly and Miaud 1993).

In contrast to the adult life stage, juvenile movements and orientation remain largely unstudied, despite their long-distance emigration and dispersal abilities (Cushman 2006). Migrating juveniles are hypothesized to have a narrow perception range and consequently rely on proximate cues for orientation in the upland habitat (Rothermel 2004, Popescu and Hunter in press). Because natal experience is linked to habitat selection at later life stages (Davis and Stamps 2004), the ability to recognize the chemical signature of the natal pond is thought to play an prominent role for juvenile orientation during emigration movements (Hepper and Waldman 1992, Ogurtsov 2004). The behavioral responses to natal pond odors are species-specific (i.e., reflect the species' affinity to aquatic or terrestrial environments), change during the ontogenetic development, and exhibit high individual variability (Bastakov 1986, Reshetnikov 1996, Shakhparonov and Ogurtsov 2003, Arhipova et al. 2005). The imprinting of specific odors in anurans can occur during the embryonic stage, as well as during two distinct stages of larval development (Gosner 19 – 21 and 31 – 41, Gosner 1960), and the discrimination between imprinted and novel odors during the aquatic stage is maintained during the early terrestrial stage (Hepper and Waldman 1992, Ogurtsov and Bastakov 2001).

Despite the strong evidence for the use of breeding/natal pond odors for orientation, little is known about the use of other olfactory cues during movements in the terrestrial environment. For the juvenile stage of our study organism, the vernal pool-breeding wood frog (*Lithobates sylvaticus*), previous research conducted in Maine, USA found evidence that forested wetlands attracted a substantial segment of the local juvenile cohort, and that directionality during emigration movements might be genetically inherited (Vasconcelos and Calhoun 2004, Patrick et al. 2007). The attraction to forested wetlands has conservation implications because forested wetlands are a scarce resource in some landscapes. Also, anthropogenic changes in land use have the potential to affect populations in which directionality of emigration movements is inherited by attracting migrants into ecological traps (*sensu* Schlaepfer et al. 2002). However, in other landscapes, the emigration of juvenile *L. sylvaticus* amphibians was either completely random (V. D. Popescu, unpublished data), or random across seasons and sites, but with evidence of local directionality that shifted on a seasonal basis (Timm et al. 2007, Homan et al. 2010). The initial orientation at pond edge does not necessarily reflect directionality farther in upland habitat (Miaud et al. 2003, Jenkins et al. 2006, Roznik and Johnson 2009); that is, it might be wrong to assume that habitat selected during upland emigration movements is consistent with orientation at the pond edge.

Given the conflicting body of evidence on the orientation of post-metamorphic *L. sylvaticus* and the need to better understand the orientation behaviors during the transition between the aquatic and terrestrial environments, the objectives of this study were: (1) to investigate whether or not newly metamorphosed *L. sylvaticus* possess innate directionality, and (2) to evaluate the use of olfactory cues during emigration movements. For the first objective, I hypothesized that if animals posses innate directionality, unrelated to the landscape context, then eliminating potential cues, such as visual (i.e., celestial, landmarks), and chemical cues (i.e., olfactory), would not affect their preferred direction of movement. For the second objective, I examined the role of forested wetland

and natal pond odors. Based on current literature, our hypotheses were: (1) juveniles would be attracted to forested wetland olfactory cues, and (2) juveniles would be able to recognize their natal pond odor.

Materials and methods

Amphibian collection

I collected egg masses from roadside ditches and other small, ephemeral pools located on the University of Maine forests in central Maine, USA (44°54’16” lat N; 68°41’55” long W). *L. sylvaticus* larvae were hatched in plastic wading pools, and reared to metamorphosis in 1500-liter self-sustaining mesocosms (cattle tanks), stocked with 1 kg of dried leaf litter, and plankton from nearby vernal pools (Kiesecker and Skelly 2001). The tanks were covered with shade cloth to prevent colonization of other aquatic organisms and placed under closed canopy; water level was maintained naturally by precipitation (Popescu and Hunter in press). For the experiment pertaining to the second objective, I also collected early stage tadpoles (Gosner 25-30, Gosner 1960) from two artificial pools on Sears Island, Maine (44°26’36” lat N; 68°53’20” long W) (Vasconcelos and Calhoun 2004), where innate directionality upon emergence was found to occur in juveniles *L. sylvaticus* (Patrick et al. 2007). Tadpoles were also added and reared to metamorphosis in cattle tanks. When the animals reached the final stage of metamorphosis (Gosner stage 46), they were transferred to plastic containers with moist leaf litter (in 2009) or moist paper towels (in 2010) for two days prior to the beginning of the experiments. Animal were returned to the site of the egg mass collection at the end of the experiments.

Circular arena design

This experimental setup was used to investigate whether or not juveniles posses innate directionality, as well as to obtain preliminary data on the use of olfactory cues during emigration. The experimental site was located on the University of Maine forests, in a 100 x 50 m clearing, where I erected a large canvas tent (6 x 4 m and 2.8 m in height). All sides of the tent were opened during the day to ensure air flow. I performed the experiment at night (starting at 21:30 EST) between 14 July and 3 August 2009. The tent eliminated the potential use of visual cues, such as celestial [Diego-Rasilla and Luengo 2002], and landmarks (i.e., forest edges [Gibbs 1998, Rothermel 2004, Popescu and Hunter in press]), and prevented the inflow of external airborne chemical cues. The test arena was a circular plastic container 1.8 m in diameter and 20 cm tall. Inside the arena, I marked a 1.5 m diameter circle at 10° intervals, which I used as the threshold for recording the direction of movement. I placed each animal under an opaque plastic container in the middle of the arena; I lifted the container after a 2-minute accommodation period using a pulley system and recorded the bearing (departure from magnetic North) of where it crossed the 1.5 m circle for each individual. Because I conducted the experiment in complete darkness and did not want to influence frog behavior I used night-vision binoculars (Rigel Optics, DeWitt, Iowa) from approximately 3 m outside the arena (Popescu and Hunter in press). The floor of the arena was wiped with paper towels and distilled water and allowed to dry between each trial in order to eliminate interference from odor trails left by previous animals (Adler 1980, Diego-Rasilla and Luengo 2002).

Circular arena experimental releases

First, I tested whether or not newly metamorphosed *L. sylvaticus* show innate directionality at emergence. I contrasted animals with assumed innate directionality from restored ponds on Sears Island (Patrick et al. 2007) and animals from University of Maine forests where they are known to be exiting without evidence of directionality (V. D. Popescu, unpublished data). The animals with innate directionality were hypothesized to orient towards the northeast (approximately 41 – 71° departure from magnetic North), in the direction of a forested wetland. Second, I conducted a preliminary test of the use of olfactory cues by juvenile wood frogs, specifically forested wetland cues, using animals from the second (random orientation) set of pools only. Previous research suggested that juvenile amphibians are attracted by forested wetlands (Vasconcelos and Calhoun 2004, Patrick et al. 2007), so the chemical signature would act as an attractant. The protocol was similar to the previous experiment, but I added a small tub (40 x 25 x 20 cm) filled with forested wetland organic soil adjacent to the circular arena, at a random bearing (220°). I then repeated the experiment with the tub located at 130° and 40° (a 90° and 180° shift from the initial location).

Y-maze experimental design

Following the preliminary investigation of the use of olfactory cues, I performed an olfactory dichotomous choice experiment. I built a Y-maze from clear acrylic tubing (5.7 cm in diameter), and a 3-way PVC connector (120° between each connection), with each arm 30 cm long (Figure 3.1). To avoid mixing of the cues before the main (releasing) arm of the olfactometer, I inserted a 5-cm long separating wall inside the connector (D in Figure 3.1) between the two arms receiving the cues.

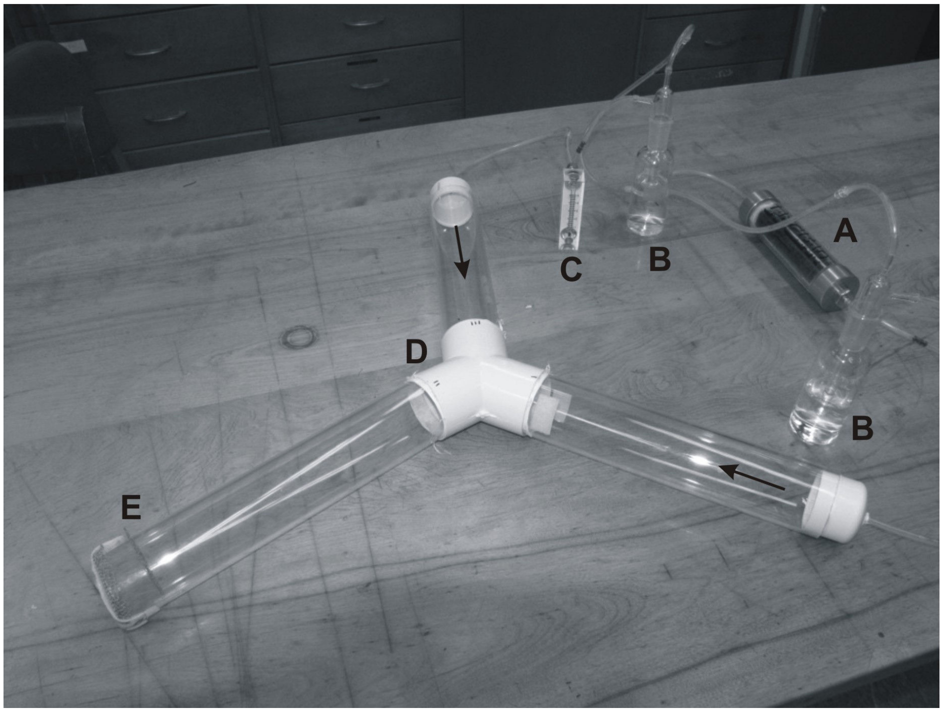


Figure 3.1. Design of the Y-maze used to test the use of olfactory cues by juvenile wood frogs (*L. sylvaticus*): (A) activated carbon filter, (B) bubbling flasks containing the olfactory cues to be tested, (C) flowmeter, (D) PVC connector, and (E) restraining wire mesh cage at the end of the arm receiving the cue airflow, used to acclimate animals prior to release; arrows indicate the direction of airflow.

I used two olfactory cues: (1) forested wetland organic soil, and (2) natal pool (cattle tank) water. I tested these cues against distilled water, a control neutral cue. I pumped ambient air into 150 ml glass bubble flasks which contained either 100 ml of testing cue or distilled water. The forested wetland cue was prepared by mixing 60 ml of saturated organic soil with 40 ml of distilled water to bring it to a consistency that allowed bubbling. The natal pool cue was cattle tank water collected 1 hour prior to the beginning of the experiment. Because I used ambient air, I attached an activated carbon filter (clear PVC pipe, 25 cm in length and 5 cm in diameter filled with 4-6 mm activated carbon pellets) to remove impurities and eliminate ambient odors (Figure 3.1). Air flow was maintained constant at 4 l/minute, measured with a Dwyer VFA-22 flowmeter (Dwyer Instruments, Inc., Michigan City, Indiana). The Y-maze was rinsed with distilled water and dried between the trials to eliminate both odors from previous animals and traces of cue (Forester and Wisnieski 1991). I randomly switched the treatment cue and the control between the left and right arms of the Y-maze to eliminate potential directional bias induced by the experimental setup itself. I also replaced the cues for every other trial in order to maintain a fresh source of odors.

Y-maze experimental releases

I conducted the experiment in low, diffuse overhead red light oriented away from the setup, during 21 – 26 July 2010, between 8:00 AM and 12:00 PM EST. After a 2-minute acclimation period, I lifted a wire mesh gate (E in Figure 3.1), and animals were allowed to move freely. An animal was considered to exhibit a preference once it moved more than halfway into one of the cue arms (Forester and Wisnieski 1991). Based on our experience with the circular arena experiment, animals that did not reach the end of one of the arms in <10 minutes (n = 6) were not counted as successful trials and were discarded from the analysis. I conducted the experiment at constant temperature (22°C) and relative humidity (80%). For all experiments, each animal was used only once.

Statistical analyses

For the circular arena trials, I used circular statistics (Batschelet 1981) to investigate whether or not juveniles exhibit innate directionality. I used Oriana 3.13 (Kovach Computing Services, Anglesey, Wales, UK) to run separate Rayleigh tests for uniformity for animals with and without hypothesized innate directionality to test whether or not there is significant departure from randomness. I also examined the circular variance for each treatment as a measure of spread of individual bearings. The circular variance takes value between 0 (clustered around a mean angle) and 1 (dispersed with respect the mean angle) (Batschelet 1981).

For the preliminary investigation of the use of olfactory cues in the circular arena, I chose not to use circular statistics, because two of the possible outcomes of the experiment -- attraction and avoidance -- are not symmetrical processes (Haila et al. 1996). Attraction, revealed by concentration of animals around the cue, is a strongly deterministic process (Haila et al. 1996) for which the use of circular statistics would be warranted. However, avoidance is likely to lead to a random distribution (i.e., not necessarily concentrated in the opposite direction of the cue) because avoidance is not a deterministic process and leaves a large margin of variation (Haila et al. 1996). Therefore, I used the bearing departure from the location of the cue (clockwise and counterclockwise), which ranged between 0° (exact location of cue) and 180° (opposite location of cue) to assess the concentration of animals within ±30, 45, 60, and 90° from the cue location. I then used a G-test for goodness of fit to test whether or not the number of individuals observed within 60, 90, 120 and 180° arcs centered on the cue, respectively, was significantly different than expected under the assumption of randomness. I pooled the bearing-departure-from-cue data for all three cue locations.

For the Y-maze experiment, I used a G-test of goodness of fit, under the assumption of equal preference of treatment cues and control. I ran separate tests for (1) forested wetland organic soil cue vs. distilled water, and (2) natal pool vs. distilled water. For all tests, I used a significance level of alpha = 0.05.

Results

Circular arena experiments

In the innate directionality experiment, both groups of juvenile wood frogs oriented at random and did not show a departure from circular uniformity; for frogs that originated from the pools where innate directionality was assumed (Patrick et al. 2007), n = 40, Rayleigh test; $Z = 1.465$, p-value = 0.232; for animals from the control, non-directional pools, n = 57, Rayleigh test; $Z = 0.267$, p-value = 0.766 (Figure 3.2). Because the orientation of both groups of animals was random, I was not able to test whether or not they shared a mean direction. The spread of bearings with regard to the mean direction was large for both trials, as expressed by the high values of the circular variance: 0.809 for animals with assumed innate directionality, 0.932 for control animals.

In the same experimental setup, the forested wetland olfactory cues acted as a deterrent, rather than as an attractant for juvenile *L. sylvaticus*. For all three locations of the wetland cue: 220° (n = 29), 130° (n = 24), and 40° (n = 28), animals tended to move away from the forested wetland cue although this pattern was statistically significant only for 220° (Figure 3.3). When all three locations were pooled, significantly fewer individuals than expected reached the edge of the arena within 60° (n = 5; *G* = 4.056, 1 d.f., p-value = 0.044), 90° (n = 7; *G* = 6.724, p-value = 0.009), and 120° arcs (n = 11; *G* = 6.952, p-value = 0.008) centered on the cue (Figure 3.4). A smaller than expected number of animals also reached the edge of the orientation arena in the half-circle centered on the cue (180° arc), but the results were not significant at α = 0.05 (n = 26; *G* = 3.187, p-value = 0.074). In addition, I observed 12 individuals that initially started moving in the general direction of the forested wetland cue, but switched direction when they were approximately 50 cm from the cue, thus appearing to show active avoidance.

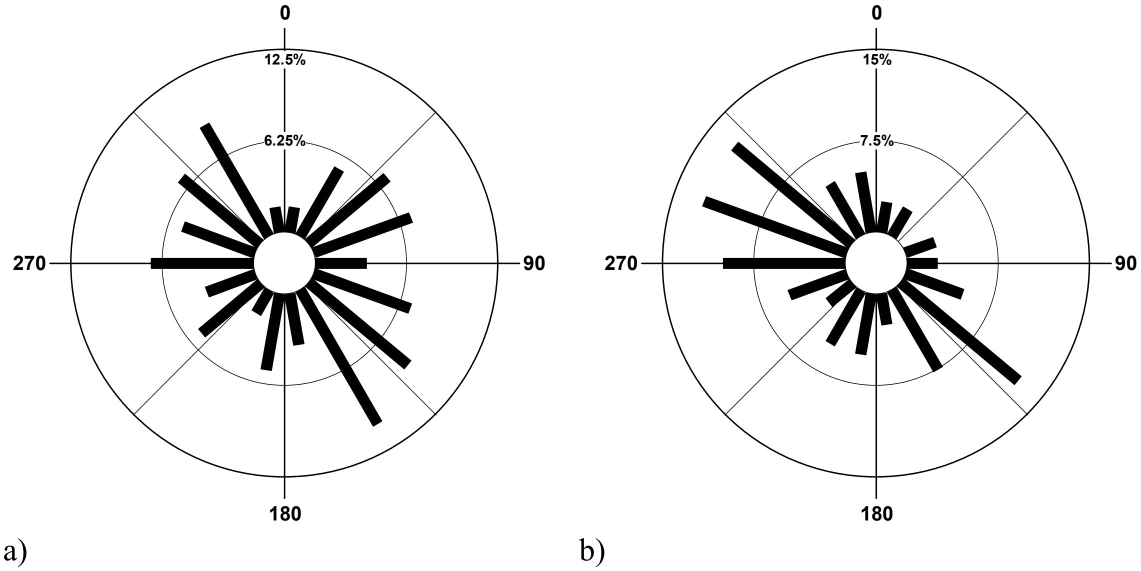


Figure 3.2. Orientation of juveniles (a) without, and (b) with assumed innate directionality (northeast [41 – 71°], Patrick et al. 2007).

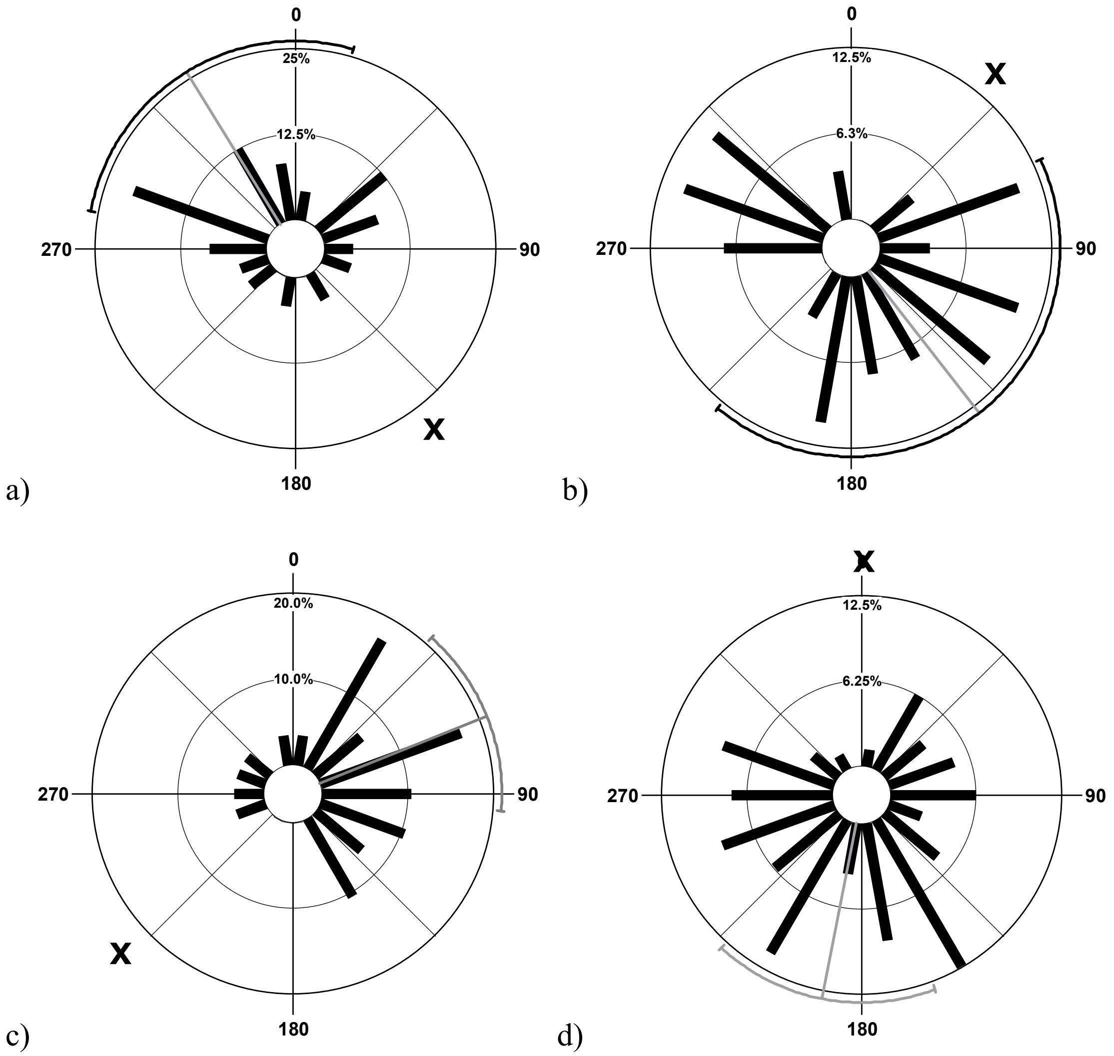


Figure 3.3. Orientation of juvenile wood frogs exposed to forested wetland olfactory cues in three circular arena trials with different locations of the cue source: (a) 130°, (b) 40°, (c) 220°, and d) all trials combined and standardized to cue = 0° bearing. Trial (c) showed the only significant departure from randomness, in the direction opposite to the cue location. (**X** = location of cue).

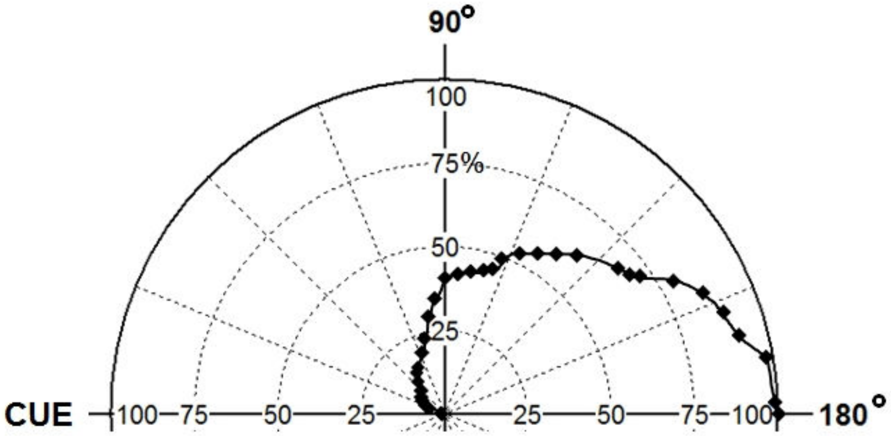


Figure 3.4. Cumulative percentage of juvenile wood frogs (n = 81) orienting with respect to forested wetland cues in a circular arena during three trials. The circular axis shows the departure from cue location (angles 0 – 180°, where 180° represents the opposite side of cue location), and the linear axes show the cumulative percentage of animals (0 – 100%); (i.e., approx. 10% of animals were within 45° of cue; 40% within 90°, etc.). Perfect attraction would be represented by a horizontal line from origin to cue; perfect avoidance would be represented by a horizontal line from origin to 180°.

Y-maze experiment

The Y-maze experiment also yielded evidence for avoidance of forested wetland cues. In 38 successful tests, 73.7% of the animals (n = 28) chose the distilled water over the forested wetland cue (G-test, G = 8.878, 1 d.f., p-value = 0.003). Frogs did not exhibit preference or avoidance of natal pool water; in 37 successful tests 40.5% (n = 15) chose the distilled water (G-test, G = 1.332, 1 d.f., p-value = 0.248).

Discussion

Innate directionality during emigration movements

I did not find evidence for innate directionality, as both control animals and those with assumed inherited directionality oriented randomly when visual and olfactory cues were removed. However, this finding does not exclude the possibility that even if directionality *per se* is not inherited, information on the strongest cues used for orientation might be transferred between successive generations. Moreover, most previous experimental evidence points at proximate cues as the main source of orientation immediately post-metamorphosis. For example, directionality of emergence in bullfrogs (*L. catesbeianus*) was found to be established during the late stages of metamorphosis, and it was influenced by the variation in the surrounding environment, both aquatic (e.g., shallow water) and terrestrial (e.g., presence of shade from adjacent trees) (Goodyear and Altig 1971). This line of evidence might explain the results of Vasconcelos and Calhoun (2004) and Walston and Mullin (2008), who found that juvenile emergence was directed towards tracts of closed-canopy forest. These studies were conducted in landscape settings where contiguous tracts of forests were concentrated in specific directions, which coincided with the direction of emigration movements.

In contrast, Miaud et al. (2003) present direct evidence for genetic control of directionality in newly metamorphosed common frogs (*R. temporaria*). Juveniles from two populations located on the edge of a large lake showed non-random directionality towards patches of suitable forest habitat and away from the lake. This directionality was maintained when tested in experimental arenas. Juveniles resulting from the crossing of male and females from the two populations oriented at random, suggesting that directionality is partly inherited across generations. However, juveniles emerging from another pond partly surrounded by suitable habitat (i.e., forest), but located away from a prominent unsuitable landscape feature (i.e., lake), showed random orientation upon emergence, ultimately orienting towards a patch of forest. Thus, animals used proximate visual cues for orientation towards suitable habitat. Overall, the findings of Miaud et al. (2003) suggests that location of suitable upland habitat alone did not select for directionality, but rather the combination of suitable habitat location and a potentially inherited cue that signaled the unsuitable habitat (e.g., water) produced the observed directionality.

For *L. sylvaticus*, research has shown high phenotypic plasticity and rapid evolutionary changes in the larval form (Skelly et al. 2002, Skelly 2004), so it is not improbable that inherited directionality based on some prominent cue can be easily acquired, but also lost, if the local conditions change. This hypothesis is also supported by Miaud et al. (2003) who concluded that the observed local adaptation for directionality of migrations in *R. temporaria* probably occurred in <100 frog generations, a period coinciding with major land cover changes in their study area.

The possibility of innate directionality also comes into play when thinking about wetland mitigation. Building artificial isolated wetlands (i.e., breeding pools mimicking the hydroperiod of natural pools) has been used as a mitigation strategy in the northeastern United States (Korfel et al. 2010). If consistent directionality due to some prominent habitat feature unavailable in all directions occurs across seasons and is inherited across generations, then populations from older pools should show consistent directionality compared to populations from newly created pools. However, evidence from field studies in forested landscapes with both new artificial (Patrick et al. 2006) and natural breeding pools (Jenkins et al. 2006, Timm et al. 2007, Homan et al. 2010) showed that juvenile vernal pool amphibians exhibit non-directional orientation at emergence across many seasons, but directional orientation might be the norm in a given year in some settings (Jenkins et al. 2006, Homan et al. 2010). A switch in directionality across years for the same breeding pool refutes the role of innate directionality toward a stable patch of upland habitat. Instead it suggests that temporally and spatially-variable environmental cues influence the orientation upon emergence. The lack of directionality exhibited in our study by both groups of juvenile wood frogs when potential orientation cues were removed fully supports this assertion. In addition, juvenile upland movements and habitat use during emigration do not always match the direction of emergence, as inferred from captures at pond edge (Miaud et al. 2003), and there is no evidence of potential movement corridors that remain consistent across multiple seasons (Jenkins et al. 2006, Roznik et al. 2009). These two lines of evidence reflect high spatial and temporal variability in upland habitat use by vernal pool-breeding amphibians and question the idea that juveniles might disperse into ecological traps (Patrick et al. 2007, Homan et al. 2010).

Role of olfaction during emigration movements

Our research suggests that olfactory cues are used by juvenile wood frogs during emigration from the natal pool into the upland. Contrary to our hypothesis, based on existing literature (notably Vasconcelos and Calhoun 2004, and Patrick et al. 2007), juveniles tended to avoid forested wetland cues, and they did so in two different experimental setups. Although attraction, rather than avoidance of forested wetlands, was expected due to juveniles' low tolerance to desiccation (Schmid 1965), our research suggests that factors not driven by physiological requirements may play a more prominent role in habitat selection post-metamorphosis. The avoidance of forested wetland olfactory cues by animals raised in cattle tanks and not previously exposed to such odors prior to the experiment, and the discordance between our findings and previous studies raises two main questions: (1) what is the relative importance of cues used for orientation during migrations, and (2) what are the mechanisms inducing the avoidance of forested wetland cues?

For the first question, the species-specific multisensory orientation system of amphibians (Ferguson 1971) suggests that the use of potential cues is hierarchical, optimizing the use of information depending on the habitat (Sinsch 1990, 2006). For example, the attraction of newly metamorphosed *L. sylvaticus* to forested wetlands suggested by Vasconcelos and Calhoun (2004) might have occurred as a result of stronger cues (i.e., lack of canopy cover in the opposite direction of the forested wetland), overriding less strong cues, such as chemical signature of forested wetlands. Moreover, the relative importance of cues used for orientation may differ between individuals from the same population or cohort (Dall'antonia and Sinsch 2001, Shakhparonov and Ogurtsov 2003). Given such variability in individual behavior and the narrow perception range of juvenile amphibians (Rothermel 2004, Popescu and Hunter in press), it is likely that olfaction comes into play only when chemical signatures used for orientation override other cues.

For the second question, one straightforward explanation for the avoidance of forested wetlands could be the affinity of *L. sylvaticus* for upland terrestrial habitat immediately post-metamorphosis. Newly metamorphosed *L. sylvaticus* quickly emigrate and settle in upland habitat up to 150 m from the natal pool (Patrick et al. 2008a). Because juveniles feed on invertebrates within the leaf litter layer, forested uplands might provide a better food resource for these animals compared to forested wetlands. Another explanation for the avoidance of forested wetland olfactory cues is that juveniles may associate this odor with that of the natal pool, given that in our landscape, vernal pools often occur in forested wetland complexes (Calhoun and deMaynadier 2007). Thus, avoidance of forested wetlands could be equivalent to avoiding the proximity of natal pools where juveniles might experience both higher predation risk and higher competition for food from individuals in the same cohort.

Previous research suggests that the natal pool experience plays an important role in orientation during initial emergence, and orientation in metamorphosing amphibians changes during ontogenetic development (Goodyear and Altig 1971, Ogurtsov 2004). For example, Bastakov (1986, 1992) found that juveniles of the highly aquatic pool frog (*Rana lessonae*) were attracted by the odor of their natal pool, while common toad juveniles (*Bufo bufo*), a semi-aquatic species, tended to avoid the odor of their natal pool. Juvenile *L. sylvaticus* and *R. temporaria* were able to discriminate between odors imprinted upon during embryonic development and novel odors, and preferentially oriented towards the known stimuli (Hepper and Waldman 1992). However, Shakhparonov and Ogurtsov (2003) and Arhipova et al. (2005) found that the behavior of juveniles of two anuran species was plastic in response to the natal pool odor and changed over a short period of time after metamorphosis. Juveniles of the semi-aquatic green toad (*Bufo viridis*) and terrestrial European common frogs (*R. temporaria*) were attracted by the natal pool odor before the onset of emigration, while during emigration movements they either avoided or were indifferent to the same cues. The fact that I did not find evidence of preference or avoidance of the natal pool cue in our experiment is consistent with these findings. The juvenile stage of *L. sylvaticus* is exclusively terrestrial, and emigration movements start immediately upon the completion of metamorphosis. In addition, Arhipova et al.(2005) found that the response of juvenile *B. viridis* and *R. temporaria* to natal pool odors was dependent on whether animals were held in laboratory setting (i.e., 9 – 12 days) or were collected from the wild prior to the experiment. That is, natal pool olfactory cues continued to elicit an attraction behavior from animals held in the laboratory, while animals caught in natural conditions were indifferent to such cues. In this context, experiments that minimize laboratory holding period (such as used in our experiment) and use animals raised in natural or semi-natural conditions (e.g., mesocosms compared to laboratory rearing) are more likely to identify real-world behaviors (Šamajová and Gvoždík 2010).

Orientation behaviors are deterministic responses to a hierarchy of environmental cues ranging from strongest to weakest. In our experiment, juvenile *L. sylvaticus* showed a clear response to olfactory cues, and the strong avoidance of forested wetland chemical signature potentially reflects upland habitat selection during emigration movements. The indifference to natal pond odors adds to the evidence that natal ponds do not elicit a behavioral response for juveniles of terrestrial and semi-terrestrial anurans after the onset of emigration movements.

However, we are still far from understanding the relative use of direct cues for orientation during juvenile upland movements and the importance of inherited directionality during the transition between the aquatic and terrestrial stages. Research aiming at differentiating between genetic variation and phenotypic variation (i.e., environmentally-driven) in orientation behavior is needed (Via and Lande 1985). Such research might be accomplished by accounting for sources of spatial and temporal variability and availability of cues, testing a hierarchy of potential cues, as well as using transplants and common garden experiments and genetic manipulations (i.e., hybrids from populations with different observed directional traits) (Mousseau et al. 2000). This would potentially lead to a better understanding of habitat selection by juvenile amphibians, a critical and data-deficient life stage.

Chapter 4

INTEGRATING MICROCLIMATE AND LOCOMOTOR PERFORMANCE OF JUVENILE AMPHIBIANS: WHY ARE CLEARCUTS NOT CONDUCIVE TO MOVEMENTS?

Abstract

Amphibian locomotor performance is dependent on the tradeoff between thermoregulation and water conservation, thus microclimates shape the patterns of habitat use by amphibians. Disturbances, such as forestry practices that completely remove the canopy, can affect the distribution and abundance of forest amphibians by creating physiologically limiting microclimates. It is hypothesized that vegetation regrowth mitigates the effects of canopy removal on microclimates, but evidence suggests that young regenerated habitats are avoided by forest amphibians. I investigated the relationship between available microclimates and locomotor performance of newly metamorphosed wood frogs (*Lithobates sylvaticus*) using a two-prong approach. First, I recorded hourly temperature and relative humidity in young regenerated stands (5-6 years old) during the emergence and emigration season (July and August). Concomitantly, I investigated the use of clearcuts and adjacent forests by juvenile wood frogs emigrating from experimental pools. Microhabitats lacking shade (i.e., with herbaceous vegetation only) were consistently warmer and drier than shrubs, saplings and forests. Low shade from shrubs and saplings mitigated the temperature above ground within 3°C of closed-canopy forests during afternoon hours, and created similar moisture conditions. Potentially lethal temperatures (>30°C) were rarely recorded in the forest, shrub, and sapling microhabitats. Despite the mitigation of microclimate by vegetation regrowth, juvenile wood frogs avoided clearcuts and preferred forested treatments during emigration. Second, I tested the locomotor performance of newly metamorphosed wood frogs in relation to temperatures likely to be experienced in the field (15-30°C) using jumping trials. Performance, expressed as the ratio between mean jump and snout-vent length, was highest at 25 and 30°C. Our results suggest that juvenile wood frogs are not limited by microclimate in well regenerated clearcuts, suggesting that such habitats are avoided for reasons other than physiological requirements. As a consequence, microclimate cannot effectively be used as a sole parameter to predict potential habitat use by amphibians.

Introduction

Spatial distribution, habitat use, and performance of amphibians are highly dependent on available microclimate conditions (Halverson et al. 2003). Many physiological systems of ectotherms (growth, locomotor ability, and reproduction) are directly regulated by their thermal environment (Huey and Stevenson 1979). Most terrestrial amphibians face a tradeoff between attaining the preferred body temperature and water conservation (Moore and Gatten 1989, Preest and Pough 1989). While behavioral thermoregulation is achieved through posture and location changes to achieve an optimal body temperature (Lillywhite 1970), this is at the cost of moisture availability (Tracy 1976, Malvin and Wood 1991, Seebacher and Alford 2002). As a consequence, understanding the mechanisms behind amphibian habitat use requires investigating the interplay between available microclimates, performance, and behavior simultaneously.

The spatial distribution and abundance of forest-dependent amphibians is affected by habitat attributes that directly control the availability of the thermal and moisture environment (Graeter et al. 2008, Blomquist and Hunter 2010). Forestry practices involving substantial canopy removal (e.g., clearcutting) create harsh microclimate conditions for many amphibians species (Keenan and Kimmins 1993). Microclimate effects of clearcutting can extend up to 240 m into the adjacent stands (Chen et al. 1999). Such impacts have a detrimental effect on amphibian populations, affecting abundance and species richness (deMaynadier and Hunter 1995, Semlitsch et al. 2009). It is hypothesized that over time, vegetation re-growth moderates the microclimate by maintaining high humidity and reducing temperatures near the ground (Weng et al. 2007, Brooks and Kyker-Snowman 2008). Coarse woody debris (CWD) left in harvested stands (Swanson and Franklin 1992) may also provide moist and cool refugia for amphibians. Silvicultural regeneration methods involving complete canopy removal (clearcutting) have longer lasting effects on microclimate, with harsher conditions compared to those using partial harvests (Chen et al. 1993, Carlson and Groot 1997, Zheng et al. 2000, Xu et al. 2002). For example, forest floor temperature and relative humidity in partially cut stands 1 – 12-years-old were similar to those of adjacent, unharvested stands (Brooks and Kyker-Snowman 2008).

Evidence from a large-scale forestry experiment replicated across three ecoregions in the United States, and looking at the immediate effects of vegetative succession post-canopy removal showed that emigrating juvenile wood frogs (*Lithobates sylvaticus*) actively avoided clearcuts (Patrick et al. 2006, Semlitsch et al. 2009, Todd et al. 2009). Specifically, experiments conducted in central Maine, USA, found that strong avoidance behavior was maintained for at least three years post-harvesting, with 3.3-fold more animals captured in the forest compared to clearcuts during the third year post-disturbance, despite substantial vegetation regrowth (Patrick et al. 2008). In addition, emigrating juvenile amphibians did not differentiate between stands with CWD-retained and stands with CWD-removed (Patrick et al. 2006). One hypothesis behind the observed avoidance of clearcuts is that harsh microclimate conditions hinder movements of juvenile amphibians, which are prone to desiccation and have a low tolerance to high temperatures. At the same time, juvenile *L. sylvaticus* show an active avoidance of regenerated stands that lack complete canopy cover, but have similar microclimates, suggesting that behavior also plays a critical role in determining habitat use (Popescu and Hunter in press). For stands undergoing complete canopy removal, the timeframe over which microclimate would recover to pre-harvest conditions, and whether such regrowth mitigates microclimate in a biologically meaningful way to amphibians are still largely unknown.

Despite the wealth of research addressing questions related to the survival of amphibians in various forest management settings (e.g. Rothermel and Luhring 2005, Rothermel and Semlitsch 2006, Rittenhouse et al. 2008, Roznik and Johnson 2009), only a few studies have directly linked the available field microclimate conditions to amphibian performance as a means to understand physiological and behavioral mechanisms behind observed distribution patterns (e.g., Walton 1988, Šamajová and Gvoždík 2010). To address the biological significance of microclimate variability for habitat use by emigrating *L. sylvaticus* juveniles, I used a combined observational/experimental approach that integrated three aspects deemed critical in understanding the physiological and behavioral consequences of habitat selection (Huey 1991): (1) measuring available field microclimates in relation to canopy removal and vegetative succession, (2) quantifying the effects of body temperature on performance, and (3) assessing microhabitat suitability by integrating the environmental and physiological data. For the second aspect, I chose to look at locomotor performance with respect to temperature for two reasons: (1) locomotor performance is critical for the survival and growth of newly metamorphosed amphibians (Watkins 2001), and (2) our study was conducted in central Maine, USA, a heavily forested region, with cool and generally moist summers, where moisture availability is arguably less important than in other parts of the species range.

When clearcut stands undergo natural vegetative succession, there can be a high degree of variability with respect to the spatial distribution, species composition, and density and height of woody regeneration (Oliver and Larson 1996). I hypothesized that the inherent patchiness of vegetative succession would create microhabitats with different thermal and moisture characteristics. Within the same experimental sites where Patrick et al. (2008) found strong avoidance of clearcuts up to three years post-clearcutting, I investigated patterns of habitat use by juvenile *L. sylvaticus* and examined microclimates of 5 – 6-year-old regenerated clearcuts and adjacent closed-canopy forests, both on the ground surface and under refugia provided by CWD. I also undertook a locomotor performance experiment to investigate whether or not field temperatures are a limiting factor for habitat use by juvenile *L. sylvaticus*.

Given this combined approach, the overarching goal was to infer physiological and behavioral mechanisms through which clearcutting influences the spatial distribution of amphibians, ultimately aiming at providing management recommendations for conserving forest-dependent amphibians. Specifically, this study addressed the following questions: (1) are emigrating juveniles thermally limited by high temperatures recorded in regenerated clearcuts, compared to mature forests? (2) does CWD mitigate the effects of clearcutting by providing refugia?, (3) does the locomotor performance of juvenile wood frogs decrease as temperatures reach their critical thermal maximum (CTM) and (4) do differences in thermal environment translate into differences in suitability between closed-canopy forest and regenerated clearcuts?

Study area and methods

Study area, vegetation, and weather

This research was conducted on the University of Maine Demerritt and Penobscot Experimental Forests in Penobscot County, Maine, where four experimental breeding pools were created in 2004. Concomitantly, four silvicultural treatments (clearcut with CWD retained, clearcut with CWD removed, partial harvest, and uncut control) were implemented adjacent to the experimental pool. These treatments extended up to 164 m from the edge of the pool, each treatment covering 2.1 ha and representing a quarter of the 164-m radius circle (the two clearcuts were opposite of each other). Drift fences (10 m long, 60 cm tall, and buried 30 cm in the ground) and associated pitfall traps were erected at four distances from the experimental pools: 16.6, 50, 100, and 150 m (1, 3, 6, and 9 fences, respectively in each treatment). Drift fences were placed in a circular manner around the breeding pools, and covered 38% of the circumference at each distance. In addition, the experimental pools were 100% encircled by drift fence (see Patrick et al. 2006 for more details on the experimental design).

This study was conducted in 2008 and 2009, representing 5 and 6 years post-harvesting, respectively. I monitored the drift fences by checking them every other day during the emergence and emigration season of juvenile *L. sylvaticus*: 30 June – 15 September 2008, and 30 June – 28 August 2009. Animals captured at the pool fence were marked with a single toe-clip representing the day of emergence. Animals recaptured in the upland at distances 16.6 – 150 m were tagged using 2.5 x 1 mm Visible Implant Alpha Tags (Northwest Marine Technologies, Shaw Island, WA), and released on the other side of the fence. I was able to mark the entire cohort of juvenile *L. sylvaticus* emerging from the pools, and individual marks of recaptured animals allowed us avoiding double-counting, and correctly assess the choice of silvicultural treatment during emigration. In addition, I captured juvenile *L. sylvaticus* that emerged from pools outside the experimental sites, and used the silvicultural treatments during their emigration movements.

I characterized the microclimate of three clearcut microhabitat treatments based on the presence and height of woody regeneration: (1) *herbaceous*, (2) *shrub* (<1 m in height), and (3) *sapling* (1 – 4 m in height), as well as a *closed-canopy forest*, used as a control. I described tree species composition and quantified stem density for all stems >25 cm in height and up to 5 cm diameter at breast height (DBH) in the sapling and shrub treatments using 10-m^2 plots. I characterized species composition and stem density, percent canopy cover, and tree height in the forested treatment using 250-m^2 plots. Percent canopy cover was measured using a densiometer (Moosehorn CoverScopes, Medford OR). I took five sightings at 2-m intervals on four transects radiating from the center of the plot for a total of 40 measurements at each location in the forested treatment. For the herbaceous treatment, I estimated the percent ground cover of vegetation (herbaceous and woody stems <25 cm in height) to the nearest 5% on 10-m^2 plots.

The vegetation in the herbaceous treatment was dominated by Poaceae and bracken fern (*Pteridium aquilinum*) but also included various low shrubs such as blueberry (*Vaccinium angustifolium*) and bristly sarsaparilla (*Aralia hispida*). The shrub plots were dominated by gray birch (*Betula populifolia*) and *Rubus* spp., while the sapling plots were dominated by big-tooth aspen (*Populus grandidentata*) and red maple (*Acer rubrum*). The closed-canopy forest was 60-80 years old, and the vegetation composition included mainly balsam fir (*Abies balsamea*), eastern white pine (*Pinus strobus*), northern white-cedar (*Thuja occidentalis*), red maple (*Acer rubrum*), and eastern hemlock (*Tsuga canadensis*). All forested locations had >90% canopy cover (Table 4.1), and no regeneration >4 m in height was present in the herbaceous, shrub, and sapling microhabitats.

Table 4.1. Vegetation and habitat structure of four microhabitats sampled in 2008 and 2009.

Treatment	**Total stem density (stems/ha)**	**Density of stems >2.54 cm diameter (stems/ha)**	**Tree height (m)**	**Canopy cover (%)**	**Ground cover (%)**	
					Moss	**Herbaceous**
Forest	4693	4693	>15	90.6	10.7	0
Sapling	30750	5370	<4	0	3.8	18.1
Shrub	46500	0	<1	0	32.5	20.2
Herbaceous	0	0	0	0	12.5	77.6

The three microhabitat treatments were represented in different proportions at the experimental sites. I quantified the extent of each microhabitat using 2007 and 2009 1-m resolution aerial imagery (U.S. Department of Agriculture – National Agricultural Inventory Project, *www.fsa.usda.gov*) in ArcGIS 9.3 (ESRI, Redlands, CA). Due to substantial vegetation regrowth , the herbaceous microhabitat had the smallest extent, covering approximately 10% of the study sites (range = 6 – 18%) The shrub microhabitat covered the most area, amounting to 53% (range = 46 – 63%), while the sapling treatment comprised the remaining 37% (range = 28 – 47%) of the regenerated clearcuts.

The two sampling seasons were different in their weather patterns, with the 2009 season cooler and wetter overall (Appendix D, *www.ncdc.noaa.gov*). The average July temperature was higher in 2008 (1.8°C above normal) compared to 2009 (3.8°C below normal). August temperatures showed an inverse relation, with a lower average in 2008 (2.8°C below normal) compared to 2009 (0.9°C above normal). Maximum daily temperatures >30°C were recorded for 3 and 5 days of the sampling 2008 and 2009 seasons, respectively (the highest temperature was 33.3°C on 18 August 2009, Appendix D). Precipitation was consistently higher during July in both 2008 and 2009 (30.2 mm and 60.2 mm above normal, respectively) compared with August (26.7 mm and 7.6 mm below normal, respectively).

Microclimate measurements

During both study years, I placed temperature and humidity data loggers (iButton, Maxim Integrated Products, Sunnyvale CA) in the three clearcut microhabitats and the forested control. All sampling locations were located >30 m away from forest edges to minimize edge effects on microclimate (Murcia 1995). In each microhabitat at each of the four experimental sites I randomly placed paired data loggers at soil level (within the leaf litter layer, above ground – AG) and at refugia (under coarse woody debris – CWD). Then, using a random direction and distance (within 15 m) from the first location, I placed another pair, in similar habitat conditions, for a total of 32 survey locations (4 study sites x 4 microhabitats x 2 locations) and 64 data loggers. Because Brooks and Kyker-Snowman (2008) found that relative humidity at refugia locations (similar to our CWD treatment) was always approximately 100%, I decided to measure only temperature at all CWD locations using Thermochron® data loggers. I measured both temperature and relative humidity at half of the AG locations (16 Hygrochron® data loggers) and temperature only at the other half. Hourly data were recorded between 28 June and 31 August 2008 (65 days) and 26 June and 31 August 2009 (67 days), encompassing the emergence and emigration season of newly metamorphosed *L. sylvaticus*.

Locomotor performance experiment

In 2009, I collected egg masses from roadside ditches located on the University of Maine forests. Animals were hatched in plastic wading pools, and reared to metamorphosis in 1500-l cattle tank mesocosms stocked with plankton from natural vernal pools, and dry leaflitter (Kiesecker and Skelly 2001). The tanks were covered with shade cloth and placed in closed-canopy conditions to prevent high water temperatures. Water level was maintained by rainfall, which was above the long-term normal for the period of the study. I measured water temperature by placing data loggers in cattle tanks, halfway in the water column. The water temperature steadily increased from 8°C on 4

May (when animals were added to mesocosm) to a maximum of 22.5°C on 30 July (the last week of emergence), and were subject to diel and seasonal variations (Appendix E). When the animals reached the final metamorphosing stage (46 Gosner, Gosner 1960), I added them to terrariums with moist leaf litter (for two days prior to the beginning of the experiment) located in an outdoor shed under closed-canopy.

I chose to use animals reared outdoors in semi-natural conditions, subject to seasonal and diel fluctuations in water temperature, rather than laboratory-rearing at constant temperatures for the following reasons: (1) the locomotor capacity on land is more likely to be influenced by fluctuating conditions, which "prepare" animals for more extreme temperatures than the ones experienced in the aquatic stage (Huey et al. 1999); (2) constant experimental temperatures tend to yield misleading conclusions about the occurrence of acclimatory performance responses (Šamajová and Gvoždík 2010); and (3) I wanted to integrate locomotor performance with field temperatures, which can be subject to extreme fluctuations during the emigration season (summer).

I investigated the locomotor performance of newly metamorphosed *L. sylvaticus* at four temperatures within the thermal tolerance of ranid frogs (Rome et al. 1992), which were likely to be experienced in the field during emergence and emigration: 15, 20, 25, and 30°C. I performed the experiment on 4 August (n = 17 animals) and 6 August 2009 (n = 19 animals) between 10:00 and 16:00 EST. I removed the animals from terrariums two hours prior to the beginning of the experiment, and transported them to an indoor experimental facility at the University of Maine. Animals achieved the desired body temperature by transferring them to a cylindrical plastic vial (5.5 cm tall and 3.5 cm in diameter) with holes to ensure water mixing and lids to prevent escaping, and immersing the lower ¾ of the vial in 10-l water baths. I prepared one water bath for each of the four experimental temperatures and monitored temperature using a MicroTherma 2 type-T thermocouple (ThermoWorks, Lindon UT) by inserting the tip of the probe (accuracy = ±0.1°C) in selected vials every 5-10 minutes.

I built two 300 x 70 cm rectangular experimental arenas using 1.5 cm-thick corrugated cardboard with 40 cm tall side walls. Experimental animals were placed at one end of the arena and allowed to jump five times (Alvarez and Nicieza 2002). Each jump was marked on the cardboard arena using an erasable pen; if animals did not move, I gently tapped their urostyle with a pen (Tejedo et al. 2000). I assessed the locomotor performance using two measures: (1) average length of the five consecutive jumps, and (2) maximum jump length (Alvarez and Nicieza 2002). Because differences in body size affect jumping ability (i.e., larger frogs jump farther than smaller frogs), I rescaled the two jumping measures by body size (snout-vent length, SVL). Thus, I expressed the jumping ability in body lengths jumped (BL index), rather than absolute jump lengths.

Each animal was used in five successive trials; the first and last were conducted at 20°C to test whether or not the multiple trials affected the locomotor performance (Huey and Stevenson 1979). After the first trial, I haphazardly assigned the animals to the other treatments. The duration of each trial was <10 seconds, and animals were allowed to rest for 1 hour between trials. I conducted the trials in two separate rooms in order to minimize the change in frog body temperature by exposure to ambient air temperature: (1) trials for 15, 20 and 25°C treatments were conducted at 20.5°C air temperature and (2) trials for and 30°C were conducted at 27°C air temperature. To determine the change in frog body temperature during trials, I measured the cloacal temperature of randomly selected animals (n = 10 for each treatment) before and after the trial, using a 1-cm blunt-tip needle thermocouple microprobe (accuracy = ±0.1°C, ThermoWorks, Lindon UT). I measured the snout-vent length (SVL) and hind limb length for all animals using a dial caliper (accuracy = 0.1 mm).

Performance of juvenile wood frogs relative to field temperatures

I built a performance curve for juvenile wood frogs using the BL index derived from the mean jump lengths recorded in the locomotion experiment. I averaged the BL index across all experimental animals for each of the four temperatures, and calculated a performance index (PI) for each temperature (15, 20, 25, and 30°C) by dividing the mean BL index for that respective temperature by the maximum of four mean values. I obtained a performance curve for juvenile wood frogs in the temperature range 15 – 30°C by fitting a second-order polynomial model (Equation 1) to the PI data.

$$PI = -0.0013*Temperature^2 + 0.063*Temperature + 0.049 \quad \text{(Eq. 1)}$$

I then calculated PI values for the hourly temperatures recorded in the field (at AG locations only) to assess whether or not juvenile wood frogs operate at their optimal performance in the four microhabitats, and whether or not the differences in temperatures recorded in the field translate into differences in performance during the emigration season. Because some of the recorded field temperatures were higher than the maximum temperature at which frogs were tested for locomotor performance, I allocated a PI value of zero to any recorded AG field temperatures >30°C. Assuming that the frogs were incapacitated at temperatures >30°C is more defensible than extrapolating Eq. 1 for two reasons: (1) prior knowledge on the critical thermal maximum (CTM) for *L. sylvaticus* (34.8°C, Brattstrom 1963), which was close to the highest experimental temperature, and (2) some of our experimental animals showed a lower performance (i.e., shorter jumps) when exposed to 30°C, compared to 25°C.

Statistical analyses

Habitat use by emigrating juveniles. I tested the differences in silvicultural treatment use by *L. sylvaticus* emerging from the experimental polls and emigrating into the upland in 2008 using a ANOVA with the number of recaptures as the dependent variable and treatment and site as factors. Due to recruitment failure, only one pool produced animals in the subsequent year, and I was not able to analyze the 2009 data. I transformed the response variable using a [log(x+1)] transformation to achieve normality. For juveniles that emerged from pools outside the experimental sites and used the silvicultural treatments during emigration movements, I adopted a different approach. Because the location of the natal pools was unknown, and this might bias the observed use of the silvicultural treatments (e.g., by chance alone one particular treatment would be used more because the natal pool was located in the immediate vicinity of that treatment), I contrasted the use of the forested and clearcut treatments by pooling together the captures across sites. I then used a G-test for goodness of fit to test whether or not the forests were used more than the clearcuts, under the assumption of equal use.

Locomotor performance. I tested for differences in locomotor performance between the four treatments using a one-way repeated measures ANOVA. If the test yielded significant differences, I performed post-hoc pairwise comparisons between treatments. I examined whether or not the duration of the experiment affected locomotor performance comparing the mean jump length for the first and last trials conducted at 20°C using a paired t-test. Significance level was $\alpha = 0.05$ for all tests.

Influence of field temperatures on performance. I investigated the overall differences in (1) thermal environment, (2) moisture availability and (3) temperature-related performance of juvenile *L. sylvaticus* between microhabitat treatments using generalized linear mixed effects (GLME) models in program R version 2.11.0 (R Development Core Team 2010). This allowed us to include a temporal autocorrelation structure that dealt with time series data, as well as a variance function to model data heterodasticity (Pinheiro and Bates 2000). I divided each day into four *time-of-day* periods: night (12:01 – 6 AM), morning (6:01 AM – 12 PM), afternoon (12:01 – 6 PM), and evening (6:01 PM – 12 AM), and averaged the hourly observations data for temperature, relative humidity, and PI values (computed using Equation 1) across each period for each individual data logger to parse out diel variations (Yang et al. 2007). To test for differences in microclimate and wood frog performance between the four microhabitat treatments, I used the interaction of microhabitat *treatment* and *time-of-day* as a fixed effect, and the nested *time-of-day* within *day* as a random effect (intercept). The autocorrelation structure consisted of a simple autoregressive function of *time-of-day* within *day* that expressed each observation as a linear function of previous observations (Pinheiro and Bates 2000). The variance was modeled using a function with different variances for each level of microhabitat *treatment.*

For field temperatures I ran models for each location (AG and CWD) and year separately; for the relative humidity and temperature-related performance index I ran different models for each year. Because the temperature data averaged across six hours did not reflect extreme changes in microclimate that could potentially adversely affect wood frog performance, I investigated the percent of the emigration season that AG temperatures reached above 25, 30, and 34.8°C, based on the hourly temperature recordings.

Results

A total of 3133 juvenile *L. sylvaticus* emerged from three experimental pools in 2008, of which 392 were recaptured into the upland habitat (n = 294 in the forested treatments; n = 98 in the clearcuts). The four silvicultural treatments differed significantly in the number of recaptures in 2008 ($F_{3,36}$ = 12.935, p-value <0.001). Post hoc comparisons revealed significantly more captures in the forested treatments (partial cut and control) compared to the clearcuts (p-value <0.001). There was no difference between the control and the partial cut, as well as between the two clearcut treatments (p-value >0.25). Only 95 animals emerged from one pool in 2009, of which seven were recaptured in the forested and only one in the clearcut treatments.

Juvenile *L. sylvaticus* that emerged from pools outside the experimental sites and were captured in the silvicultural treatments showed a significantly higher use of the forests compared to the clearcuts (2008: 749 captures in forest and 280 in clearcuts; 2009: 149 captures in forest and 48 in clearcuts; G-test, p-value <0.001 for both years).

Locomotor performance

Temperature affected the locomotor performance of juvenile *L. sylvaticus* (one-way repeated measures ANOVA, $F_{3,33} = 27.072$, p-value < 0.001). The body length index (BLI) representing the ratio between jump length and SVL was greatest at 25°C and 30°C (Figure 4.1). Performance at these temperatures was significantly higher than in the 20°C treatment (p-value = 0.008). Locomotor performance of juvenile wood frogs was lowest at 15°C, with a significantly smaller BLI compared to the 20°C treatment (p-value <0.001) (Figure 4.1). BLI derived from the maximum jump length followed the same pattern as the mean jump length (15°C < 20°C < 25°C = 30°C). There was no difference in the BLI between the first and last trial conducted at 20°C (paired t-test, p-value = 0.317), suggesting that the duration of the experiment did not affect the locomotor performance of juveniles.

The average body size was 18.1 ± 0.2 cm and it was strongly correlated with hind limb length (29.7 ± 0.3 cm) (Pearson $r = 0.934$). Mean and maximum jump length were correlated with hind leg length and body size for all temperatures (Pearson $r = 0.48 – 0.74$, p-value <0.01 for all pairwise correlations). I found only a small change in body temperature towards the room temperature of experimental animals during the trials. The largest change occurred for animals in the 30°C treatment (mean = 0.17°C) and the smallest for the 20°C treatment (mean = 0.05°C).

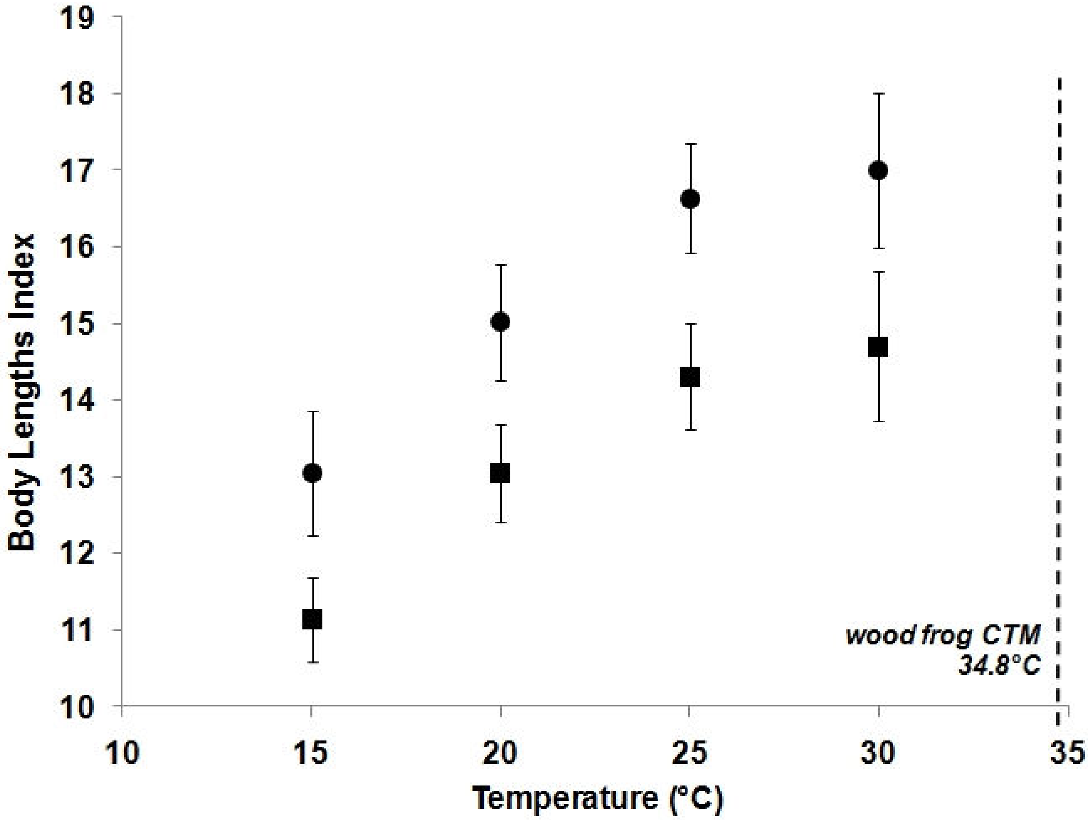


Figure 4.1. Jumping performance of juvenile wood frogs exposed to four temperatures. Body Length Index represents the ratio between mean (squares) and maximum (circles) jump length and body length (SVL) (error bars are 95% CI).

Microclimate of forest and regenerated clearcuts during emigration season

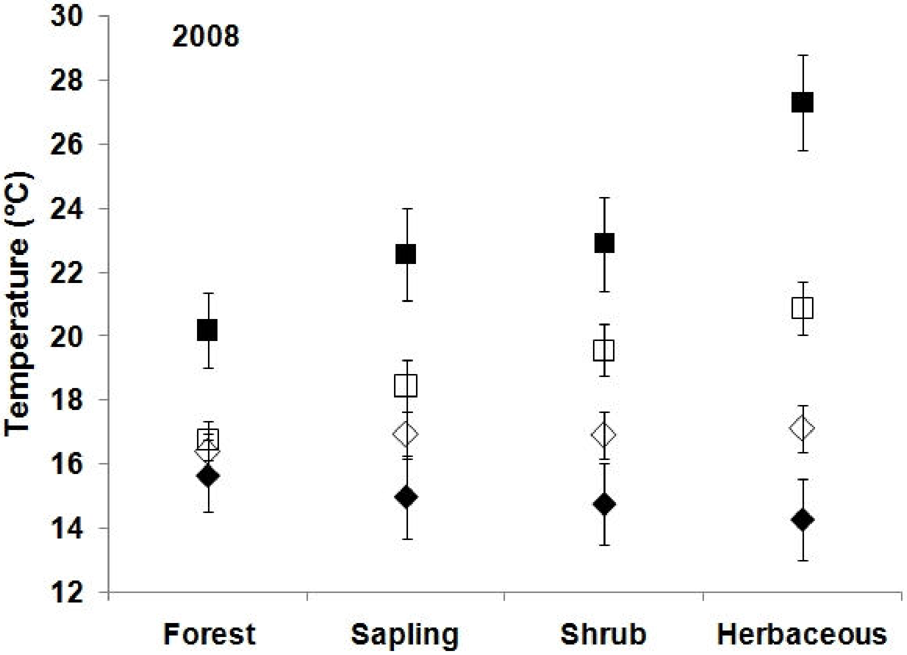
I found significant differences in the thermal characteristics of the forested and the clearcut microhabitat treatments, as well as between herbaceous and young woody regeneration (shrub and sapling), manifested mainly during afternoon hours. Mean afternoon AG temperatures in the forest were lower than each of the other treatments in both years, but these differences were larger in 2008 compared to 2009 (Figure 4.2). In 2008, the herbaceous treatment was in average 7.2°C warmer than the forest and 2.3 – 2.7°C warmer than the sapling and shrub treatments, respectively, during afternoon hours

(Figure 4.2a). In 2009, these differences amounted to only 3.3, 1.7, and 0.7°C, respectively (Figure 4.2b). There was no difference in temperature between the shrubs and saplings at any time of day during both years. Night temperatures in the four treatments were within 1°C in both years.

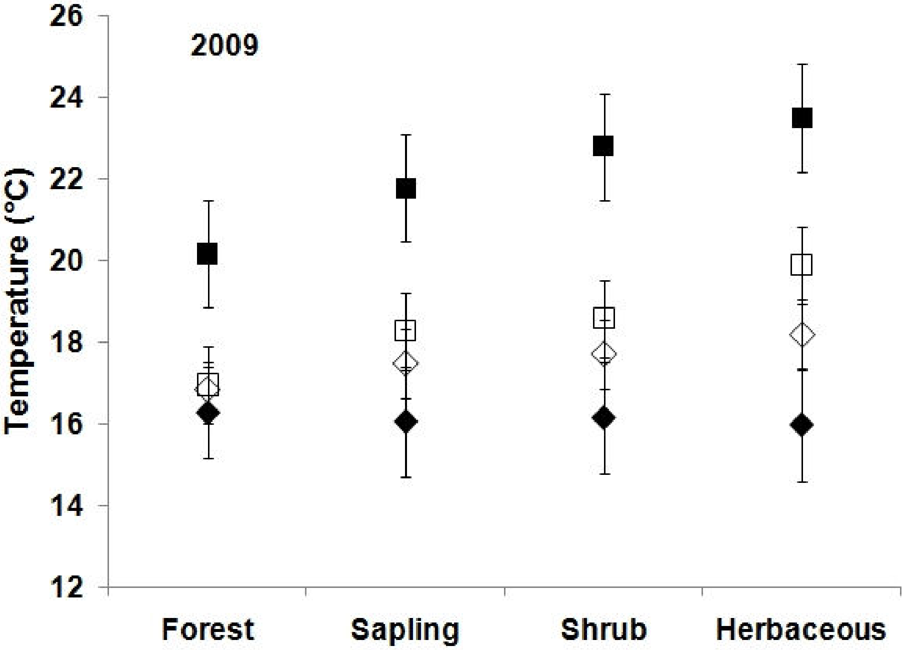
Potentially lethal temperatures were recorded at AG locations in all treatments during both years, but temperatures >30°C were relatively common only in the herbaceous microhabitat (11.3% of the time in 2008, Table 4.2). Above-ground temperatures rarely reached the CTM for wood frogs during the 2009 emigration season (0.3 – 1.8% of the season).

Coarse woody debris mitigated the effects of clearcutting on microclimate. There were still differences between the herbaceous and the forested treatments during afternoon hours – 4.1°C in 2008 and 2.9°C in 2009 – but at a lower overall temperature (Figure 4.2). Nighttime temperatures at CWD locations were slightly higher than the AG locations in the clearcut microhabitats.

Differences in moisture availability between the four microhabitats, expressed by relative humidity measurements, were evident during afternoon hours, but only in 2008. The herbaceous treatment had the lowest average relative humidity (Figure 4.3a), while the forested, shrub and sapling microhabitats had similar, high average moisture availability during both years (>90%, Figure 4.3a, b).

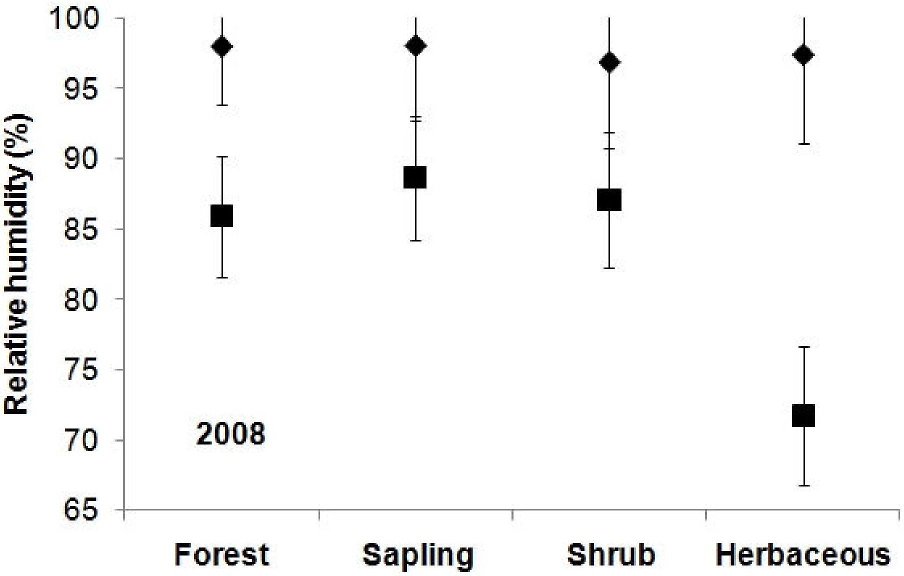


a)

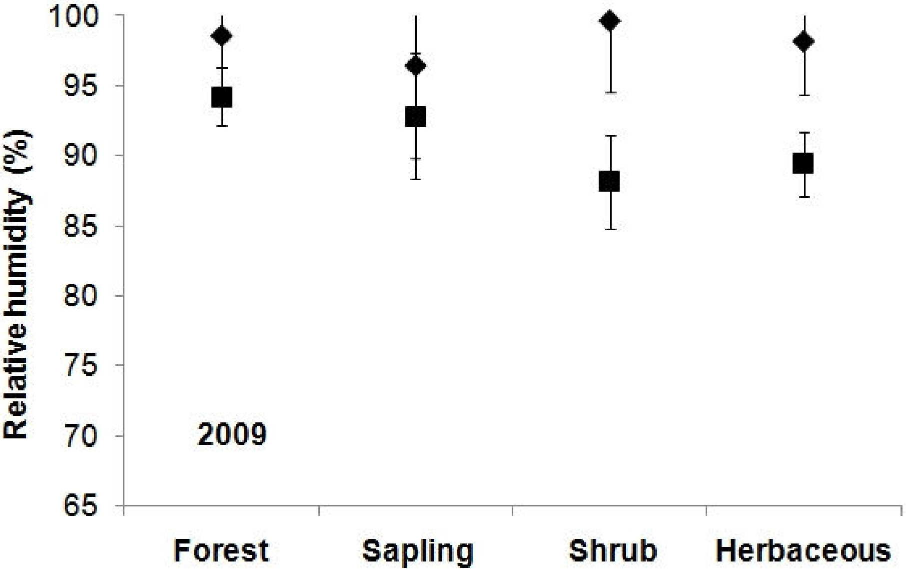


b)

Figure 4.2. Average predicted afternoon (squares) and night (diamonds) field temperatures at above ground (solid symbols) and under CWD (empty symbols) locations; error bars are 95% confidence intervals; note the difference in Y-axis scale between 2008 and 2009.



a)



b)

Figure 4.3. Average predicted afternoon (squares) and night (diamonds) field relative humidity at above ground locations in 2008 and 2009; error bars are 95% confidence intervals.

Table 4.2. Mean percent (± 1 SE) of juvenile wood frog emigration season in 2008 (28 June – 31 August) and 2009 (26 June – 31 August) above three temperature thresholds recorded at above-ground level in forest and clearcut stands. (34.8°C is the critical thermal maximum [CTM] for *L. sylvaticus*).

Habitat	**Percent of season**		
	>25°C	**>30°C**	**>34.8°C**
2008			
Forest	3.3 ± 1.1	0.4 ± 0.3	0.1 ± 0.0
Sapling	10.2 ± 1.3	1.2 ± 0.3	0.1 ± 0.1
Shrub	10.8 ± 1.8	1.8 ± 0.4	0.2 ± 1.1
Herbaceous	25.1 ± 1.7	11.3 ± 1.5	5.0 ± 0.9
2009			
Forest	2.1 ± 2.5	0.3 ± 0.3	0.1 ± 0.1
Sapling	4.7 ± 1.5	0.4 ± 0.3	0.1 ± 0.0
Shrub	7.2 ± 3.2	1.8 ± 1.4	0.4 ± 0.4
Herbaceous	8.6 ± 2.7	1.4 ± 1.0	0.4 ± 0.4

Microhabitat suitability - integrating field temperatures and performance

The average performance (PI) of juvenile wood frogs at the lowest experimental temperature (15°C) was 0.75 of the maximum performance (recorded at 30°C); within this temperature range, the trend was non-linear (Figure 4.4). Microhabitat suitability was highest (PI >0.9) in the shrub and sapling microhabitats during afternoon hours, but differences between microhabitats were generally small (ΔPI <0.1 for each time of day separately) (Figure 4.5). The only significant difference in suitability occurred during 2008, when the herbaceous treatment had a potentially negative effect on overall juvenile performance (Figure 4.5a), mainly as a result of high afternoon temperatures at ground level (Table 4.2). For each time of day, suitability was similar across microhabitats in 2009 (Figure 4.5b). The smaller differences between microhabitats (ΔPI <0.03) were recorded during evening and night, as a result of similar microclimate conditions across the experimental sites (Figure 4.5).

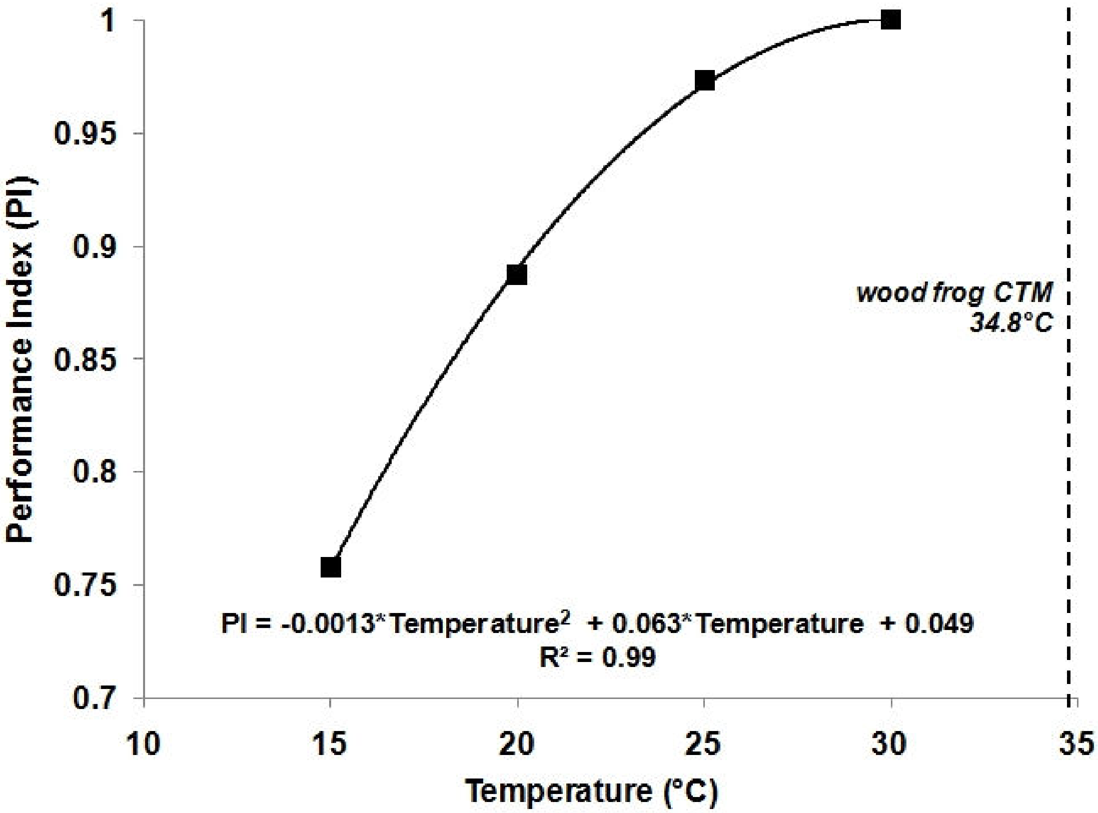
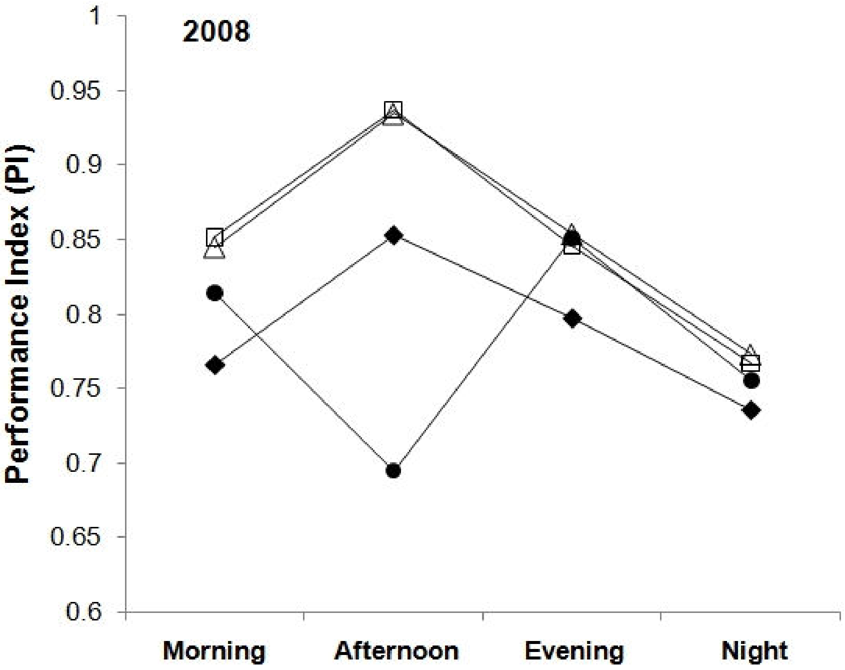
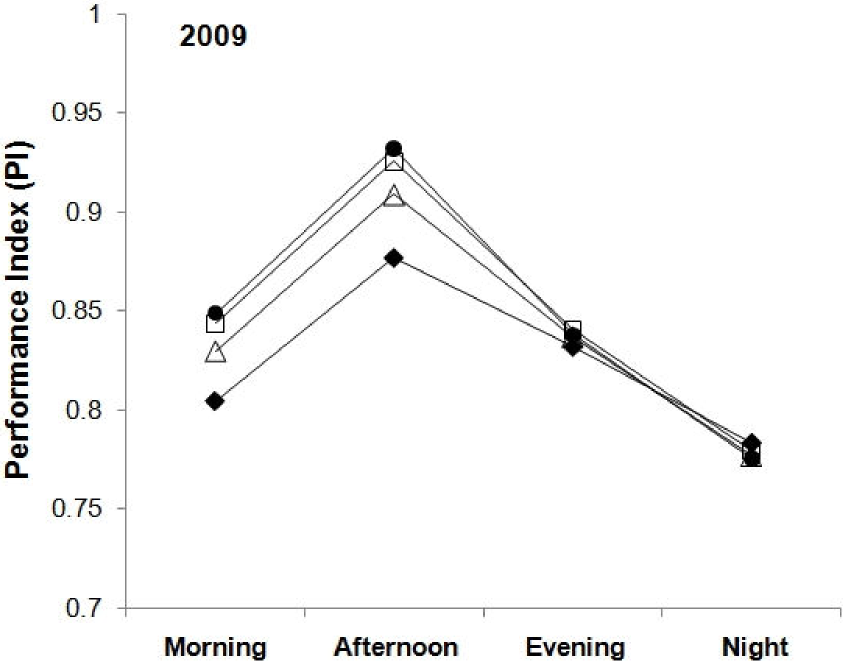


Figure 4.4. Performance index of juvenile wood frogs at temperatures between 15 and 30°C derived from the locomotion experiment.



a)



b)

Figure 4.5. Predicted suitability of four microhabitat treatments based on the average performance of juvenile wood frogs during the emigration season (end June – end August) in 2008 and 2009. Symbols: diamond = forest; circle = herbaceous; square = shrubs <1 m; triangle = saplings 1-4 m.

Discussion

Our study aimed to explain the avoidance of regenerated clearcut habitats by emigrating juvenile *L. sylvaticus* by relating available microclimates to physiological mechanisms. Despite differences in thermal and moisture conditions between the forest and clearcut microhabitats during afternoon hours, I found no clear evidence that avoidance of clearcuts is primarily a result of physiology (i.e., high temperatures and low moisture conditions limiting the presence of juvenile wood frogs in clearcuts). The presence of low shade and retained CWD in clearcuts effectively moderated temperature and relative humidity such that shrub, sapling, and CWD microhabitats were as suitable as the closed-canopy forest.

The locomotor performance experiment showed that juvenile *L. sylvaticus* performed best at the highest temperatures tested: 25 and 30°C. Temperatures above 30°C (which might be debilitating given the CTM for *L. sylvaticus*, 34.8°C) were rarely recorded in the closed-canopy forest and amounted to a very small proportion of the emigration season in the shrub and sapling microhabitats with low shade present (Table 4.2). As a consequence, despite the differences in microclimate, habitat suitability based on the locomotor performance index was similar across years and microhabitat treatments (Figure 4.5). The only exception was the herbaceous treatment in 2008, which was overall less suitable during afternoon (25% of the season). Moisture availability was similar across all microhabitats, except for the herbaceous treatment, which was consistently drier during daytime, but only in 2008 (Figure 4.3).

The afternoon ground-level temperatures in the regenerated microhabitats (shrubs and saplings) were alleviated by the presence of low shade. These treatments were only approximately 3°C warmer than the closed-canopy forest (Figure 4.2), and covered approximately 80% of the studied clearcuts. Higher ground-level temperatures were recorded in both years in the herbaceous treatment during afternoon hours, and these differences were more substantial in 2008 than in 2009 (Figure 4.2). The 2009 season in particular was characterized by cool temperatures and consistent rainfall. Despite the small average differences in the thermal and moisture characteristics (0.7 – 3.3°C [Figure 4.2], and <5%, relative humidity [Figure 4.3], respectively) of our treatments in 2009, the observed patterns of habitat use by juvenile *L. sylvaticus* in relation to clearcuts did not reflect habitat selection based on performance. Emigrating *L. sylvaticus* selected for forested habitat over clearcuts during both years of study. This supports our hypothesis that temperatures are not likely to be a limiting factor in our study area, unlike in drier/warmer parts of the species range (Rittenhouse et al. 2008). In fact, the similarity of PI in the forested and clearcut microhabitats (except *herbaceous* in 2008, Figure 4.5) and the discrepancy between the field temperatures and PI values suggests that in our system wood frogs might be "heat-starved", and rarely attain their optimal body temperature. This finding has implications for conservation strategies based on the mitigation of microclimate by vegetative succession.

Our results suggest that emigrating juvenile wood frogs do not tend to select habitats primarily due to their microclimatic characteristics. Other factors, such as structural attributes of the vegetation, risk of predation, and food availability are likely to influence the distribution of juvenile wood frogs in relation to disturbance. For example, in a parallel study (Popescu and Hunter in press) found that *L. sylvaticus* emigrating juveniles preferred and settled in a 20-year-old stand (>90% canopy cover and an average height of 9.2 m), but actively avoided a 11-year-old stand (averaging 3.8 m in height and lacking continuous canopy cover) even though they had similar microclimates. One explanation for the avoidance of younger stands might also involve the physical resistance of vegetation to movements. Vegetation was dense in all regeneration treatments (Table 4.1) and a study of habitat permeability to movements of various land cover types showed that juvenile wood frogs moved less frequently and over shorter distances in hayfields that were not mowed (with dense and >0.5 m tall grasses) compared to mowed hayfields and lawns (B.B. Cline, unpublished data). Furthermore, potential predators, such as garter snakes (*Thamnophis sirtalis*), were common in the clearcuts, and predation *per se* or avoidance due to predation pressure could contribute to low wood frog abundance in clearcut habitats. Another potential factor influencing avoidance of young regenerated clearcuts is food availability. Because wood frog juveniles feed on invertebrates within the leaf litter layer, and leaf litter cover in young regenerating stands is generally sparse owing to rapid decomposition and limited input (Oliver and Larson 1996), animals might be food-limited in these stands.

A study of habitat selection by adult *L. sylvaticus* conducted at the same experimental sites as our study found that individuals selected for warmer microhabitats in the forested treatments (Blomquist and Hunter 2010). The same study found that some adult wood frogs did not show avoidance of clearcuts and established home ranges in 1-2 year-old clearcuts, selecting microhabitats based on moisture availability. These two lines of evidence suggest that: (1) animals are not physiologically stressed and seek to enhance their overall performance by selecting warmer microhabitats in forested conditions, and (2) suitable clearcut microhabitats are available at our experimental sites during summer.

Higher use of open habitats by *L. sylvaticus* was also observed in other northern locations of the species range (e.g., central Alberta, Canada, C.A. Paszkowski, personal communication). Such findings might be explained by the genetic adaptation of thermal sensitivity to varied thermal environments, observed for other species with large geographic distributions (Wilson 2001). For example, juvenile striped marsh frogs (*Limnodynastes peronii*) from cooler-temperate environments jumped farther at lower temperatures, compared to animals from warmer-tropical parts of the range (Wilson 2001).

The herbaceous microhabitat was the only treatment that consistently differed from the forested and regenerated treatments in both temperature and moisture availability, but only during afternoon hours. High temperatures and lower moisture conditions during daytime substantially lowered the suitability of this treatment to wood frog movements and settling (*sensu* (Patrick et al. 2008)). These conditions are likely to be characteristic of the microclimate in clearcuts immediately post-harvesting (1 – 2 years), before woody regeneration becomes reestablished. However, the herbaceous treatment was poorly represented at our sites after 5 – 6 years of natural vegetative succession. These conditions should have provided good quality habitat for movements, especially when considering the abundant CWD retained in our treatments (Patrick et al. 2006), which provided moist cool conditions during daytime (Figure 4.2). Nighttime humidity and temperature conditions were similar across all treatments (Figures 4.2, 4.3) and given that emigration movements of juvenile amphibians occur during nighttime and are usually associated with rain events (Semlitsch 1985), extreme daytime microclimate alone cannot explain the avoidance of regenerated clearcuts. Furthermore, despite mitigation of microclimate by vegetative succession, the effects of clearcutting on amphibian distribution and abundance are long-lasting ((Petranka et al. 1993, Ash 1997, Karraker and Welsh 2006, Homyack and Haas 2009), but see (Chazal and Niewiarowski 1998)).

One of the limitations of our study is that I did not account for the effects of dehydration, as I tested only fully hydrated animals. Moisture and temperature interact to determine performance in terrestrial anurans (Preest and Pough 1989). Although highly sensitive to desiccation (Schmid 1965), anurans are able to tolerate certain levels of water loss (e.g., 20% of body mass (Gatten and Clark 1989, Moore and Gatten 1989)). Experimental evidence suggests that the risk of dehydration is considerably greater in clearcut habitats and dry forests compared to moist forest conditions (Rothermel and Semlitsch 2002, Rothermel and Luhring 2005, Rittenhouse et al. 2008). However, these experiments were conducted using terrestrial enclosures, and did not account for the behavioral avoidance of open canopy habitats (Popescu and Hunter in press), as well as for microhabitat selection based on thermal characteristics and moisture availability (Lillywhite 1970, Tracy et al. 1993). For example, cane toads (*Bufo marinus*) and American toads (*A. americanus*) have been found to select for lower temperatures given low moisture conditions and for higher temperatures in humid environments, a process known as behavioral hypothermia (Malvin and Wood 1991, Tracy et al. 1993). Because moisture availability during the emigration season was not an issue in our study (especially in 2009), avoidance of clearcuts cannot be explained by behavioral hypothermia.

In conclusion, our study showed that microclimate cannot be used as a sole parameter to predict potential habitat use by amphibians. Microclimate measurements should be complemented by studies investigating amphibian behavior in relation to structural attributes of the vegetation (e.g., stand age, edges (Popescu and Hunter in press)), risk of predation, and food availability. Furthermore, studies aimed at identifying mechanisms and cues used by amphibians for orientation during movements would provide useful information for amphibian conservation. From a forest management standpoint, it is clear that practices involving complete canopy removal are not conducive to juvenile movements. Given that juveniles in our landscape might be heat-starved, partial canopy removal might actually provide better habitat and enhance juvenile performance by improving the microclimate conditions. However, it is unclear what minimum amount of canopy cover must be retained, and thus further investigations on habitat use and behavior of juvenile amphibians in relation to various amounts of canopy cover are warranted.

REFERENCES

Adler, K. 1980. Individuality in the use of orientation cues by green frogs. Animal Behaviour 28:413-425.

Altwegg, R. 2003. Multistage density dependence in an amphibian. Oecologia 136:46-50.

Alvarez, D. and A. G. Nicieza. 2002. Effects of induced variation in anuran larval development on postmetamorphic energy reserves and locomotion. Oecologia 131:186-195.

Arhipova, K. A., V. V. Borisov, V. Yuanvan, G. Danian, A. S. Kazakov, E. S. Mehova, K. V. Minin, O. V. Nilmaer, T. A. Skvortsov, J. A. Tretiakova, S. S. Ukolova, V. V. Shakhparonov, and S. V. Ogurtsov. 2005. Parameters determining the type of reaction to the native pond odour in juveniles of the common grass frog (*Rana temporaria*) and of the green toad (*Bufo viridis*). Pages 131-141 Flora and Fauna of the Western Part of Moscow Region. Moscow State University, Moscow.

Ash, A. N. 1997. Disappearance and return of plethodontid salamanders to clearcut plots in the Southern Blue Ridge Mountains. Conservation Biology 11:983-989.

Aubin, I., C. Messier, and A. Bouchard. 2008. Can plantations develop understory biological and physical attributes of naturally regenerated forests? Biological Conservation 141:2461-2476.

Baldwin, R. F., A. J. K. Calhoun, and P. G. deMaynadier. 2006. Conservation planning for amphibian species with complex habitat requirements: a case study using movements and habitat selection of the wood frog *Rana sylvatica*. Journal of Herpetology 40:442-453.

Baldwin, R. F., and P. G. deMaynadier. 2009. Assessing threats to pool-breeding amphibian habitat in an urbanizing landscape. Biological Conservation 142:1628-1638.

Bastakov, V. A. 1986. Preference by youngs-of-the-year of the edible frog (*Rana esculenta* complex) for their own reservoir ground smell. Zoologichesky Zhurnal 65:1864 – 1868.

Bastakov, V. A. 1992. Experimental study of the memorizing of pond odor during larval development of two anuran species. Zoologichesky Zhurnal 71:123 - 127.

Bates, D. and M. Maechler. 2009. lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-32. http://CRAN.R-project.org/package=lme4

Batschelet, E. 1981. Circular statistics in biology. Academic Press, London.

Bell, G. 2001. Neutral macroecology. Science 293:2413-2418.

Berven, K. A., and T. A. Grudzien. 1990. Dispersal in the wood frog (*Rana sylvatica*): implications for genetic population structure. Evolution 44:2047-2056.

Birchfield, G. L., and J. E. Deters. 2005. Movement paths of displaced northern green frogs (*Rana clamitans melanota*). Southeastern Naturalist 4:63-76.

Blomquist, S. M., and M. L. Hunter. 2010. A multi-scale assessment of amphibian habitat selection: wood frog response to timber harvesting. Ecoscience 17:251-264.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24:127-135.

Brattstrom, B. H. 1963. A preliminary review of the thermal requirements of amphibians. Ecology 44:238-255.

Brooks, R. T., and T. D. Kyker-Snowman. 2008. Forest floor temperature and relative humidity following timber harvesting in southern New England, USA. Forest Ecology and Management 254:65-73.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multi-model inference. 2nd edition. Springer, Berlin.

Calhoun, A. J. K., and P. G. deMaynadier. 2004. Forestry habitat management guidelines for vernal pool wildlife. Metropolitan Conservation Alliance, Wildlife Conservation Society, Bronx, New York.

Calhoun, A. J. K. and P. G. deMaynadier. 2007. Science and conservation of vernal pools. CRC Press, Boca Raton.

Calhoun, A. J. K., N. A. Miller, and M. W. Klemens. 2005. Conserving pool-breeding amphibians in human-dominated landscapes through local implementation of Best Development Practices. Wetlands Ecology and Management 13:291-304.

Campbell, S. P., J. W. Witham, and M. L. Hunter. 2010. Stochasticity as an alternative to deterministic explanations for patterns of habitat use by birds. Ecological Monographs 80:287-302.

Carlson, D. W. and A. Groot. 1997. Microclimate of clear-cut, forest interior, and small openings in trembling aspen forest. Agricultural and Forest Meteorology 87:313-329.

Chan-McLeod, A. C. A. 2003. Factors affecting the permeability of clearcuts to red-legged frogs. Journal of Wildlife Management 67:663-671.

Chazal, A. C., and P. H. Niewiarowski. 1998. Responses of mole salamanders to clearcutting: using field experiments in forest management. Ecological Applications 8:1133-1143.

Chelgren, N. D., D. K. Rosenberg, S. S. Heppell, and A. I. Gitelman. 2006. Carryover aquatic effects on survival of metamorphic frogs during pond emigration. Ecological Applications 16:250-261.

Chen, J., J. F. Franklin, and T. A. Spies. 1993. Contrasting microclimates among clearcut, edge, and interior of old-growth Douglas-fir forest. Agricultural and Forest Meteorology 63:219-237.

Chen, J., S. C. Saunders, T. R. Crow, R. J. Naiman, K. D. Brosofske, G. D. Mroz, B. L. Brookshire, and J. F. Franklin. 1999. Microclimate in forest ecosystem and landscape ecology. BioScience 49:288-297.

Compton, B. W., K. McGarigal, S. A. Cushman, and L. R. Gamble. 2007. A resistant-kernel model of connectivity for amphibians that breed in vernal pools. Conservation Biology 21:788-799.

Cushman, S. A. 2006. Effects of habitat loss and fragmentation on amphibians: A review and prospectus. Biological Conservation 128:231-240.

Dall'antonia, P., and U. Sinsch. 2001. In search of water: orientation behaviour of dehydrated natterjack toads, *Bufo calamita*. Animal Behaviour 61:617-629.

Davis, J. M., and J. A. Stamps. 2004. The effect of natal experience on habitat preferences. Trends in Ecology & Evolution 19:411-416.

deMaynadier, P. G. and M. L. Hunter. 1995. The relationship between forest management and amphibian ecology: a review of the North American literature. Environmental Reviews 3:230-261.

deMaynadier, P.G., and M. L. Hunter. 1998. Effects of silvicultural edges on the distribution and abundance of amphibians in Maine. Conservation Biology 12:340-352.

Diego-Rasilla, J. and R. Luengo. 2002. Celestial orientation in the marbled newt (*Triturus marmoratus*). Journal of Ethology 20:137-141.

Doak, D. F., P. C. Marino, and P. M. Kareiva. 1992. Spatial scale mediates the influence of habitat fragmentation on dispersal success: Implications for conservation. Theoretical Population Biology 41:315-336.

Eggert, C. 2002. Use of fluorescent pigments and implantable transmitters to track a fossorial toad (*Pelobates fuscus*). Herpetological Journal 12:69-74.

Ewers, R. M., and R. K. Didham. 2006. Confounding factors in the detection of species responses to habitat fragmentation. Biological Reviews 81:117-142.

Fahrig, L. 1992. Relative importance of spatial and temporal scales in a patchy environment. Theoretical Population Biology 41:300-314.

Ferguson, D. E. 1971. The sensory basis of orientation in amphibians. Annals of the New York Academy of Sciences 188:30-36.

Fisher, N. I. 1993. Statistical analysis of circular data. Cambridge University Press, New York.

Forester, D. C. and A. Wisnieski. 1991. The significance of airborne olfactory cues to the recognition of home area by the dart-poison frog *Dendrobates pumilio*. Journal of Herpetology 25:502-504.

Gatten, R. E., Jr. and R. M. Clark. 1989. Locomotor performance of hydrated and dehydrated frogs: recovery following exhaustive exercise. Copeia 1989:451-455.

Gibbs, J. P. 1998. Amphibian movements in response to forest edges, roads, and streambeds in Southern New England. The Journal of Wildlife Management 62:584-589.

Goodyear, C. P. and R. Altig. 1971. Orientation of bullfrogs (*Rana catesbeiana*) during metamorphosis. Copeia 1971:362-364.

Gosner, K. L. 1960. A simplified key for staging anuran embryos and larvae with notes on identification. Herpetologica 16 183-190.

Graeter, G. J., B. B. Rothermel, and J. W. Gibbons. 2008. Habitat selection and movement of pond-breeding amphibians in experimentally fragmented pine forests. Journal of Wildlife Management 72:473-482.

Grubb, J. C. 1973. Olfactory orientation in *Bufo woodhousei fowleri*, *Pseudacris clarki* and *Pseudacris streckeri*. Animal Behaviour 21:726-732.

Haila, Y., A. O. Nicholls, I. K. Hanski, and S. Raivio. 1996. Stochasticity in bird habitat selection: year-to-year changes in territory locations in a boreal forest bird assemblage. Oikos 76:536-552.

Halverson, M. A., D. K. Skelly, J. M. Kiesecker, and L. K. Freidenburg. 2003. Forest mediated light regime linked to amphibian distribution and performance. Oecologia 134:360-364.

Harper, E. B., and R. D. Semlitsch. 2007. Density dependence in the terrestrial life history stage of two anurans. Oecologia 153:879-889.

Hepper, P. G. and B. Waldman. 1992. Embryonic olfactory learning in frogs. The Quarterly Journal of Experimental Psychology Section B: Comparative and Physiological Psychology 44:179 - 197.

Hershey, J. L. and D. C. Forester. 1980. Sensory orientation in *Notophthalmus v. viridescens* (Amphibia: Salamandridae). Canadian Journal of Zoology 58:266–276.

Hill, J. K., and K.C. Hamer. 2004. Determining impacts of habitat modification on diversity of tropical forest fauna: the importance of spatial scale. Journal of Applied Ecology 41:744-754.

Homan, R. N., B. S. Windmiller, and J. M. Reed. 2004. Critical thresholds associated with habitat loss for two vernal pool-breeding amphibians. Ecological Applications 14:1547-1553.

Homan, R. N., M. A. Atwood, A. J. Dunkle, and S. B. Karr. 2010. Movement orientation by adult and juvenile wood frogs (*Rana sylvatica*) and american toads (*Bufo americanus*) over multiple years. Herpetological Conservation and Biology 5:64-72.

Homyack, J. A. and C. A. Haas. 2009. Long-term effects of experimental forest harvesting on abundance and reproductive demography of terrestrial salamanders. Biological Conservation 142:110-121.

Huey, R. B. 1991. Physiological consequences of habitat selection. The American Naturalist 137:S91-S115.

Huey, R. B., D. Berrigan, G. W. Gilchrist, and J. C. Herron. 1999. Testing the adaptive significance of acclimation: A strong inference approach. American Zoologist 39:323-336.

Huey, R. B., and R. D. Stevenson. 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. American Zoologist 19:357-366.

Hunter, M. L., and F. A. Schmiegelow. 2010. Wildlife, forests and forestry: Principles of managing forests for biological diversity, Second edn. Prentice Hall, New Jersey.

Jaeger, R. G., J. A. Wicknick, M. R. Griffis, and C. D. Anthony. 1995. Socioecology of a terrestrial calamander: juveniles enter adult territories during stressful foraging periods. Ecology 76:533-543.

Janin, A., J.-P. Léna, N. Ray, Delacourt, P. Allemand, and P. Joly. 2009. Assessing landscape connectivity with calibrated cost-distance modelling: predicting common toad distribution in a context of spreading agriculture. Journal of Applied Ecology 46:833-841.

Jenkins, C. L., K. McGarigal, and B. C. Timm. 2006. Orientation of movements and habitat selection in a spatially structured population of Marbled Salamanders (*Ambystoma opacum*). Journal of Herpetology 40:240-248.

Joly, P. and C. Miaud. 1993. How does a newt find its pond? The role of chemical cues in migrating newts (*Triturus alpestris*). Ethology, Ecology and Evolution 5:447-455.

Joly, P., M. Claire, and A. Cohas. 2003. Habitat fragmentation and amphibian conservation: building a tool for assessing landscape matrix connectivity. Comptes rendus Biologies 326:132-139.

Karraker, N. E., and H. H. J. Welsh. 2006. Long-term impacts of even-aged timber management on abundance and body condition of terrestrial amphibians in Northwestern California. Biological Conservation 131:132-140.

Karraker, N. E., and J. P. Gibbs. 2009. Amphibian production in forested landscapes in relation to wetland hydroperiod: A case study of vernal pools and beaver ponds. Biological Conservation 142:2293–2302.

Keenan, R. J., and J. P. Kimmins. 1993. The ecological effects of clear-cutting. Environmental Reviews 1:121-144.

Kiesecker, J. M., and D. K. Skelly. 2001. Effects of disease and pond drying on gray tree frog growth, development, and survival. Ecology 82:1956-1963.

Korfel, C. A., W. J. Mitsch, T. E. Hetherington, and J. J. Mack. 2010. Hydrology, physiochemistry, and amphibians in natural and created vernal pool wetlands. Restoration Ecology 18:843-854.

Laan, R., and B. Verboom. 1990. Effects of pool size and isolation of amphibian communities. Biological Conservation 54:251-262.

Lillywhite, H. B. 1970. Behavioral temperature regulation in the bullfrog, *Rana catesbeiana*. Copeia 1970:158-168.

Lindenmayer, D. B. 2009. Forest wildlife management and conservation. Annals of the New York Academy of Sciences 1162:284-310.

Lindenmayer, D. B., J. F. Franklin, and J. Fischer. 2006. General management principles and a checklist of strategies to guide forest biodiversity conservation. Biological Conservation 131:433-445.

Lindenmayer, D. B., and R. J. Hobbs. 2004. Fauna conservation in Australian plantation forests – a review. Biological Conservation 119:151-168.

Liu, W., C. Song, T. A. Schroeder, and W. B. Cohen. 2008. Predicting forest successional stages using multitemporal Landsat imagery with forest inventory and analysis data. International Journal of Remote Sensing 29:3855-3872.

Lund, U. and C. Agostinelli. 2007. circular: Circular Statistics. R package version 0.3-8.

Malvin, G. M., and S. C. Wood. 1991. Behavioral thermoregulation of the toad, *Bufo marinus*: effects of air humidity. Journal of Experimental Zoology 258:322-326.

Marsh, D. M. 2001. Fluctuations in amphibian populations: a meta-analysis. Biological Conservation 101:327-335.

Marsh, D. M., and P. C. Trenham. 2001. Metapopulation dynamics and amphibian conservation. Conservation Biology 15:40-49.

May, R. M. 1973. Stability in randomly fluctuating versus deterministic environments. The American Naturalist 107:621-650.

McGregor, J. H. and W. R. Teska. 1989. Olfaction as an orientation mechanism in migrating *Ambystoma maculatum*. Copeia 1989:779-781.

McRae, B. H. 2006. Isolation by resistance. Evolution 60:1551-1561.

Miaud, C., J. Sérandour, R. Martin, and N. Pidancier. 2003. Preliminary results on the genetic control of dispersal in common frog *Rana temporaria* froglets. Pages 193-197 *in* Herpetologia Petropolitana: Proceedings of the 12th Ordinary Meeting of Societas Europaea Herpetologica. Russian Journal of Herpetology, St. Petersburg.

Moore, F. R. and R. E. Gatten. 1989. Locomotor performance of hydrated, dehydrated, and osmotically stressed anuran amphibians. Herpetologica 45:101-110.

Mousseau, T. A., B. Sinervo, and J. Endler. 2000. Adaptative genetic variation in the wild. Oxford University Press, Oxford, USA.

Murcia, C. 1995. Edge effects in fragmented forests: implications for conservation. Trends in Ecology & Evolution 10:58-62.

Newman, R.A., and T. Squire. 2001. Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). Molecular Ecology 10:1087-1100.

Newton, M., E. C. Cole, D. E. White, and M. L. McCormack. 1992. Young spruce-fir forests released by herbicides I. Response of hardwoods and shrubs. Northern Journal of Applied Forestry 9:126-130.

Nichols, E., T. Larsen, S. Spector, A. L. Davis, F. Escobar, M. Favila, and K. Vulinec. 2007. Global dung beetle response to tropical forest modification and fragmentation: A quantitative literature review and meta-analysis. Biological Conservation 137:1-19.

Ogurtsov, S. V. 2004. Olfactory orientation in anuran amphibians. Russian Journal of Herpetology 11:35-40.

Ogurtsov, S. V. and V. A. Bastakov. 2001. Imprinting on native pond odour in the pool frog, *Rana lessonae*. Pages 433-438 *in* A. Marchlewska-Koj, J. J. Lepri, and D. Müller-Schwarze, editors. Chemical signals in vertebrates 9. Springer-Verlag, New York.

Oliver, C. D. and B. C. Larson. 1996. Forest stand dynamics. Update edition. John Wiley & Sons, New York.

Papi, F. 2006. Navigation of marine, freshwater and coastal animals: concepts and current problems. Marine and Freshwater Behaviour and Physiology 39:3-12.

Parris, K. M., and D. B. Lindenmayer. 2004. Evidence that creation of a *Pinus radiata* plantation in south-eastern Australia has reduced habitat for frogs. Acta Oecologica 25:93-101.

Patrick, D. A., A. J. K. Calhoun, and M. L. Hunter. 2007. Orientation of juvenile wood frogs, *Rana sylvatica*, leaving experimental ponds. Journal of Herpetology 41:158-163.

Patrick, D. A., E. B. Harper, M. L. Hunter, and A. J. K. Calhoun. 2008a. Terrestrial habitat selection and strong density-dependent mortality in recently metamorphosed amphibians. Ecology 89:2563-2574.

Patrick, D. A., A. J. K.Calhoun, and M. L. Hunter. 2008b. The importance of understanding spatial population structure when evaluating the effects of silviculture on spotted salamanders (*Ambystoma maculatum*). Biological Conservation 141:807-814.

Patrick, D. A., M. L. Hunter, and A. J. K. Calhoun. 2006. Effects of experimental forestry treatments on a Maine amphibian community. Forest Ecology and Management 234:323-332.

Petranka, J. W., M. E. Eldridge, and K. E. Haley. 1993. Effects of timber harvesting on Southern Appalachian salamanders. Conservation Biology 7:363-370.

Pinheiro, J. C. and D. M. Bates. 2000. Mixed effects models in S and S-Plus. Springer-Verlag, New York.

Popescu, V. D., and J. P. Gibbs. 2009. Landscape ecology and GIS methods. Pages 339-360 *in* K. C. Dodd, Jr., editor. Amphibian ecology and conservation. Oxford University Press, New York.

Popescu, V. D., and M. L. Hunter. in press. Clearcutting affects habitat connectivity for a forest amphibian by decreasing permeability to juvenile movements. Ecological Applications.

Pough, F. H., E. M. Smith, D. H. Rhodes, and A. Collazo. 1987. The abundance of salamanders in forest stands with different histories of disturbance. Forest Ecology and Management 20:1-9.

Preest, M. R. and F. H. Pough. 1989. Interaction of temperature and hydration on locomotion of toads. Functional Ecology 3:693-699.

R Development Core Team. 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org.

Ramovs, B. V., and M. R. Roberts. 2003. Understory vegetation and environment responses to tillage, forest harvesting, and conifer plantation development. Ecological Applications 13:1682-1700.

Ray, N., A. Lehmann, and P. Joly. 2002. Modeling spatial distribution of amphibian populations: a GIS approach based on habitat matrix permeability. Biodiversity and Conservation 11:2143-2165.

Reshetnikov, A. N. 1996. Hygrotactic and olfactory orientation in juvenile common toads (*Bufo bufo*) during the postmetamorphic period. Advances in Amphibian Research in the Former Soviet Union 1:181-190.

Richter-Boix, A., G. A. Llorente, and A. Montori. 2007. Structure and dynamics of an amphibian metacommunity in two regions. Journal of Animal Ecology 76:607-618.

Ricketts, T. H. 2001. The matrix matters: effective isolation in fragmented landscapes. The American Naturalist 158:87-99.

Rittenhouse, T. A. G., E. B. Harper, L. R. Rehard, and R. D. Semlitsch. 2008. The role of microhabitats in the desiccation and survival of anurans in recently harvested oak-hickory forest. Copeia:807-814.

Roe, A. W., and K. L. Grayson. 2008. Terrestrial movements and habitat use of juvenile and emigrating adult Eastern Red-Spotted Newts, *Notophthalmus viridescens*. Journal of Herpetology 42:22-30.

Rome, L. L., E. D. Stevens, and H. B. John-Alder. 1992. The influence of temperature and thermal acclimation on physiological function. Pages 183-205 *in* M. E. Feder and W. W. Burggren, editors. Environmental Physiology of the Amphibians. University of Chicago Press, Chicago.

Rosenvald, R., and A. Lõhmus. 2008. For what, when, and where is green-tree retention better than clear-cutting? A review of the biodiversity aspects. Forest Ecology and Management 255:1-15.

Ross-Davis, A. L., and K. A. Frego. 2002. Comparison of plantations and naturally regenerated clearcuts in the Acadian Forest: forest floor bryophyte community and habitat features. Canadian Journal of Botany 80:21-33.

Rothermel, B. B. 2004. Migratory success of juveniles: a potential constraint on connectivity for pond-breeding amphibians. Ecological Applications 14:1535-1546.

Rothermel, B. B. and T. M. Luhring. 2005. Burrow availability and desiccation risk of mole salamanders (*Ambystoma talpoideum*) in harvested versus unharvested forest stands. Journal of Herpetology 39:619-626.

Rothermel, B. B. and R. D. Semlitsch. 2002. An experimental investigation of landscape resistance of forest versus old-field habitats to emigrating juvenile amphibians. Conservation Biology 16:1324-1332.

Rothermel, B. B. and R. D. Semlitsch. 2006. Consequences of forest fragmentation for juvenile survival in spotted (*Ambystoma maculatum*) and marbled (*Ambystoma opacum*) salamanders. Canadian Journal of Zoology 84:797-807.

Rozhok, A. 2008. Orientation and navigation in vertebrates. Springer-Verlag, Berlin.

Roznik, E. A. and S. A. Johnson. 2009a. Burrow use and survival of newly metamorphosed gopher frogs (*Rana capito*). Journal of Herpetology 43:431-437.

Roznik, E. A. and S. A. Johnson. 2009b. Canopy closure and emigration by juvenile gopher frogs. Journal of Wildlife Management 73:260-268.

Roznik, E. A., S. A. Johnson, C. H. Greenberg, and G. W. Tanner. 2009. Terrestrial movements and habitat use of gopher frogs in longleaf pine forests: A comparative study of juveniles and adults. Forest Ecology and Management 259:187-194.

Russell, R. E., R. K. Swihart, and Z. Feng. 2003. Population consequences of movement decisions in a patchy landscape. Oikos 103:142-152.

Šamajová, P. and L. Gvoždík. 2010. Inaccurate or disparate temperature cues? Seasonal acclimation of terrestrial and aquatic locomotor capacity in newts. Functional Ecology 24:1023-1030.

Sanuy, D. and P. Joly. 2009. Olfactory cues and breeding habitat selection in the natterjack toad, *Bufo calamita*. Amphibia-Reptilia 30:555-559.

Saunders, M. and R. Wagner. 2008. Long-term spatial and structural dynamics in Acadian mixedwood stands managed under various silvicultural systems. Canadian Journal of Forest Research 38:498-517.

Schlaepfer, M. A., M. C. Runge, and P. W. Sherman. 2002. Ecological and evolutionary traps. Trends in Ecology and Evolution 17:474-480.

Schmid, W. D. 1965. Some aspects of the water economies of nine species of amphibians. Ecology 46:261-269.

Seebacher, F. and R. A. Alford. 2002. Shelter microhabitats determine body temperature and dehydration rates of a terrestrial amphibian (*Bufo marinus*). Journal of Herpetology 36:69-75.

Semlitsch, R. D. 1985. Analysis of climatic factors influencing migrations of the salamander *Ambystoma talpoideum*. Copeia 1985:477-489.

Semlitsch, R.D. 1998. Biological delineation of terrestrial buffer zones for pond-breeding salamanders. Conservation Biology 12:1113-1119.

Semlitsch, R. D. 2000. Principles for management of aquatic-breeding amphibians. Journal of Wildlife Management 64:615-631.

Semlitsch, R. D. 2002. Critical elements for biologically based recovery plans of aquatic-breeding amphibians. Conservation Biology 16:619-629.

Semlitsch, R. D. 2008. Differentiating migration and dispersal processes for pond-breeding amphibians. Journal of Wildlife Management 72:260-267.

Semlitsch, R. D., C. A. Conner, D. J. Hocking, T. A. G. Rittenhouse, and E. B. Harper. 2008. Effects of timber harvesting on pond-breeding amphibian persistence: testing the evacuation hypothesis. Ecological Applications 18:283-289.

Semlitsch, R. D., B. D. Todd, S. M. Blomquist, A. J. K. Calhoun, J. W. Gibbons, J. P. Gibbs, G. J. Graeter, E. B. Harper, D. J. Hocking, M. L. Hunter, D. A. Patrick, T. A. G. Rittenhouse, and B. B. Rothermel. 2009. Effects of timber harvest on amphibian populations: understanding mechanisms from forest experiments. BioScience 59:853-862.

Shakhparonov, V. V. and S. V. Ogurtsov. 2003. The role of native pond odor in orientation of the green toad (*Bufo viridis* Laur.) youngs-of-the-year. Pages 209 – 212 *in* Herpetologia Petropolitana: Proceedings of the 12th Ordinary Meeting of Societas Europaea Herpetologica. Russian Journal of Herpetology, St. Petersburg.

Sinsch, U. 1990. Migration and orientation in anuran amphibians. Ethology, Ecology and Evolution 2:65-79.

Sinsch, U. 1991. Mini-review: the orientation behaviour of amphibians. Herpetological Journal 1:541-544.

Sinsch, U. 2006. Orientation and navigation in Amphibia. Marine & Freshwater Behaviour & Physiology 39:65-71.

Sjögren, P. 1991. Extinction and isolation gradients in metapopulations: the case of the pool frog (*Rana lessonae*). Biological Journal of the Linnean Society 42:135-147.

Sjögren-Gulve, P. 1994. Distribution and extinction patterns within a northern metapopulation of the pool frog, *Rana lessonae*. Ecology 75:1357-1367.

Skelly, D. K. 2004. Microgeographic countergradient variation in the wood frog, *Rana sylvatica*. Evolution 58:160-165.

Skelly, D. K., L. K. Freidenburg, and J. M. Kiesecker. 2002. Forest canopy and the performance of larval amphibians. Ecology 83:983-992.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787-792.

Smith, A. M., and D. M. Green. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography 28:110-128.

Stephens, S. E., D. N. Koons, J. J. Rotella, D. W. Willey. 2004. Effects of habitat fragmentation on avian nesting success: a review of the evidence at multiple spatial scales. Biological Conservation 115:101-110.

Stevens, V., E. Polus, R. Wesselingh, N. Schtickzelle, and M. Baguette. 2005. Quantifying functional connectivity: Experimental evidence for patch-specific resistance in the Natterjack toad (*Bufo calamita*). Landscape Ecology 19:829-842.

Swanson, F. J. and J. F. Franklin. 1992. New forestry principles from ecosystem analysis of Pacific Northwest forests. Ecological Applications 2:262-274.

Tejedo, M., R. D. Semlitsch, and H. Hotz. 2000. Differential morphology and jumping performance of newly metamorphosed frogs of the hybridogenetic *Rana esculenta* complex. Journal of Herpetology 34:201-210.

Tews, J., U. Brose, V. Grimm, K.Tielbörger, M. C.,Wichmann, M. Schwager, and F. Jeltsch. 2004. Animal species diversity driven by habitat heterogeneity/diversity: the importance of keystone structures. Journal of Biogeography 31:79-92.

Timm, B. C., K. McGarigal, and C. L. Jenkins. 2007. Emigration orientation of juvenile pond-breeding amphibians in western Massachusetts. Copeia:685-698.

Todd, B. D., T. M. Luhring, B. B. Rothermel, and J. W. Gibbons. 2009. Effects of forest removal on amphibian migrations: implications for habitat and landscape connectivity. Journal of Applied Ecology 46:554-561.

Torsten H., K. Hornik, M. A. van de Wiel, A. Zeileis. 2008. Implementing a Class of Permutation Tests: The coin Package. Journal of Statistical Software 28(8), 1-23. URL http://www.jstatsoft.org/v28/i08/.

Tracy, C. R. 1976. A model of the dynamic exchanges of water and energy between a terrestrial amphibian and its environment. Ecological Monographs 46:293-326.

Tracy, C. R., K. A. Christian, M. P. O'Connor, and C. R. Tracy. 1993. Behavioral thermoregulation by *Bufo americanus*: The Importance of the hydric environment. Herpetologica 49:375-382.

Tracy, C. R. and J. W. Dole. 1969. Orientation of displaced California toads, *Bufo boreas*, to their breeding sites. Copeia 1969:693-700.

Trenham, P.C. and H. B. Shaffer. 2005. Amphibian upland habitat use and its consequences for population viability. Ecological Applications 15:1158-1168.

Vasconcelos, D. and A. J. K. Calhoun. 2004. Movement patterns of adult and juvenile *Rana sylvatica* (LeConte) and *Ambystoma maculatum* (Shaw) in three restored seasonal pools in Maine. Journal of Herpetology 38:551-561.

Veysey, J. S., K. J. Babbitt, and A. Cooper. 2009. An experimental assessment of buffer width: Implications for salamander migratory behavior. Biological Conservation 142:2227-2239.

Via, S. and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39:505-522.

Vonesh, J. and O. De la Cruz. 2002. Complex life cycles and density dependence: assessing the contribution of egg mortality to amphibian declines. Oecologia 133:325-333.

Vos, C. C., P. W. Goedhart, D. R. Lammertsma, and A. M. Spitzen-Van der Sluijs. 2007. Matrix permeability of agricultural landscapes: an analysis of movements of the common frog (*Rana temporaria*). Herpetological Journal 17:174-182.

Waldick, R. C., B. Freedman, and R. J. Wassersug. 1999. The consequences for amphibians of the conversion of natural, mixed-species forests to conifer plantations in southern New Brunswick. Canadian Field-Naturalist 113:408-418.

Walston, L. J., and S. J. Mullin. 2008. Variation in amount of surrounding forest habitat influences the initial orientation of juvenile amphibians emigrating from breeding ponds. Canadian Journal of Zoology 86:141-146.

Walton, M. 1988. Relationships among metabolic, locomotory, and field measures of organismal performance in the Fowler's toad (*Bufo woodhousei fowleri*). Physiological Zoology 61:107-118.

Watkins, T. B. 2001. A quantitative genetic test of adaptive decoupling across metamorphosis for locomotor and life-history traits in the Pacific tree frog, *Hyla regilla*. Evolution 55:1668-1677.

Weng, S.-H., S.-R. Kuo, B. T. Guan, T.-Y. Chang, H.-W. Hsu, and C.-W. Shen. 2007. Microclimatic responses to different thinning intensities in a Japanese cedar plantation of northern Taiwan. Forest Ecology and Management 241:91-100.

Werner, E. E., K. L. Yurewicz, D. K. Skelly, and R. A. Relyea. 2007. Turnover in an amphibian metacommunity: the role of local and regional factors. Oikos 116:1713-1725.

Wiens, J.A. 1989. Spatial scaling in ecology. Functional Ecology 3:385-397.

Wilson, R. S. 2001. Geographic variation in thermal sensitivity of jumping performance in the frog *Limnodynastes peronii*. Journal of Experimental Biology 204:4227-4236.

Winfree, R., J. Dushoff, Elizabeth E. Crone, Cheryl B. Schultz, Robert V. Budny, Neal M. Williams, and C. Kremen. 2005. Testing simple indices of habitat proximity. The American Naturalist 165:707-717.

Xu, M., J. Chen, and Y. Qi. 2002. Growing-season temperature and soil moisture along a 10 km transect across a forested landscape. Climate Research 22:57-72.

Yang, J., Y. Wang, and D. R. Miller. 2007. Estimating air temperature profiles in forest canopy using empirical models and Landsat data. Forest Science 53:93-99.

Zamudio, K. R., and A. M.Wieczorek. 2007. Fine-scale spatial genetic structure and dispersal among spotted salamander (*Ambystoma maculatum*) breeding populations. Molecular Ecology 16:257-274.

Zellmer, A. J., and L. L. Knowles. 2009. Disentangling the effects of historic vs. contemporary landscape structure on population genetic divergence. Molecular Ecology 18:3593-3602.

Zheng, D., J. Chen, B. Song, M. Xu, P. Sneed, and R. Jensen. 2000. Effects of silvicultural treatments on summer forest microclimate in southeastern Missouri Ozarks. Climate Research 15:45-59.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer, New York.

APPENDIX A

Sex ratios of breeding adults at experimental sites

Table A.1. Sex ratios of *L. sylvaticus* and *A. maculatum* breeding in four experimental pools (Pools = number of pools sampled each year).

Year	***L. sylvaticus***			***A. maculatum***			**Pools**
	Female	Male	F:M	Female	Male	F:M	
2005	158	191	0.8:1	275	64	4.3:1	4
2006	37	92	0.4:1	187	113	1.65:1	3
2007	86	155	0.5:1	73	64	0.9:1	2
2008	103	183	0.6:1	192	129	1.5:1	4
2009	86	85	1:1	93	88	1.05:1	3

APPENDIX B

Captures of juvenile amphibians at experimental sites

Table B.1. Number of newly metamorphosed *L. sylvaticus* and *A. maculatum* emerging from experimental pools and recaptured in the upland habitat (n = number of pools that produced animals).

Year	**Total emerged from experimental pools**		**Total recaptured in upland habitat**	
	L. sylvaticus	*A. maculatum*	*L. sylvaticus*	*A. maculatum*
2004	2342 (n=4)	272 (n=4)	81	32
2005	6085 (n=4)	589 (n=3)	1061	35
2006	2083 (n=4)	319 (n=4)	410	14
2007	328 (n=1)	211 (n=4)	41	4
2008	3133 (n=3)	122 (n=3)	392	2
2009	95 (n=1)	8 (n=1)	8	0

APPENDIX C

Habitat permeability to juvenile wood frog movements

Table C.1. Proportion of released juvenile *L. sylvaticus* reaching 10, 20, 30, and 40 m distance from the forest edge in four forestry treatments in 2008 and 2009.

Distance from edge			**Treatment**			
			Clearcut	**11-yr stand**	**20-yr stand**	**Mature forest**
10 m	*mean ± SE**	2008	0.32 ± 0.08	0.56 ± 0.06	-	0.74 ± 0.08
		2009	0.29 ± 0.03	0.32 ± 0.07	0.60 ± 0.05	0.59 ± 0.04
	*Coefficient ± SE ***		-0.87 ± 0.21	-0.30 ± 0.20	0.61 ± 0.25	0.62 ± 0.21
	*Predicted 95% CI ****		(-1.30, -0.45)	(-0.71, 0.10)	(0.12, 1.11)	(0.21, 1.04)
20 m	*mean ± SE*	2008	0.28 ± 0.06	0.30 ± 0.05	-	0.61 ± 0.11
		2009	0.17 ± 0.03	0.16 ± 0.03	0.54 ± 0.06	0.60 ± 0.04
	Coefficient ± SE		-1.26 ± 0.22	-1.27 ± 0.22	0.29 ± 0.24	0.41 ± 0.20
	Predicted 95% CI		(-1.69, -0.83)	(-1.71, -0.83)	(-0.19, 0.77)	(0.015, 0.81)
30 m	*mean ± SE*	2008	0.27 ± 0.06	0.28 ± 0.04	-	0.56 ± 0.10
		2009	0.11 ± 0.03	0.13 ± 0.03	0.40 ± 0.07	0.51 ± 0.04
	Coefficient ± SE		-1.55 ± 0.24	-1.50 ± 0.24	-0.19 ± 0.26	0.11 ± 0.22
	Predicted 95% CI		(-2.04, -1.06)	(-1.99, -1.02)	(-0.72, 0.32)	(-0.32, 0.50)
40 m	*mean ± SE*	2008	0.25 ± 0.05	0.26 ± 0.06	-	0.55 ± 0.11
		2009	0.07 ± 0.2	0.11 ± 0.03	0.25 ± 0.05	0.45 ± 0.05
	Coefficient ± SE		-1.83 ± 0.30	-1.68 ± 0.29	-0.81 ± 0.32	-0.04 ± 0.27
	Predicted 95% CI		(-2.43, -1.24)	(-2.26, -1.10)	(-1.40, -0.17)	(-0.56, 0.48)

* Observed proportions of released animals reaching each tracking station

** Untransformed logit coefficients ± 1SE are those of the fixed effects from the best (lowest AIC) mixed effects binomial models fitted for each tracking station distance for 2008 and 2009.

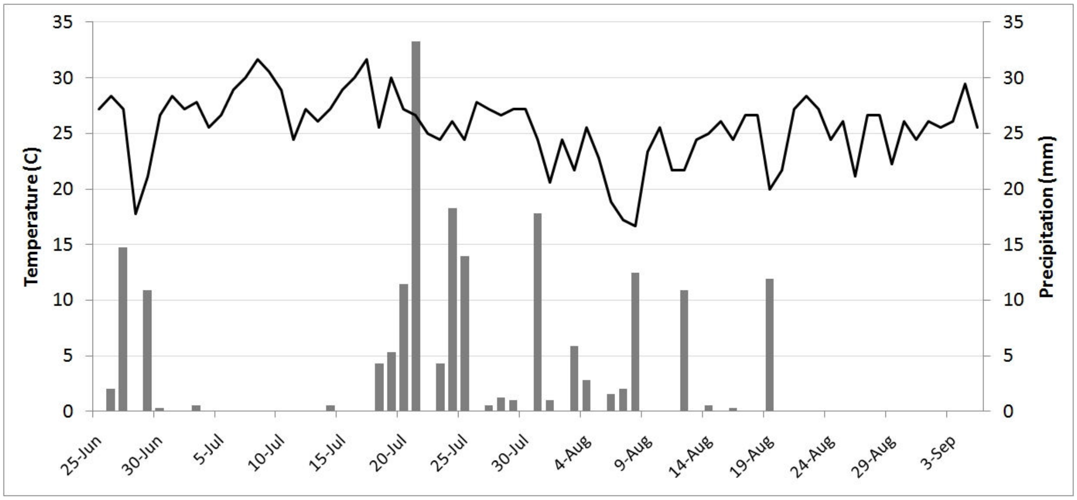
*** 95% confidence intervals for the predicted logit coefficients

APPENDIX D

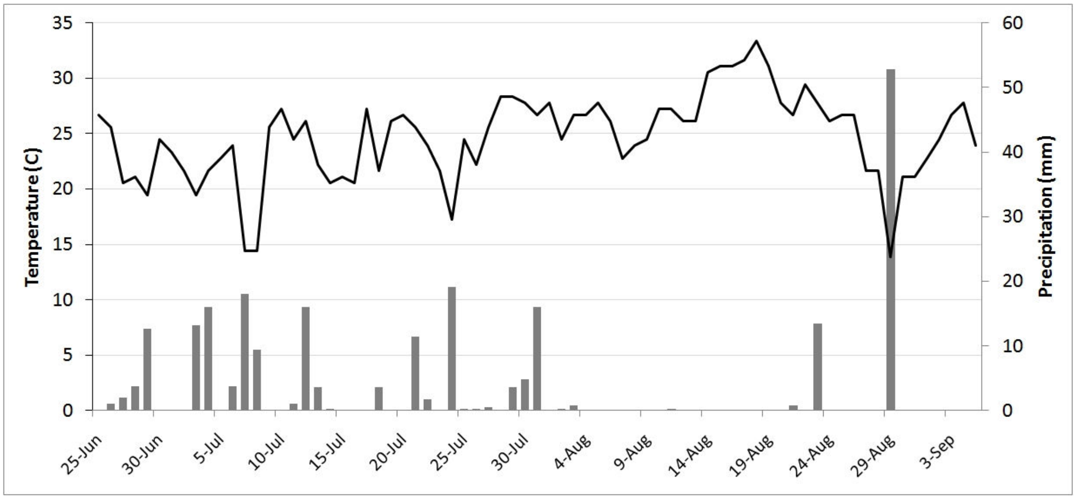
Weather patterns during the 2008 and 2009 juvenile emigration seasons

Figure D.1. Maximum daily temperatures (black line) and total daily precipitation (gray bars) recorded during the 2008 (a) and 2009 (b) sampling seasons (data from *www.ncdc.noaa.gov* for Bangor International Airport, located 15-20 km from the experimental sites).

a)



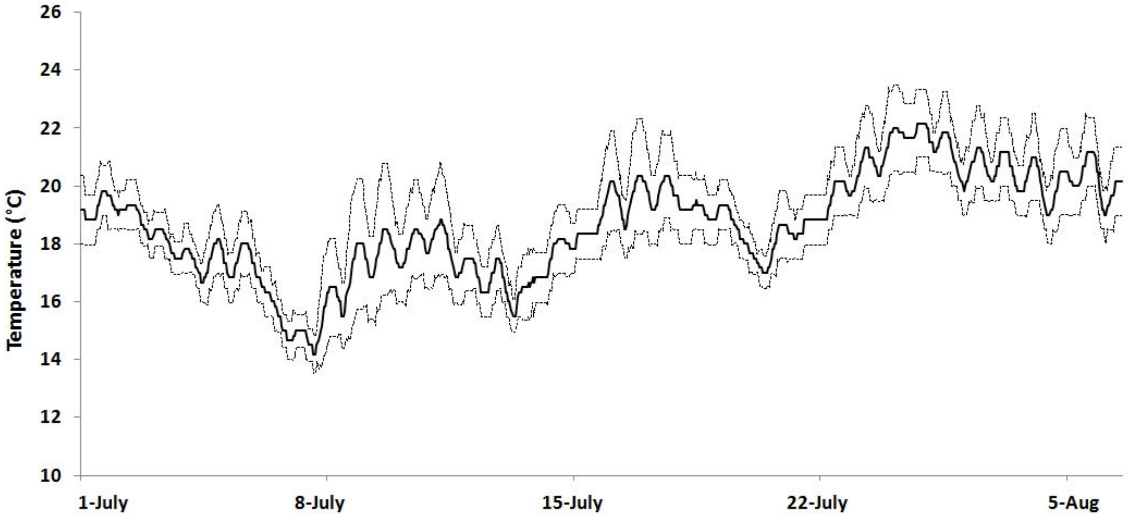
b)



APPENDIX E

Water temperature in wood frog larvae-rearing mesocosms

Figure E.1. Average water temperature of mesocosms (1500-l cattle tanks) used to rear wood frog larvae during the emergence season of experimental animals in 2009 (dotted lines represent 95% confidence intervals).



BIOGRAPHY OF THE AUTHOR

Viorel Dan Popescu was born in Braila, Romania on 29 April 1979. He received his Bachelor of Science in Environmental Sciences from the University of Bucharest, Romania. During his undergraduate studies, he became involved in environmental research conducted at the Centre for Environmental Research – University of Bucharest, and in 2000 he was offered a research assistant job under the supervision of Dr. Maria Patroescu. During this tenure, he worked on a variety of biological conservation problems in Romania, spanning from species conservation (reptiles, large carnivores, amphibians) to environmental education and community outreach. In 2005 he was awarded a Fulbright Scholarship for pursuing a Master of Science in conservation biology at SUNY-College of Environmental Science and Forestry, Syracuse, NY. For his Masters thesis he worked with Dr. James Gibbs on the relation between pond occupancy by mink frogs, beaver disturbance, and climate change in the Adirondack Mountains, NY.

Viorel entered the Department of Wildlife Ecology at the University of Maine in 2007 to work with Dr. Mac Hunter on the effects of forest management on amphibian communities. He was awarded the Maine Economic Improvement Fund Doctoral Fellowship in 2010, and was recognized by the College of Natural Sciences, Forestry and Agriculture with the George Dow Award for excellence in research in 2010. After receiving his degree Viorel will join University of California – Berkeley as a postdoctoral researcher to work on hierarchical modeling of wildlife data. Viorel is a candidate for the Doctor of Philosophy degree in Wildlife Ecology from the University of Maine in May, 2011.