

8-13-2014

# Manipulation of Larval and Winter Habitat Reveals Potential Effects of Urbanization and Climate Change on Wood Frogs in Connecticut

Jason H. O'Connor

*University of Connecticut - Storrs*, [jason.h.oconnor@uconn.edu](mailto:jason.h.oconnor@uconn.edu)

---

## Recommended Citation

O'Connor, Jason H., "Manipulation of Larval and Winter Habitat Reveals Potential Effects of Urbanization and Climate Change on Wood Frogs in Connecticut" (2014). *Master's Theses*. 644.  
[https://opencommons.uconn.edu/gs\\_theses/644](https://opencommons.uconn.edu/gs_theses/644)

This work is brought to you for free and open access by the University of Connecticut Graduate School at OpenCommons@UConn. It has been accepted for inclusion in Master's Theses by an authorized administrator of OpenCommons@UConn. For more information, please contact [opencommons@uconn.edu](mailto:opencommons@uconn.edu).

# Manipulation of Larval and Winter Habitat Reveals Potential Effects of Urbanization and Climate Change on Wood Frogs in Connecticut

Jason Harding O'Connor

B.S., University of Miami, 2011

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

At the

University of Connecticut

2014

# APPROVAL PAGE

Masters of Science Thesis

## Manipulation of Larval and Winter Habitat Reveals Potential Effects of Urbanization and Climate Change on Wood Frogs in Connecticut

Presented by

Jason Harding O'Connor, B.S.

Major Advisor\_\_\_\_\_ Tracy A.G. Rittenhouse

Associate Advisor\_\_\_\_\_ Isaac M. Ortega

Associate Advisor\_\_\_\_\_ Mark C. Urban

University of Connecticut

2014

## **Acknowledgments**

I first and foremost thank my advisor Tracy Rittenhouse. This research project would not have been possible without the Tracy's support and I am deeply indebted to Tracy for deciding to make me part of her inaugural lab. The research topics investigated herein were undoubtedly seeded in my thoughts during extensive discussion with Tracy during the early stages of my studies. Over the past two years, Tracy has offered advice, criticism and encouragement which have challenged me to grow as a scientist and professional, for which I am very grateful.

I also thank my committee members, Morty Ortega and Mark Urban for providing resources necessary to carry out portions of this study and for helpful comments and suggestions during both planning and writing stages of this project. I am also grateful for helpful suggestions and insightful discussions with Jason Vokoun which have challenged me to consider the broader context and implications of my research.

I have benefited greatly from the NRE graduate community. I am extremely thankful for field assistance provided by Sarah Banker, Lindsey Dreiss, Emily Hall, Rachael Hyland, Jaron Kolek, David Rosa, and Annie Stupik. From 9am Monday morning presentation critiques to midnight frog catching excursions, my lab mates Michael Evans, Jan-Michael Hessenauer, Cristina Macklem, and Kelly O'Connor, have been invaluable over the past two years.

Finally, for supporting me, encouraging me to pursue my passions and tolerating my chronic absence at family events I thank my parents, my brother Adam, and my sisters Kaitlyn and Nadya.

## Table of Contents

<b>Introduction.....</b>	<b>1</b>
<b>Chapter 1: Silt additions increase wood frog growth and speed development in mesocosms</b>	
Abstract .....	5
Introduction .....	6
Methods .....	9
<i>Animal Collection and Husbandry</i> .....	9
<i>Experimental Design</i> .....	9
<i>Data Analysis</i> .....	11
Results .....	11
Discussion .....	12
References .....	17
Tables .....	20
Figures .....	21
<b>Chapter 2: Survival of a terrestrially overwintering amphibian in experimentally altered microhabitats</b>	
Abstract .....	25
Introduction .....	26
Methods .....	30
<i>Animal Collection</i> .....	31
<i>Radio-Telemetry</i> .....	32
<i>Survival Monitoring</i> .....	32
<i>Data Analysis</i> .....	34
Results .....	37

<i>Tracking</i> .....	37
<i>Habitat Selection</i> .....	38
<i>Overwinter Microhabitat Conditions</i> .....	39
Discussion .....	40
<i>Winter Survival</i> .....	40
<i>Late Fall Movement</i> .....	44
<i>Conclusion</i> .....	46
References .....	47
Tables .....	50
Figures .....	56

## **Introduction**

Globally, amphibian populations are threatened by habitat loss and fragmentation, disease, invasive species, over-collection and climate change (Beebee and Griffiths 2005). Many of these problems stem from human activity, and are growing as a result of rapid human population growth. Habitat loss in particular is driven by conversion of natural lands for urban and agricultural use. The effect of human development is further compounded by the effects of human activity in developed areas. Once an area has been converted to urban or agricultural land, human activity can result in degradation of the surrounding habitat by increasing the consumption of resources such as water, and by producing and exporting pollutants and waste products.

Human population growth is increasing demand for both housing and agriculture, thus loss of natural lands will loom as an ever growing threat for wildlife species in the future. Given that many areas around the world are rapidly developing, learning how to maintain species diversity in human-influenced landscapes is a growing challenge in conservation biology. Understanding how organisms respond to typical patterns of habitat alteration resulting from development will aid development of conservation actions to maintain biodiversity in developed areas.

Urban and agricultural activity have been shown to affect water quality by increasing turbidity and pollutant levels (Paul & Meyer 2001, Matthaei et al. 2010). High turbidity generally results from increased transport of sediment into water bodies and has been shown to deleteriously affect fish and invertebrate communities. While amphibians are major components of many wetland ecosystems, amphibian responses to augmented sediment loads are relatively

unknown. Understanding amphibian response to anthropogenic habitat change will allow for better conservation of wetland communities in urban landscapes.

In addition to affecting local habitats, human activity has caused a precipitous rise in greenhouse gas emissions which are causing changes in global climate. As a result, winter temperatures in the northeastern United States have increased by 0.7°C over the past century and this trend is expected to continue (Rustad et al. 2012). Climate change is also expected to increase precipitation in the northeast (Hayhoe et al. 2007). Amphibian responses to climate change have already been observed in some areas, but much remains unknown. In order to develop effective conservation measures, it will be necessary to predict how amphibians will respond to predicted changes. In the northeastern United States climate change may have the greatest potential to directly affect amphibian populations during winter. Overwintering amphibians enter a period of reduced activity, leaving them vulnerable to changes in temperature and moisture conditions, without the ability to behaviorally locate new, tolerable microhabitats.

Wood frogs *Lithobates sylvaticus* are a widely distributed ranid that range from north of the Arctic circle in Alaska, east to the Atlantic coast of North America and south to North Carolina. By virtue, of their wide range and explosive spring breeding behavior, Wood Frogs have been a model organism for understanding community dynamics in seasonal wetlands, effects of anthropogenic pollutants on aquatic organisms, and the effects of forest management actions affect amphibians. Since, much is known about wood frog response to variety of environmental conditions, they are a particularly suitable species for studying how human induced habitat changes affect pond breeding amphibians. Wood frog physiology has also been extensively studied due to their ability to survive freezing. Despite numerous physiological studies on Wood Frog freeze tolerance over the past 30 years, very little is known about the



conditions Wood Frogs actually experience during winter. The dearth of information on overwintering ecology is a problem for most northern amphibian species. As a freeze tolerant organism, Wood Frogs use habitat during the winter that is more exposed than species that overwinter underwater or in deep burrows. Therefore, Wood Frogs and other freeze tolerant amphibians may be most susceptible to changes in winter climate. Information on this understudied life history stage will be useful for understanding the risk that climate change poses to northeastern amphibians.

## References

- Beebee TJC and Griffiths RA. 2005. The amphibian decline crisis: A watershed for conservation biology? *Biol Conserv* 125(3):271-85.
- Hayhoe K, Wake C, Huntington T, Luo L, Schwartz M, Sheffield J, Wood E, Anderson B, Bradbury J, DeGaetano A, et al. 2007. Past and future changes in climate and hydrological indicators in the US northeast. *Clim Dyn* 28(4):381-407.
- Matthaei CD, Piggott JJ, Townsend CR. 2010. Multiple stressors in agricultural streams: Interactions among sediment addition, nutrient enrichment and water abstraction. *J Appl Ecol* 47(3):639-49.
- Paul MJ and Meyer JL. 2001. Streams in the urban landscape. *Annu Rev Ecol Syst* :333-65.
- Rustad L, Campbell J, Dukes JS, Huntington T, Lambert KF, Mohan J, Rodenhouse N. 2011. Changing climate, changing forests: The impacts of climate change on forests of the northeastern united states and eastern canada. Newtown Square, PA: U.S. Forest Service. Report nr NRS-99.

**Silt additions increase wood frog growth and speed development in  
mesocosms**

Jason H. O'Connor and Tracy A.G. Rittenhouse

Department of Natural Resources and the Environment

University of Connecticut, Storrs, CT, USA.

**Abstract:**

Runoff from urban development and agricultural activity increases sediment input and water turbidity in many aquatic systems. These factors are known to affect fish and invertebrate communities but effects on amphibians are poorly understood. Runoff can transport nitrogenous compounds, heavy metals, pesticides, and other pollutants in addition to sediment. Our goal was to isolate the effect of sediment input from these other potential aquatic stressors. We manipulated silt addition in mesocosms to determine if sediment input affected survival, growth, or development of larval wood frogs (*Lithobates sylvaticus*). We also crossed our silt addition treatment with a predator presence treatment to assess interactive effects of multiple stressors. We found no effect of silt addition or predator presence on survival. Furthermore, addition of a large amount of silt during the early larval period resulted in earlier metamorphosis ( $F_{1,30} = 5.111$ ,  $p = 0.031$ ) at a larger size ( $F_{1,30} = 36.244$ ,  $p < 0.001$ ), traits generally viewed as positive for population dynamics. Non-lethal predator presence did not affect either mass at or time to metamorphosis. Results suggest that suspended sediment by itself is not directly harmful to wood frogs and potentially may serve as an additional food resource. Manipulating turbidity in mesocosms has advantages over studying turbidity in natural systems because treatments can be controlled and replicated sufficiently. Future research should investigate the relationship between sediment organic content and tadpole growth and survival and the interaction between water turbidity and other wetland stressors.

## Introduction

Sediment deposition and associated increases in water turbidity are major water quality concerns in areas affected by agriculture and urban development (Paul & Meyer 2001, Göbel et al. 2007, Matthaei et al. 2010). Increased suspended sediment and turbidity levels can be detrimental to aquatic organisms. Invertebrate species richness and density tend to decline with increasing sediment loads and turbidity (Roy et al. 2003, Sharma and Rawat 2009, Shilla & Shilla 2011). Similarly, declines in fish diversity have been observed in streams affected by human development (Richardson & Jowett 2002, Mol & Ouboter 2004). However, deleterious effects are not ubiquitous across aquatic taxa. For instance, oligochaete worm abundance increases with increasing turbidity and sedimentation (Freeman & Schorr 2004, Suttle et al. 2004). Likewise, sedimentation due to human activity can cause an increase in abundance of fish that spawn on fine sediment (Sutherland 2002).

Increased water turbidity has also been shown to reduce growth rates in fish and invertebrates (Sutherland & Meyer 2007, Gonzalez et al. 2010). Reduced growth may be directly caused by ingestion of sediment particles which can reduce assimilation efficiency (Broekhuizen et al. 2001). Turbidity can also affect primary productivity (Ryan 1991, Schallenberg & Burns 2004) which can alter the amount of food available to consumers. Additionally, turbidity conditions can affect behaviors that influence growth rates. For instance, prey species in a turbid environment may be more active and feed longer than in a non-turbid environment because the turbidity may provide cover from predators (Van de Muetter et al. 2005, Chivers et al. 2013).

Amphibians are major components of aquatic habitats and are sensitive to habitat changes, but relatively little is known about how amphibians respond to anthropogenic sediment inputs (Wood & Richardson 2009). Wood frogs (*Lithobates sylvaticus*) are a wide ranging

species, which occur across much of northern North America. They are explosive early spring breeders and utilize diverse breeding habitat, although they are often associated with ephemeral wetlands. Like many pond breeding amphibians, wood frog tadpoles are sensitive to conspecific density, predator presence, and pollution (Wilbur 1976, Relyea 2002, Snodgrass et al. 2008). Given their sensitivity to environmental conditions and wide range, wood frogs are an excellent model species to test effects of water turbidity and sedimentation.

We manipulated water turbidity using silt additions and predator presence in replicate cattle tank mesocosms. We wanted to know if a large sediment pulse during early larval development affects the survival, growth or time to metamorphosis of wood frogs. We also wanted to know if sediment addition and predator presence interact to affect tadpole performance. Wood frog tadpoles are typically less active in the presence of predators, and grow and develop slower than in predator free environments (Relyea 2002). We included this known stressor because water turbidity may influence how tadpoles respond to predators. We hypothesized that silt addition and the resulting high water turbidity could affect wood frog tadpoles in one of three ways:

(1) *Direct Mortality* - Large amounts of suspended sediment could cause direct mortality by damaging gills or other vital organs and reducing feeding efficiency. If direct effects are important, we expected tadpoles in the high turbidity treatments to experience greater mortality than tadpoles from low turbidity treatments.

(2) *Reduced primary productivity*- If silt addition does not directly affect tadpoles, we expected sediment treatment differences to result from effects of elevated water turbidity on other aspects of mesocosms community. High turbidity reduces light penetration and therefore may reduce primary productivity, thus limiting tadpole food

supply. In this case we expected that survival would be similar among treatments but high turbidity treatments would experience reduced growth and slower development compared to low turbidity treatments as a result of reduced primary productivity in mesocosm.

- (3) *Sediment as a food resource*- Alternatively, suspended silt particles may serve as a food resource. In this case we also expected no survival differences, but we expected tadpoles in the high turbidity treatment to experience increased growth and faster development than tadpoles from the low turbidity treatments.

We enclosed predators in cages to provide visual and chemical cues to while preventing actual predation within mesocosms. Based on previous research, we expected that tadpoles from predator-present treatments would metamorphose later than tadpoles from predator-free treatments, but would emerge at a similar size. We anticipated two possible turbidity-predator interaction scenarios:

- (1) *Reduced response* - High turbidity could reduce tadpole response to predators if suspended sediment provides perceived cover or affects the dispersion of predator chemical cues. In this event, we predicted that tadpoles in high turbidity with predator present treatments would grow larger and metamorphose earlier than tadpoles in the low turbidity with predator treatment, and would develop at a similar rate as tadpoles in the no predator treatments.
- (2) *Amplified response* - Alternatively, if turbidity is an environmental stressor for tadpoles, the combined influence of predator presence and high turbidity could cause a synergistic response. In this event, we expected decreased growth and slower

developmental rate in high turbidity with predator treatments, than in high turbidity with no predator or low turbidity with predator treatments.

## **Methods**

### ***Animal Collection and Husbandry***

We conducted the study in 2013 at an outdoor mesocosm facility in Storrs, CT. Five wood frog egg masses were collected from a vernal pool in the University of Connecticut Forest in Storrs, CT on 17 April. Egg masses were divided and kept in plastic containers filled with natal pond water in a cold room at 10°C until embryos had developed into free swimming tadpoles. On 23 April, tadpoles were transferred to the experimental mesocosms. We collected late instar *Libellula cyanea* naiads to use as tadpole predators from a permanent wetland in the UConn forest on 29 April. Dragonfly larvae were kept in 5-gallon buckets filled with pond water until added to experimental enclosures on 1 May.

### ***Experimental design***

We initiated the experiment on 23 April and it concluded on 13 July, a week after the last metamorph emerged from tanks. It was a 2 X 2 full factorial experiment testing the effect of silt addition and predator presence on larval wood frog mass and age at metamorphosis and survival. Each treatment combination was replicated 10 times. Treatments were randomly assigned to tanks within five spatial blocks.

Tanks were filled with well water two weeks prior to the start of the experiment. We added 1 kg leaf litter, collected from forest adjacent to mesocosm facility and 3 mL homogenized and concentrated phytoplankton/zooplankton inoculate collected from a wetland in the UConn Forest to each tank on 11 April. Each tank received an additional 3 mL concentrated phytoplankton/zooplankton on April 15 and April 20. All tanks were fitted with a 50% shade-

cloth top to prevent insect predators and other amphibian species from colonizing tanks.

Turbidity and predator treatments were started on May 1.

Dragonfly larvae were caged in plastic cups capped with window screening, which allowed tadpoles to sense predator chemical cues but prevented actual predation. Tanks either received 4 empty plastic cups (no predator treatment) or 4 plastic cups, each containing a single larval *L. cyanea* (predator treatment). All dragonfly larvae were fed weekly a single larval wood frog from a single non-experimental tank.

We added silt, purchased from a local sand quarry, to mesocosms to manipulate water turbidity. Silt was applied to each tank by hand, and care was taken to ensure that additions were distributed evenly across the entire water surface. To avoid confounding nutrient addition with turbidity treatment, we added the same quantity of sediment to all tanks, but manipulated the timing of the sediment addition. The experiment tested the effect of a single large sediment addition versus multiple small sediment additions. This created two very different turbidity profiles between the two treatments over the course of the experiment (Figure 1). The single large addition treatment received 20-kg silt in a single addition on May 1. This produced an extremely high initial turbidity level (Overall mean =  $4.8 \pm 0.5$  NTU, Max Daily Mean =  $19.2 \pm 2.3$  NTU) that steadily decreased over the course of the experiment. The pulsed addition treatment received five 4-kg silt additions, added every five days starting on May 1. This created a fairly constant low turbidity treatment (Overall mean =  $2.7 \pm 0.4$  NTU, Max Daily Mean =  $4.0 \pm 1.6$  NTU).

We monitored turbidity levels in all tanks every other day from the beginning of the experiment until tadpoles began to metamorphose. To measure turbidity, we collected a water sample at a standard depth from the center of each tank and then obtained a turbidity reading



using a DRT100B turbidimeter (HF Scientific, Inc.). On June 6, we also measured water temperature, pH, specific conductivity, chlorophyll a concentration, and dissolved oxygen concentration using a Hydrolab MS5 Multiparameter Sonde. We removed tadpoles from tanks after front legs emerged. Metamorphs were kept in the lab (range: 1-5 days) until their tails were fully absorbed at which point we weighed them to the nearest 0.01g and placed them in outdoor enclosures for subsequent experiments.

### ***Data Analysis***

Age and mass at metamorphosis were averaged for each tank and tank averages were used in analyses because the tank was the unit of replication. Tank 34 (pulsed addition, no predator) and tank 39 (single addition, no predator) were removed from analyses because they received the incorrect number of tadpoles at the beginning of the experiment.

We used ANOVA to analyze tank survival, age and mass at metamorphosis. Block, predator treatment and turbidity treatment were treated as fixed effects and the model also included an interaction term between predator and turbidity treatment. Tank survival did not differ among treatments and thus was not used as a covariate for age and mass analyses. Where necessary, we used Box-Cox transformations to ensure that data met assumptions of normality and homoscedasticity. We also used ANOVA to determine if block, turbidity treatment, predator treatment or an interaction between turbidity treatment and predator treatment affected water conductivity, dissolved oxygen, pH, or chlorophyll concentration. All data analyses were carried out in R version 3.0.2 (R Development Core Team 2013).

### **Results**

Survival was high experiment wide (mean: 87%, range: 62.5% - 100%) and only three mesocosms had less than 80% survival. Survival was not affected by block ( $F_{4, 30} = 1.018, p =$

0.414), turbidity treatment ( $F_{1,30} = 0.541$ ,  $p = 0.468$ ), predator presence ( $F_{1,30} = 0.023$ ,  $p = 0.879$ ) or the turbidity\*predator interaction ( $F_{1,30} = 1.405$ ,  $p = 0.245$ ). Turbidity treatment affected mass ( $F_{1,30} = 36.244$ ,  $p < 0.001$ ) and age at metamorphosis ( $F_{1,30} = 5.111$ ,  $p = 0.031$ ). Metamorphs from the single silt addition treatment reached metamorphosis earlier (Figure 3) and were significantly larger than tadpoles from the pulsed addition treatment (Figure 2). Predator presence did not significantly affect either age ( $F_{1,30} = 0.843$ ,  $p = 0.366$ ) or mass at metamorphosis ( $F_{1,30} = 2.050$ ,  $p = 0.163$ ) and there were no interaction effects between turbidity and predator presence on age ( $F_{1,30} = 0.329$ ,  $p = 0.571$ ) or mass ( $F_{1,30} = 0.589$ ,  $p = 0.449$ ) at metamorphosis.

Turbidity treatment did not significantly affect any of the water quality variables measured (Table 1). There were significant effects of block on water temperature ( $F_{4,30} = 25.058$ ,  $p < 0.001$ ), and pH ( $F_{4,30} = 2.899$ ,  $p < 0.0385$ ) and there was a significant effect of predator treatment on dissolved oxygen concentration ( $F_{1,30} = 4.884$ ,  $p = 0.0349$ ), with reduced oxygen in tanks containing predators.

## **Discussion**

Anthropogenic sediment input has the potential to affect wetland community dynamics by altering water quality, species-specific vital rates, and interspecific interactions. Ephemeral wetlands, which are important breeding habitat for many amphibians and invertebrates, may be particularly affected by sediment inputs because they are typically characterized by clear, low nutrient water with leaf litter substrate. For this reason, we expected sediment input would be detrimental to organisms that use these habitats. However, we found that a large sediment pulse during the early larval period was not directly harmful to wood frog tadpoles. Average survival among mesocosms was 87% and did not vary among sediment input treatments. The highest

suspended sediment concentrations occurred at the beginning of the experiment, following the initial sediment addition. Due to their small size, tadpoles would have been most vulnerable to direct effects of sediment during this period, so the lack of an effect on survival is particularly informative. A large early sediment pulse not only had no effect on survival, but actually resulted in an increase in mass at metamorphosis. Tadpoles exposed to the single large silt addition were nearly 25% larger at metamorphosis than tadpoles from the pulsed small addition treatment.

We expected primary productivity to decrease in the large sediment addition treatment because the average turbidity was nearly double the average turbidity of the small pulsed addition treatment. At the time frogs started to metamorphose, there was a small, but not statistically significant difference between chlorophyll *a* concentration in large and small sediment addition tanks. That metamorphs in the highest turbidity tanks grew the largest, suggests that suspended silt particles served as an additional food resource, which offset reduced primary productivity, or silt particles increased primary productivity despite high turbidity by providing limiting nutrients. Mass at metamorphosis is an important trait in amphibians because larger metamorphs typically experience higher survival as juveniles than smaller metamorphs (Berven 1990). Tadpoles from the single large addition treatment also tended to reach metamorphosis earlier than tadpoles from the pulsed addition treatment, however given that the mean difference was less than a day, the effect may not be ecologically meaningful.

Considering the large body of literature documenting effects of predator presence on tadpole growth, development, and morphology, we were surprised predator presence had no effect on either growth or development in our study. Aeschnid naiads are typically used as predators in mesocosms experiments (Relyea & Hoverman 2003, Takahara et al. 2012, Middlemis Maher et al. 2013), however, we used a Libellulid species because they were the most

common dragonfly naiad encountered our study area. The *Libellula cyanea* naiads that we used exhibited low predation rates even when confined in a small plastic cup with a tadpole. This may have contributed to the lack of tank response because wood frog tadpoles respond to both kairomones released by predators and alarm cues released by conspecifics (Chivers & Mizral 2001). Therefore low predation rates may have resulted in low concentrations of alarm cues. Predator density may have been too low to affect tadpole response, because we detected the predator at higher densities in natural wetlands than were used in the experiment (4 naiads/3m<sup>2</sup>). Unfortunately, the lack of response to predator presence limits our ability to make a conclusion regarding the interaction between predator presence and turbidity conditions.

Our results might lead to the conclusion that urban and agricultural inputs to wetland habitat are beneficial to wood frogs. However, our results notably contrast the findings of Richardson and Wood (2009), who found that western toad tadpoles reared in mesocosms experienced greater mortality and slower growth when exposed to high sediment input. They concluded that survival and growth effects were due to decreased availability of high quality food (periphyton) and increased consumption of low-nutrient sediment, which resulted in greater intraspecific competition. A possible explanation for the difference between our findings, is that silt used in our study may have higher organic content. Sediment with high organic content has been shown to increase growth of *Rana palmipes* tadpoles (Flecker et al. 1999). This suggests that when assessing the impact of sediment input on amphibians, sediment organic content should be an important consideration.

When wetlands receive runoff from urban and agricultural land, the influx of material includes not only sediment, but also an array of pollutants that may affect aquatic life, including nitrogenous compounds, pesticides, and heavy metals. Nitrogen and heavy metals can cause

developmental abnormalities, alter behavior, and increase mortality of tadpoles (Rouse et al. 1999, Blaustein et al. 2003). Additionally, pesticides including atrazine, glyphosate, carbaryl and endosulfan, have been shown to harm amphibians (Berrill et al. 1998, Boone and James 2003, Storrs and Kiesecker 2004, Relyea 2005). Pollutants also have the potential to cause non-additive effects on mortality when combined with other stressors, like predator presence or pond drying (Relyea 2003, Boone and James 2003). Wood frogs may be particularly sensitive to pollution. For example, wood frog embryos exposed to sediment from storm water drainage ponds suffered almost complete mortality (Snodgrass 2008) and wood frog tadpoles exposed to low levels of endosulfan for as little as 96 hours experienced high mortality (Berrill et al. 1998). Although we have shown that sediment input alone is not detrimental to wood frogs, in a natural setting the effects of pollutants could outweigh any beneficial effects of sediment addition.

Our understanding of the effects of sediment input on lentic communities lags behind that of lotic communities. Mesocosm studies offer an excellent way to bridge this gap, by allowing the experimenter to control extraneous factors, such as wetland size, community composition and isolate sediment input from potentially co-occurring pollutants. The drawbacks are that the mesocosm system is less complex than a natural system, so certain processes important in nature may not occur in mesocosms. Additionally, since experimental treatments are applied by the experimenter, care must be taken to ensure that treatments represent conditions that are found in natural systems. This is not always an easy task, and one of the more challenging aspects of conducting this study involved selecting an appropriate method to manipulate turbidity. We considered two main alternatives including: 1) stirring tanks periodically to suspend sediments, 2) adding silt periodically to ensure a constant turbidity. The benefit to stirring tanks is that you can have the exact same sediment inputs in all tanks, but manipulate how much sediment is

suspended by altering stirring frequency. However, we chose not to stir tanks because that would create water movement that is unnatural for the small wetlands in which wood frogs breed. We reasoned that periodic silt additions would more closely match the way surface runoff increases turbidity in wetlands following large rain events.

The main drawback of manipulating turbidity by adding sediment is that each time an addition is made, the quantity of sediment in experimental tanks changes relative to control tanks. Sediment amount could affect ecosystem processes within mesocosms by altering the quantity of nutrients in the tanks. Although imperfect, our solution to this problem was to ensure that each tank received the same quantity of sediment and instead alter turbidity levels by changing the timing and frequency of sediment delivery. This ensured that by experiment end the total nutrient input was the same for all tanks, the only difference across treatments was the average turbidity level. Our water quality measurements (Table 1), taken near the end of the experiment, confirm that there was little to no difference in water quality between turbidity treatments.

Sediment input clearly affects aquatic communities, but not all species are equally vulnerable. Here we show that large sediment pulses do not cause increased mortality in wood frog tadpoles and may actually increase growth. We suggest that silt increased available food resources for tadpoles and that suspended particulates are not directly harmful to tadpoles. However, we argue against concluding that sediment input is beneficial to amphibians, because surface runoff often contains pollutants in addition to sediment. The effect of sediment input may also vary substantially depending on the amount of organic material that it contains. Future research should investigate the relationship between sediment organic content and amphibian

larval performance. Additionally, a better understanding of the effect of turbidity on interspecific interactions will help predict the effect of sediment input in natural systems.

### **Acknowledgements**

We thank John Clausen for providing resources for collecting water samples and measuring turbidity. Mark Urban provided equipment for measuring water quality and helpful comments on the manuscript. Isaac Ortega also provided helpful comments. We are also grateful for field assistance provided by Michael Evans, Jan-Michael Hessenauer, Cristina Macklem, Kelly O'Connor, and Annie Stupik.

### **References**

- Berrill M, Coulson D, McGillivray L, Pauli B. 1998. Toxicity of endosulfan to aquatic stages of anuran amphibians. *Environ Toxicol Chem* 17(9):1738-44.
- Berven K. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology* 71(4):1599-608.
- Blaustein A, Romansic J, Kiesecker J, Hatch A. 2003. Ultraviolet radiation, toxic chemicals and amphibian population declines. *Divers Distrib* 9(2):123-40.
- Boone M and James S. 2003. Interactions of an insecticide, herbicide, and natural stressors in amphibian community mesocosms. *Ecol Appl* 13(3):829-41.
- Broekhuizen N, Parkyn S, Miller D. 2001. Fine sediment effects on feeding and growth in the invertebrate grazers *Potamopyrgus antipodarum* (gastropoda, hydrobiidae) and *Deleatidium* sp. (ephemeroptera, leptophlebiidae). *Hydrobiologia* 457(1-3):125-32.
- Chivers DP, Al-Batati F, Brown GE, Ferrari MCO. 2013. The effect of turbidity on recognition and generalization of predators and non-predators in aquatic ecosystems. *Ecol Evol* 3(2):268-77.
- Chivers DP and Mirza RS. 2001. Importance of predator diet cues in responses of larval wood frogs to fish and invertebrate predators. *J Chem Ecol* 27(1):45-51.
- Flecker A, Feifarek B, Taylor B. 1999. Ecosystem engineering by a tropical tadpole: Density-dependent effects on habitat structure and larval growth rates. *Copeia* (2):495-500.

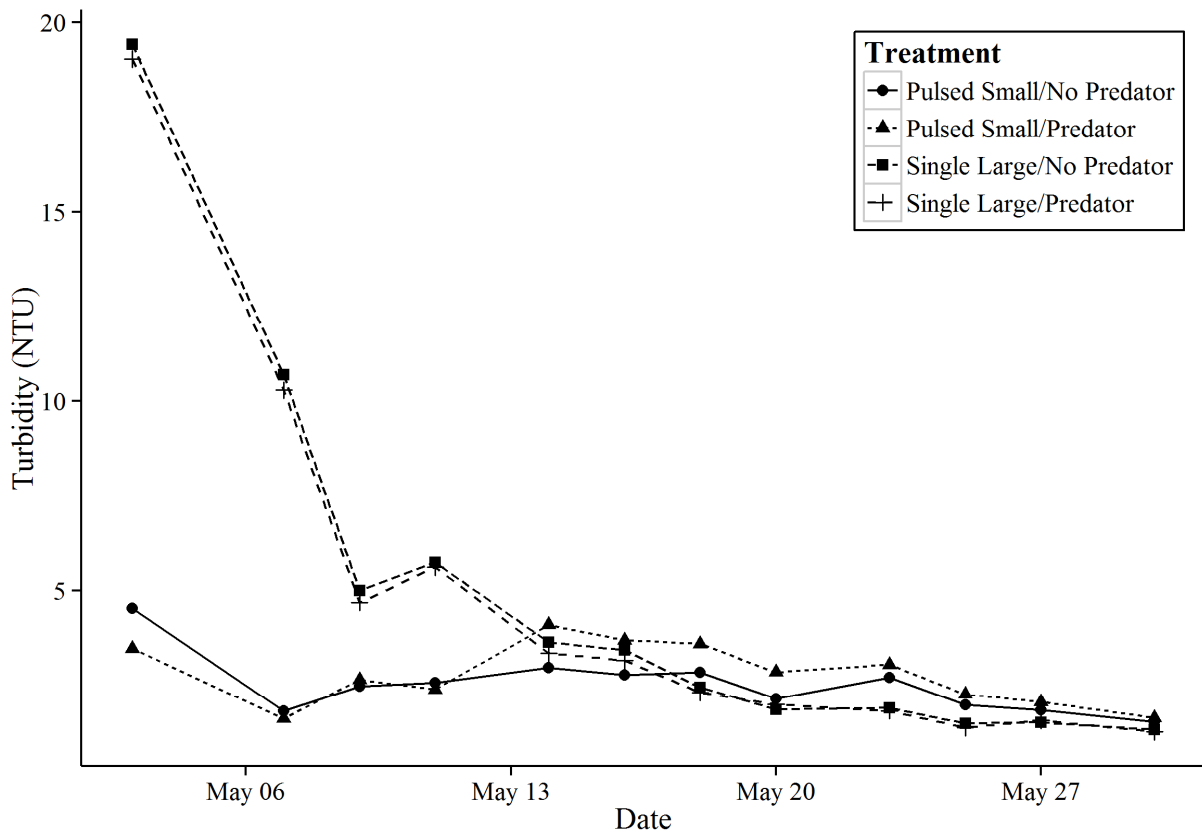
- Freeman P and Schorr M. 2004. Influence of watershed urbanization on fine sediment and macroinvertebrate assemblage characteristics in Tennessee ridge and valley streams. *J Freshwat Ecol* 19(3):353-62.
- Göbel P, Dierkes C, Coldewey W. 2007. Storm water runoff concentration matrix for urban areas. *J Contam Hydrol* 91(1):26-42.
- Gonzalez MJ, Knoll LB, Vanni MJ. 2010. Differential effects of elevated nutrient and sediment inputs on survival, growth and biomass of a common larval fish species (*Dorosoma cepedianum*). *Freshwat Biol* 55(3):654-69.
- Kröger R, Holland M, Moore M, Cooper C. 2008. Agricultural drainage ditches mitigate phosphorus loads as a function of hydrological variability. *J Environ Qual* 37(1):107-13.
- Matthaei CD, Piggott JJ, Townsend CR. 2010. Multiple stressors in agricultural streams: Interactions among sediment addition, nutrient enrichment and water abstraction. *J Appl Ecol* 47(3):639-49.
- Middlemis Maher J, Werner EE, Denver RJ. 2013. Stress hormones mediate predator-induced phenotypic plasticity in amphibian tadpoles. *Proc R Soc B* 280(1758).
- Mol J and Ouboter P. 2004. Downstream effects of erosion from small-scale gold mining on the instream habitat and fish community of a small neotropical rainforest stream. *Conserv Biol* 18(1):201-14.
- Paul MJ and Meyer JL. 2001. Streams in the urban landscape. *Annu Rev Ecol Syst* :333-65.
- Relyea R. 2002. Local population differences in phenotypic plasticity: Predator-induced changes in wood frog tadpoles. *Ecol Monogr* 72(1):77-93.
- Relyea RA and Jason T. Hoverman. 2003. The impact of larval predators and competitors on the morphology and fitness of juvenile treefrogs. *Oecologia* 134(4):596-604.
- Relyea R. 2005. The lethal impact of roundup on aquatic and terrestrial amphibians. *Ecol Appl* 15(4):1118-24.
- Richardson J and Jowett I. 2002. Effects of sediment on fish communities in East Cape streams, North Island, New Zealand. *N Z J Mar Freshwat Res* 36(2):431-42.
- Rouse J, Bishop C, Struger J. 1999. Nitrogen pollution: An assessment of its threat to amphibian survival. *Environ Health Perspect* 107(10):799-803.
- Roy AH, Rosemond AD, Paul MJ, Leigh DS, Wallace JB. 2003. Stream macroinvertebrate response to catchment urbanisation (Georgia, U.S.A.). *Freshwat Biol* 48(2):329-46.



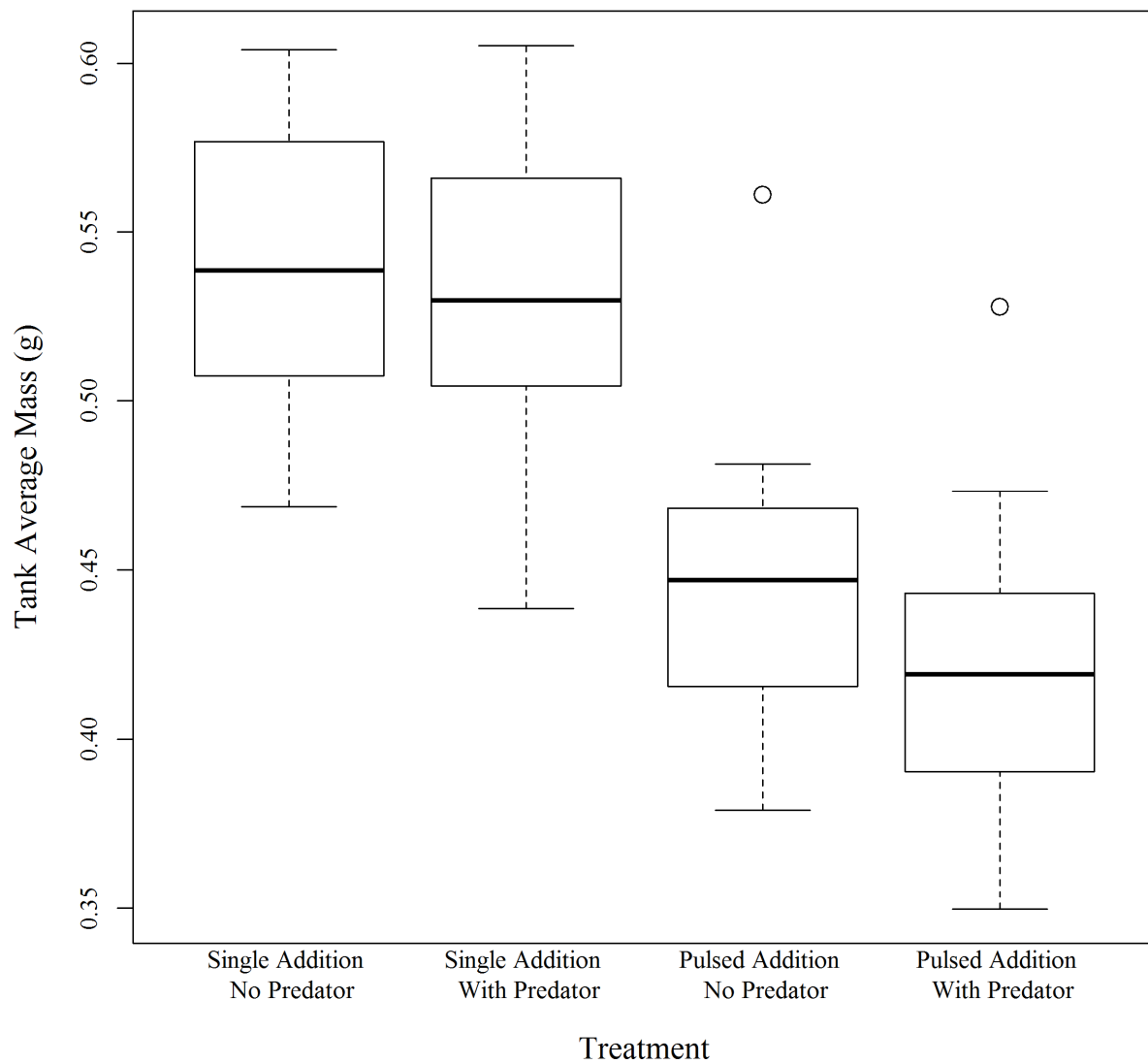
- Ryan P. 1991. Environmental-effects of sediment on New Zealand streams - a review. *N Z J Mar Freshwat Res* 25(2):207-21.
- Schallenberg M and Burns C. 2004. Effects of sediment resuspension on phytoplankton production: Teasing apart the influences of light, nutrients and algal entrainment. *Freshwat Biol* 49(2):143-59.
- Sharma RC and Rawat JS. 2009. Monitoring of aquatic macroinvertebrates as bioindicator for assessing the health of wetlands: A case study in the central Himalayas, India. *Ecol Ind* 9(1):118-28.
- Shilla DJ and Shilla DA. 2011. The effects of catchment land use on water quality and macroinvertebrate assemblages in Otara creek, New Zealand. *Chem Ecol* 27(5):445-60.
- Snodgrass JW, Casey RE, Joseph D, Simon JA. 2008. Microcosm investigations of stormwater pond sediment toxicity to embryonic and larval amphibians: Variation in sensitivity among species. *Environ Pollut* 154(2):291-7.
- Storrs S and Kiesecker J. 2004. Survivorship patterns of larval amphibians exposed to low concentrations of atrazine. *Environ Health Perspect* 112(10):1054-7.
- Sutherland A, Meyer J, Gardiner E. 2002. Effects of land cover on sediment regime and fish assemblage structure in four southern Appalachian streams. *Freshwat Biol* 47(9):1791-805.
- Sutherland AB and Meyer JL. 2007. Effects of increased suspended sediment on growth rate and gill condition of two southern Appalachian minnows. *Environ Biol Fishes* 80(4):389-403.
- Suttle KB, Power ME, Levine JM, McNeely C. 2004. How fine sediment in riverbeds impairs growth and survival of juvenile salmonids. *Ecol Appl* 14(4):969-74.
- Takahara T, Kohmatsu Y, Maruyama A, Doi H, Yamanaka H, Yamaoka R. 2012. Inducible defense behavior of an anuran tadpole: Cue-detection range and cue types used against predator. *Behav Ecol* 23(4):863-8.
- Van de Meutter F, De Meester L, Stoks R. 2005. Water turbidity affects predator-prey interactions in a fish-damselfly system. *Oecologia* 144(2):327-36.
- Wilbur H. 1976. Density-dependent aspects of metamorphosis in *Ambystoma* and *Rana sylvatica*. *Ecology* 57(6):1289-96.
- Wood SL and Richardson JS. 2009. Impact of sediment and nutrient inputs on growth and survival of tadpoles of the western toad. *Freshwat Biol* 54(5):1120-34.

**Table 1: Water quality measurements: All measurements were taken on 6 June 2013. Values represent mean  $\pm$  one standard deviation. Differences are not statistically significant ( $p > 0.05$ ).**

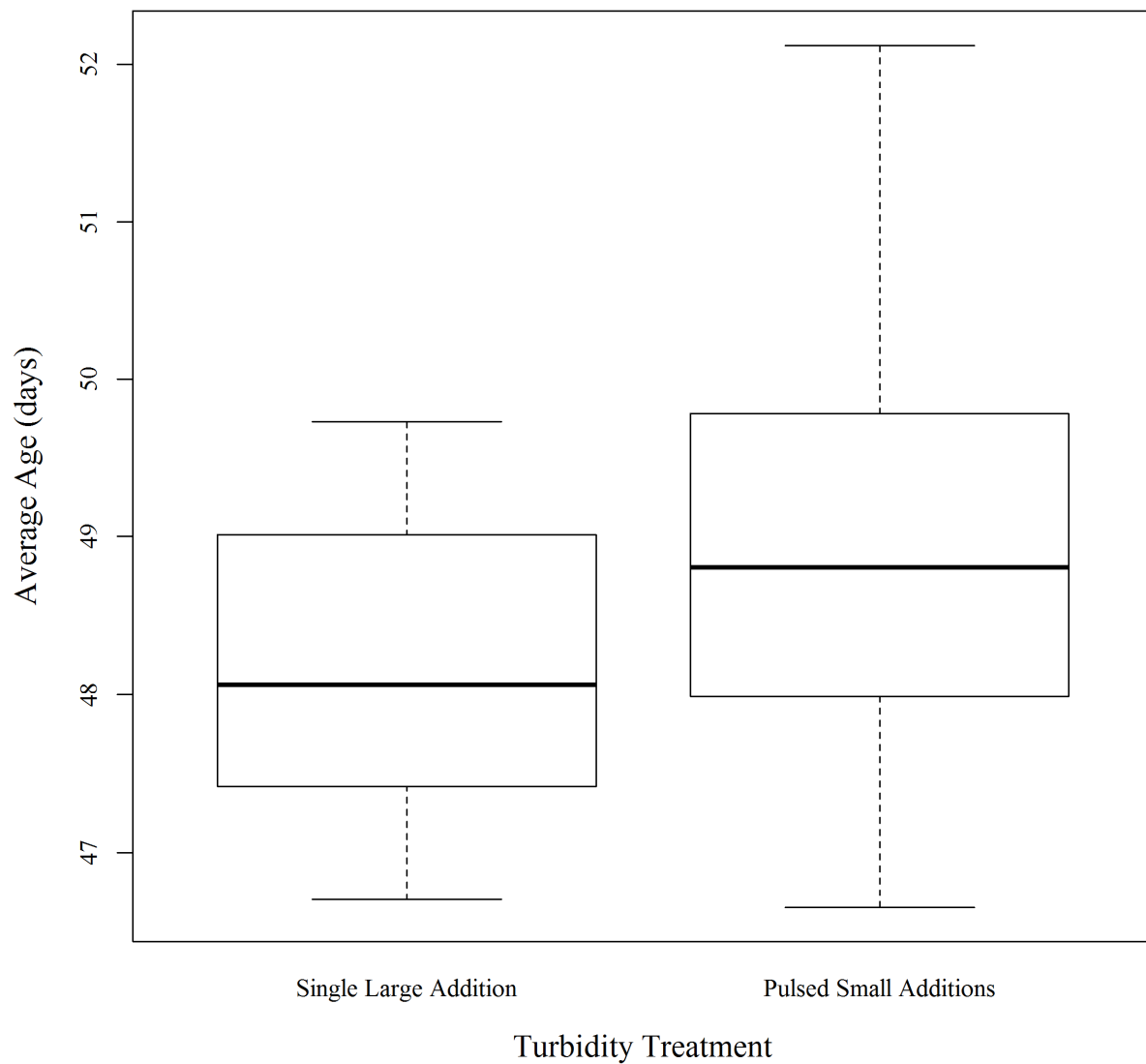
	<b>Single Large Addition</b>	<b>Pulsed Small Additions</b>
<b>Temperature (°C)</b>	21.46 $\pm$ 0.82	21.70 $\pm$ 0.82
<b>Specific Conductivity (<math>\mu</math>S/cm)</b>	112.19 $\pm$ 26.80	105.02 $\pm$ 40.71
<b>Total Dissolved Solids (g/L)</b>	0.09 $\pm$ 0.02	0.08 $\pm$ 0.04
<b>pH</b>	7.78 $\pm$ 0.17	7.78 $\pm$ 0.18
<b>Chlorophyll a (<math>\mu</math>g/l)</b>	4.57 $\pm$ 2.71	6.18 $\pm$ 3.94
<b>Dissolved Oxygen (mg/L)</b>	7.18 $\pm$ 0.54	7.32 $\pm$ 0.92



**Figure 1: Change in mesocosm water turbidity among treatments, over the course of the experiment.**



**Figure 2: Average mass at metamorphosis based on turbidity treatment and predator presence. Tadpoles from mesocosms with single large sediment addition were significantly larger than tadpoles from mesocosms with pulsed small sediment additions ( $F_{1,30} = 36.244$ ,  $p < 0.001$ ).**



**Figure 3: Effect of turbidity treatment on tank average number of days to metamorphosis. Tadpoles from mesocosms with a single large sediment addition reached metamorphosis significantly earlier than tadpoles from mesocosms with pulsed small sediment additions ( $F_{1,30} = 5.111, p = 0.031$ ).**

**Survival of a terrestrially overwintering amphibian in experimentally altered  
microhabitats**

Jason O'Connor

Department of Natural Resources and the Environment

University of Connecticut, Storrs, CT, USA

**Abstract:**

Freezing temperatures during winter pose a significant challenge for ectothermic animals, which must behaviorally avoid lethal temperatures or physiologically adapt to survive freezing conditions. Wood frogs (*Lithobates sylvaticus*) are freeze tolerant amphibians that overwinter terrestrially in shallow hibernacula, however little is known about what temperature and moisture conditions they experience throughout the winter and how microhabitat conditions affect survival. Using radio-telemetry, I tracked 34 wood frogs to their overwintering sites and enclosed them with small circular fences to monitor survival over the course of the winter. I also enclosed an additional 34 frogs at sites that had been used by tracked frogs prior to late fall movement to test whether survival was higher in locations selected immediately before the onset of winter. Frogs were also randomly assigned to a snow removal treatment or a natural snow cover control to test whether reduced snow cover has the potential to affect wood frog survival. I used cox proportional hazards models to estimate survival over the course of the winter. 28 of the 68 frogs monitored survived the winter. Estimated survival rate was highest (78%) for tracked frogs that received natural snow cover and lowest for paired frogs (6%) that experienced snow removal. The hazard ratio for paired frogs relative to tracked frogs was 2.74 and was 15.55 for frogs in the snow removal treatment relative to the natural snow cover treatment during periods with complete snow cover. I used information theoretic approach to evaluate support for a set of competing models to explain survival differences as a function of soil temperature and moisture conditions. The top ranked model included frog depth in substrate and minimum temperature experienced. The models with the greatest support suggest that acute exposure to cold temperatures are likely responsible for wood frog mortality during the winter. Experimental manipulations of temperature and moisture conditions would be useful for further refining estimates of the relationship between these fundamental abiotic parameters and survival of wood frogs.

## **Introduction**

Temporal variation in weather is ubiquitous in natural systems. Air temperature and precipitation patterns vary on scales ranging from hourly to millennially. In general, daily and seasonal weather variations are most relevant to individual organisms. In order to thrive in a given climate organisms must be able to function effectively under the full range of typical weather conditions and possess the capacity to tolerate infrequent atypical weather patterns. In temperate regions, air temperatures fluctuate dramatically over the course of a year. In late spring, summer and early fall air temperatures are generally warm enough to permit normal feeding and reproductive behaviors. During winter however, temperatures may approach or drop below the freezing point of water and force organisms to respond physiologically or behaviorally to survive.

While interactions between organisms and biotic and abiotic components of their environment are well defined during spring and summer, ecological relationships during winter are often less clear. For many taxa, such as amphibians, little is known about even basic population vital rates such as survival over winter (James et al. 2004). This is due in part to the challenge of finding and studying these organisms during periods of inactivity. Yet, it is inactivity that makes this life stage uniquely interesting. While predation risk is high for amphibians during active periods (Rittenhouse et al. 2009, Heemeyer and Lannoo 2012), winter mortality is likely due to abiotic conditions (Schmidt 2007).

Amphibians are ectothermic, making them especially vulnerable to cold temperatures. Cold temperatures reduce metabolic rate, thus reducing the organism's scope for physical activity. Furthermore, if temperatures drop below zero centigrade, water within the body may begin to freeze, which can have lethal consequences. Amphibians that live in regions where air temperature regularly drops below freezing must rely on behaviorally avoiding freezing



temperatures or tolerating limited freezing of body water. Behavioral avoidance is typically accomplished by burrowing below the frost-line or submerging in water bodies unlikely to freeze solid (Tattersal and Ultsch 2008). The alternative to behavioral freeze avoidance is physiological freeze tolerance, which is a strategy employed by wood frogs (*Lithobates sylvaticus*) and a few other species (Schmid 1982). Freeze tolerance in amphibians is a result of adaptations that control the rate of extracellular ice formation and prevent intracellular ice-formation. Before the onset of winter wood frogs accumulate urea, and during freeze events, release glucose stored in the liver and transport it to vital organs such as the brain and heart (Storey and Storey 1984, Costanzo and Lee 2005). Both urea and glucose act as cryoprotectants, which slow the rate of freezing and prevent intracellular ice-formation, limiting tissue damage due to ice expansion (Costanzo et al. 2013).

While these adaptations allow wood frogs to survive some freezing conditions, laboratory experiments have revealed that temperatures below  $-6^{\circ}\text{C}$  (Costanzo and Lee 2013), freezes that last longer than several weeks (Layne et al. 1998), and rapid freeze events can be lethal (Costanzo et al. 1991). Even in the warmer parts of the wood frog range, winter temperatures regularly exceed these physiological limits, thus acute exposure may be an important source of winter mortality for wood frogs. Enduring a freeze-thaw cycle may also entail a significant energetic cost (Sinclair et al. 2013), which could increase the risk of energy depletion. Since feeding activity is greatly reduced, if not ceased completely, frogs rely on metabolizing stored lipids during the winter (Reading 2007). Thus, they enter winter with a limited amount of energy with which to maintain life processes. Factors which increase energy consumption, including winter duration and temperature, may increase the likelihood of mortality due to energy exhaustion.

Moisture conditions are another important component of the terrestrial ecosystem that may affect overwinter survival. Maintaining water balance can be a challenge for amphibians because they have highly permeable skin. While many ranid frogs overwinter underwater, wood frogs prefer unsaturated soil over both saturated soil and water when temperatures approach freezing (Licht 1991). This strategy may limit risk of winterkill due to anoxia in wetlands that experience prolonged periods of ice cover, or prevent becoming trapped in ice in the spring following wetland recharge (Baldwin et al. 2006). However, wood frogs in upland habitat may face a greater risk of desiccation during winter because air temperatures may prevent frogs from selecting a new location in response to low moisture availability. The potential for soil moisture to interact with temperature conditions further complicates predictions of the effect of soil moisture on winter survival of wood frogs. A typical response to dehydrating conditions for many amphibians, including wood frogs, is to retain and accumulate urea in the body (Costanzo and Lee 2008). Since urea is also a cryoprotectant, wood frogs overwintering in drier sites may be more likely to survive freeze events than those that over winter in saturated locations.

The majority of studies linking winter climate to amphibian survival fit linear models with winter climate covariates to estimates of annual survival based on mark-recapture of individuals congregated at breeding sites (Table 1). While breeding site mark-recapture might be the most efficient way to estimate population size, it does not allow one to separate post-breeding mortality that occurs during the active period, from the relatively inactive period that occurs during overwintering. Another less commonly used method to estimate winter survival, which to my knowledge has only been used with metamorph juveniles, is to stock terrestrial enclosures in an experimental array and conduct mark-recapture or simple recapture to estimate overwinter survival. In these studies overwinter survival is typically measured as a response variable to an

experimental manipulation (i.e. density, contaminant exposure, body mass), therefore these estimates may not reflect survival in natural systems.

Understanding how winter conditions affect survival of amphibians is particularly important given predicted changes in winter temperature and precipitation patterns resulting from climate change. Over the past century, average winter air temperature has increased 0.7°C and the number of days with snow cover per year has decreased by over a week in the northeastern United States (Rustad et al. 2012). Climate models predict that by 2100 average winter temperature could increase by up to 5.4°C and the number of snow cover days could decrease by 3.8 per winter month (Hayhoe et al. 2007). In light of these predictions, information is needed to inform conservation planning and prioritization. To accurately predict how these changes will affect amphibian populations, it will be important to have baseline data on current winter survival rates and to understand the relationship between survival rates and temperature and moisture conditions. The goal of this research is to better understand what factors influence the overwinter survival of terrestrially overwintering amphibians to gain insight into how climate change will affect amphibian populations. My main research objectives were to:

- 1) Describe late fall movement of wood frogs and assess whether they select habitat based on soil moisture or temperature.
- 2) Describe temperature and moisture conditions at wood frog overwintering locations.
- 3) Test the effect of snow removal and late fall movement on wood frog survival.
- 4) Identify microhabitat variables that affect survival.
- 5) Evaluate support for competing hypothesis about overwinter mortality in temperate amphibians.

I expected snow removal to decrease wood frog survival by exposing wood frogs to colder and more variable temperature conditions. I expected late fall movement would increase wood frog survival by allow frogs to select favorable overwintering habitat. Based on previous studies linking amphibian populations and winter conditions, I hypothesized three alternative ways winter conditions could increase wood frog mortality. First, wood frog mortality could result from acute exposure to temperature conditions exceeding physiological tolerances. If mortality results from acute exposure, I expected that colder temperatures experienced, longer freeze durations, and faster freeze events would be linked to higher winter mortality. Alternatively, winter mortality could result from energy depletion. If energy depletion is responsible for mortality, I predicted that higher average temperatures would increase mortality by increasing energy consumption. I also predicted that mortality would increase with the number of freeze/thaw cycles experienced due to the associated energy cost, and that larger individuals would be more likely to survive than smaller individuals due to having greater energy reserves. The third potential way wood frog mortality could result from winter conditions is due to desiccation resulting from low moisture availability. If desiccation is major factor affecting winter survival, I predicted that wood frog survival would be lower for frogs that selected locations with lower soil moisture.

## **Methods**

The study was conducted at Nathan Hale State Forest in Coventry, CT and the Fifty-Foot Cliff Preserve in Mansfield, CT between 4 October 2013 and 2 April 2014. Both sites consist of primarily upland oak/hickory forest. Nathan Hale State Forest is a 1,529 acre property that is actively managed by the state of Connecticut to maintain timber production and wildlife habitat.

66% of the forest is actively managed for timber, 13% has been designated “Old Forest” and is set aside to develop without management, and the rest of the forest is not managed due to physical features such as slopes or wetlands that prevent management operations. A small warm water stream bisects the southern portion of the property and empties into Bear Swamp, a scrub shrub wetland at the southern border of the forest. A variety of other wetland types, including forested vernal pools, open canopy permanent ponds, and hardwood wetlands, are interspersed throughout the property and account for 5% of the forest area.

The Fifty-Foot Cliff Preserve is a 102-acre tract that has been owned by the town of Mansfield since 1998 and is used primarily for recreational hiking. The entire site slopes towards the Fenton River. Frogs were tracked in the upslope areas, which consists primarily of mature oak and maple. The understory is mostly open, but a few areas are extensively covered in invasive Japanese Barberry. Birch is more common in the downslope areas, and ferns dominate the understory. The main hydrologic feature of the site is a seasonally inundated seep situated in the downhill area. The seep drains into a nearby first order stream.

### ***Animal Collection***

I collected 70 adult wood frogs by actively searching during rainy evenings between 4 October and 31 October. I also collected frogs from roads along the perimeter of Nathan Hale State forest by road cruising. Capture location was marked with a wire flag and a handheld GPS (Garmin eTrex 10). Frogs were weighed with a pesola<sup>®</sup> scale and sexed. The first 36 frogs captured were fitted with a radio transmitter (model BD-2, Holohil Systems Ltd.) and released at capture location. Three additional frogs were later fitted with transmitters after loss of original frogs. Transmitters were attached using stretch bead cord belts. Handling time was usually about 5 minutes. 20 frogs were tracked at Nathan Hale State Forest and 18 frogs were tracked at the

Fifty-Foot Cliff Preserve. The additional 34 frogs collected were temporarily housed in outdoor enclosures in Storrs, CT for a maximum of 3 weeks before being transferred to experimental enclosures.

### ***Radio-Telemetry***

I located radio tagged frogs daily using a R410 Scanning Receiver and Yagi antenna (Advanced Telemetry Systems, Inc., Isanti, Minnesota, USA). All relocations were made during daylight hours. Upon locating a frog, I marked its location with a wire flag and handheld GPS, recorded if it was active (observed moving or in active position above litter), and whether it was above or beneath litter. I recorded any structural features used for shelter (rocks, downed logs, etc.). When frogs were found to have moved greater than 1m since previous day, I also measured air temperature, soil temperature and soil moisture at the new location, the previous location, and an unused location equidistant from the previous location but in a random direction. This randomly selected point was taken to get a measurement of available but unused habitat. If a transmitter was found without a frog I recorded the location of the transmitter and looked for evidence of predation. When possible, recovered transmitters were reused on new frogs. When frogs were believed to be in the overwintering location and batteries were near failure (average life = 42 days), I removed the transmitter, weighed the frog, returned the frog to its forum, and placed a 0.3m diameter circular enclosure around it. Enclosures were built using E-Fence (ERTEC® Environmental Systems). I installed enclosures between 12 November and 10 December.

### ***Survival Monitoring***

Many of the tracked frogs made considerable (>100m) movements in late November. To test whether these movements increased likelihood of surviving the winter, each tracked frog was paired with a similarly sized, same sex, non-tracked frog. The paired frog was enclosed at a

location previously used by the tracked frog. For the paired location I selected the most recent location prior to a movement greater than 30m, where the tracked frog had resided for at least a week. For 10 individuals these conditions were not met. This occurred when frogs did not move greater than 30m in single night or had not resided in location for at least a week prior to movement greater than 30m. These frogs either did not move very far during the entire tracking period or made frequent small distance movement. For these individuals I enclosed paired frogs at the initial capture location of the tracked frog.

Additionally, within each pair of frogs (Tracked and its paired non-tracked frog) one was randomly assigned to a natural snow cover treatment and the other to a snow removal treatment to test the effect of reduced snow cover on overwinter survival. Following all snow events, snow within the enclosure and 1 m surrounding the enclosure was removed in the snow removal treatment, while snow cover was not manipulated in the natural snow cover treatment.

Temperature within enclosures was measured hourly throughout the winter using data loggers (Thermochron ibutton model: DS1921fG, Maxim Integrated Products, Sunnyvale, CA), placed at the soil-leaf litter interface. Data loggers were placed in the center of the enclosure no further than 10cm from frog location and were pushed into the soil so that the top surface of the ibutton was flush with the ground level. Soil moisture was measured during survival checks using a FieldScout TDR 100 soil moisture meter (Spectrum® Technologies, Inc.).

Enclosures were checked opportunistically throughout the winter to determine survival status of frogs. Reliable checks could only be conducted when there was no snow cover and when air temperatures were above 4°C. This resulted in six survival checkpoints over the course of the winter. Checks were conducted on 6, 11, and 18 January, 2 February, 20 March, and 1 April. A period of consistent snow cover occurred between 2 February and 20 March, preventing

survival checks in the natural snow cover treatment and thus I did not check the frogs in the snow removal treatment. During checks, survival status was determined by visual observation. Activity (if any), location within the enclosure (edge, center, or against enclosure wall), microhabitat (above leaf litter, under leaf litter, on soil not under litter, or on top of ice) and depth in soil (measured from soil surface to dorsum) were also recorded. I obtained daily air temperature maxima, minima, precipitation and snow depth data from NOAA National Climatic Data Center Climate Data Online for a weather station in Storrs, CT.

### ***Data Analysis***

For each tracked frog I calculated average daily distance moved, maximum single day movement, total distance moved, and average direction moved. I used two-way ANOVA to test the effect of sex and site on average daily distance moved, maximum single day movement and average direction moved. Average daily distance moved and maximum single day movement data were log-transformed to meet the assumptions of normality and homoscedasticity. I used Spearman's rank correlation tests to determine the relationship between average daily movement distance and minimum air temperature and precipitation.

I used conditional logistic regression to assess whether wood frogs select habitat based on air temperature, soil temperature, or soil moisture during late fall. I ran two analyses, the first analysis compared used locations with the location used the previous day and the second analysis compared used location with a randomly selected location. I used the 'clogit' function in the "survival" package (Therneau 2014) for program R to fit global models with air temperature, soil temperature and soil moisture. I also included interaction terms between each of these covariates and time to assess whether selection changed over the tracking period. Models were stratified by sampling occasion and frog ID to account for paired samples and non-independence of multiple



samples for the same frog. I used the 'stepAIC' function in the "MASS" package (Venables and Ripley 2002) to perform a stepwise selection using AIC to select the best model. For models that included time interactions, I used the "simPH" package (Gandrud 2014) to produce plots of relative hazard over time to show how the effect of the covariate changes over time.

Using the hourly temperature data from data loggers in enclosures, I calculated average temperature, minimum temperature, maximum temperature decrease over an hour, and maximum temperature decrease over 24 hours for each interval between survival checks. I also determined the number of freeze/thaw cycles and the maximum freeze duration experienced during each interval. Freeze events were defined as periods where the soil temperature dropped below  $-2^{\circ}\text{C}$  and thaw events were defined as periods where the soil temperature rose above  $-2^{\circ}\text{C}$ . Sinclair et al. (2013) used a similar threshold ( $-1.4^{\circ}\text{C}$ ) to define freeze/thaw events because wood frog body fluids are capable of super cooling to  $-2^{\circ}\text{C}$ . I used two-way ANOVA to test for treatment effects on temperature variables, soil moisture, and frog depth in substrate.

I modelled survival using Cox proportional hazards models (Cox 1972). Tied survival times were handled using the Efron method (Efron 1977). First, I modeled survival based on tracking group and snow cover group to determine the effect of the experimental treatments on survival. I plotted Cox-Snell residuals to assess model fit and examined standardized Schoenfeld residuals plotted against time for each variable to assess the proportional hazards assumption. Although the model fit the data reasonably well, the hazard for snow cover was non-proportional. This non-proportionality was expected because the snow removal treatment should differ from the natural snow cover treatment only during periods in where snow cover was present. To account for changes in snow cover over time I included an interaction term between snow cover treatment and the percentage of days during a survival interval with snow cover.

I used an information theoretic approach to investigate support for four competing hypotheses regarding overwintering survival: acute exposure, energy exhaustion, desiccation, or differences among individuals. I developed a candidate set of *a priori* models to represent each hypothesis using temperature and moisture covariates. Covariates that were highly correlated were not included in the same model to avoid problems with collinearity. This resulted in a total of 15 *a priori* models including a null model containing no covariates and a global model containing all covariates in the model set. Under the acute exposure hypothesis I expected that colder minimum temperature, longer maximum freeze duration, greater decrease in temperature in a day, and/or shallower frog depth would result in reduced survival. Under the energy exhaustion hypothesis I expected lower survival under higher average temperature, greater number of freeze-thaw cycles endured, and/ or lower initial body weight. Under the desiccation hypothesis I expected lower survival in locations with lower soil moisture. I included frog sex under the individual differences to test for overwinter survival differences between males and females, since overwintering site selection may differ between the sexes (Regosin 2003).

Using the *a priori* model set, I built Cox proportional hazards models stratified by treatment group. Stratification treats the experimental treatment groups (tracking group and snow cover group) as nuisance variables and controls for them by allowing their baseline hazard rates to vary. Under a stratified model, hazard rates vary between strata, but the hazard ratio for covariates is the same across strata. Thus if a one unit change in the covariate doubles the hazard in one strata, the one unit change also doubles the hazard in all other strata. All temperature variables were treated as time-varying covariates in the survival models. Since survival was determined at irregular intervals throughout the winter, interval specific temperature averages, maxima, minima and counts were calculated from the raw temperature data so that survival

status within a given interval reflects only temperature conditions experienced in that time interval.

I examined plots of Schoenfeld residuals for each variable plotted against time in the global model to assess the assumption of proportional hazards. I ranked models by Akaike Information Criterion corrected for small sample size (AICc) and Akaike weights ( $w$ ). I calculated model averaged parameter and unconditional variance estimates for each covariate used in the *a priori* candidate set. Model averaging reduces bias and increases precision of parameter estimates by basing inference on all models in the set and by accounting for model selection uncertainty (Burnham and Anderson 2002). I calculated hazard ratios for the covariates included in the top ranking model and created plots of survival over the entire winter period (X # of days) as a function of model covariates. I created a plot of smoothed instantaneous hazard rates for each strata using the smooth macro in SAS.

## **Results**

### ***Tracking***

I tracked 38 wood frogs between 4 October and 10 December. Mean tracking time per frog was  $38 \pm 10$  SD days. Two frogs were predated and one frog shed its transmitter after a week. The transmitter from the first predation event and the shed transmitter were recovered within a week of activation and reused on newly capture frogs. The first predated frog was consumed by an owl (transmitter found functioning, ~ 15m from last known location in owl pellet) on 11 October and the second predated frog was consumed by a mammalian predator (functioning transmitter found ~ 10m from last known location with tooth marks and mangled antenna) following a large rain event on 19 November.

Frogs moved an average total distance of  $221 \pm 113$  m (max = 524 m, median = 215 m) over the course of the tracking period. The average daily distance moved per frog was  $6 \pm 3$  m (max = 314m, average max per frog = 69m). Neither sex nor site significantly affected average daily distance moved, maximum single day distance moved, or average direction moved (Table 1). Average distance moved per day was positively correlated with both minimum daily air temperature ( $r_s = 0.824$ ,  $p < 0.0001$ ) and precipitation ( $r_s = 0.325$ ,  $p = 0.0107$ ).

### ***Habitat Selection***

Soil temperature and a soil temperature by time interaction were the only two covariates retained in the final model comparing current and previous locations. The model indicates that during the early tracking period wood frogs selected cooler locations ( $\beta_{\text{SoilTemp}} = -0.8394 \pm 0.6060$  SE, Table 2) but later in the tracking period, frogs were more likely to select warmer locations (Figure 1). The final model comparing current locations and random locations included soil temperature, soil moisture and the interaction terms between soil temperature and time and soil moisture and time. This model also suggested that frogs were more likely to use warmer locations later in the tracking period (Table 2, Figure 2). Wood frogs were also more likely to use moister locations and this effect became stronger later in the tracking period (Table 2, Figure 3).

### ***Overwintering Microhabitat Conditions***

Experiment-wide average soil temperature at wood frog overwintering sites was  $-0.10 \pm 0.50$  (Range:  $-0.99 - 1.00$ ). The average minimum soil temperature experienced was  $-3.34 \pm 1.67$  (Range:  $-8.5 - -0.5$ ). Overwintering locations experienced an average of  $11.2 \pm 10.9$  (Range:  $0 - 43$ ) freeze-thaw cycles over the winter and the average maximum freeze duration experienced was  $0.5 \pm 0.9$  days (Range:  $0 - 4.6$  days). Average soil moisture at overwintering locations was

34.4 ± 12.3 %VMC and average minimum soil moisture was 22.2 ± 13.2 % VMC. Temperature conditions in the natural snow cover treatments and snow removal treatments were similar during periods without snow cover, but differed when there snow cover, especially later in the season (Figure 4). Frogs in snow removal treatments experienced lower minimum ( $F_{1,63} = 55.37$ ,  $p < 0.0001$ ) and average temperatures ( $F_{1,63} = 9.40$ ,  $p = 0.0032$ ), more freeze-thaw cycles ( $F_{1,63} = 54.24$ ,  $p < 0.0001$ ) and longer maximum freeze durations ( $F_{1,63} = 72.75$ ,  $p < 0.0001$ ) than frogs in the natural snow cover treatments (Figure 5, Table 3). Tracked frogs were in deeper hibernacula than paired frogs ( $F_{1,63} = 12.38$ ,  $p = 0.0008$ ). Soil moisture did not differ among treatment groups (Figure 5).

### ***Survival Monitoring***

Of the 38 frogs tracked, I enclosed 35 frogs before transmitter failure (two lost to predation, one transmitter shed during tracking period). Additionally, one enclosure was removed by a hiker (enclosure found wedged in tree root ~ 300 m away), resulting in 34 tracked frogs for the survival study. The 34 tracked frogs were paired with 34 non-tracked frogs resulting in a total sample size of 68 frogs.

A total of 28 of the 68 frogs monitored survived the winter. One paired frog was removed from the survival analysis despite surviving because it experienced atypical conditions due to rainwater filling its enclosure with >10cm of water for the majority of winter. Estimated survival probability for tracked frogs was 78.2% in the natural snow cover treatment and 36.1% in the snow removal treatment. Estimated survival probability for paired frogs was 51.0% in the natural snow cover treatment and 6.1% in the snow removal treatment. The base Cox proportional hazards model indicated that paired frogs had a 2.74 times greater risk of death (Table 4). A significant interaction between snow cover treatment and the percent of days with snow cover

during a survival interval indicated that during intervals with 100% snow cover, frogs in the snow removal treatment were at 15.55 times greater risk of death than frogs in the natural snow cover treatment (Table 4).

The four top ranking microhabitat survival models all support the acute exposure hypothesis (Table 5). The total Akaike weight of models representing the acute exposure hypothesis was 0.711, compared with 0.133 for the energy exhaustion hypothesis, 0.047 for the desiccation hypothesis, and 0.028 for the individual differences hypothesis. The top ranking model ( $w = 0.246$ ) included both minimum temperature experienced and frog depth in substrate. Parameter estimates from the top model indicate that for every 1°C decrease in minimum temperature experienced, risk of death increases by 22% (Figure 7) and for every 1cm increase in depth in the soil (increasing depth meaning deeper), risk of death decreases by 54.8% (Figure 8).

## **Discussion**

### ***Winter Survival***

Winter in temperate regions pose a significant challenge to many organisms, and ectothermic species in particular. While many amphibians spend the winter underwater or in burrows that extend below the frost line, wood frogs reside in shallow terrestrial hibernacula. This is the first study to report overwinter survival of adult wood frogs monitored *in situ*. My results demonstrate that despite being freeze tolerant, wood frogs depend on avoiding extreme temperatures to survive the winter and likely spend the majority of the winter unfrozen. Snow cover and hibernacula depth provide important buffers from low air temperatures.

Both snow cover and tracking group treatments had large effects on survival. Reduced snow cover has been linked to higher mortality in *Rana lutiventris* (McCafferey and Maxwell 2010), but this is the first study to report direct winter survival effects of experimental snow

removal on an amphibian. Snow removal elevated the risk of death by up to 15.55 times during periods with complete snow cover and paired frogs were 2.74 times more likely to die than tracked frogs. Relative to the natural snow cover treatment, probability of surviving the entire winter was 42% and 45% lower in the snow removal tracked and paired groups respectively. The effect of snow removal is undoubtedly due to the loss the buffer between soil and air temperature. Snow removal resulted in a 0.50°C decrease in mean soil temperature at tracked frog locations and 0.68°C decrease in mean soil temperature at paired frog locations. Furthermore, minimum temperatures experienced were lower, number of freeze thaw cycles endured were higher and average freeze duration was longer in snow removal treatments relative to natural snow cover treatments.

I found that minimum temperature and frog depth explained some of the within group variation in survival. This provides additional evidence that mortality in this study resulted from exposure to temperature conditions that exceeded physiological tolerances. Colder winters have been linked to lower annual survival rates in both Boreal Toads *Bufo boreas* (Scherer et al. 2008) and Common Frogs *Rana lessonae*. This study suggests that in addition to inter-annual temperature variation, spatial variation in temperatures conditions within a winter season can influence wood frog survival.

Of the variables associated with acute exposure mortality, frog depth and minimum temperature explain survival patterns better than maximum freeze duration or maximum decrease in temperature over 24 hours. Although, there are limits to the length of time wood frogs can survive in the frozen state, they are physiologically capable of surviving frozen periods much longer than the relatively short freeze durations experienced during this study (Layne et al. 1998). On the other hand, frogs experienced minimum temperatures between -3.5 and -8.5 which

are much closer to the biologically survivable limits (Costanzo et al. 2013). The strong influence of frog depth was somewhat surprising considering that the deepest recorded depth in this study was 3cm from soil surface to frog dorsum. By creating a small depression, frogs likely benefit from increasing the body surface area in contact with the soil. This may be particularly important during periods without snow cover.

Amphibian winter mortality has also been suggested to result from energy depletion (Pope and Matthews 2002, James et al. 2004, Harper and Semlitsch 2007). While energy exhaustion and acute temperature exposure are not mutually exclusive, I did not find any evidence that energy exhaustion affected winter mortality in this study. The strong effect of acute exposure mortality may have obscured my ability to detect an effect of energy exhaustion. It is also conceivable that conditions did not reach thresholds that would increase risk of energy exhaustion. Winter conditions during this study were neither unusually warm nor cold, while previous research has generally attributed energy exhaustion mortality to abnormal winter seasons. The winter conditions that contribute to energy exhaustion may vary depending on the length of winter a given area. Evidence from habitats with short growing seasons suggest that energy depletion is most likely during severe winters (Pope and Matthews 2002, McCaffery and Maxwell 2010). However, Connecticut winters are more similar to conditions in the United Kingdom, where Reading (2007) found that mild winters were associated lower winter survival rates and poorer body condition of common toads in the United Kingdom. By allowing comparison of survival across years of varying winter conditions, longitudinal studies may be better suited for assessing risk of mortality due to energy depletion.

Soil moisture did not appear to be a major factor affecting survival in this study. Consistent with the findings of Licht (1991), average soil moisture measured at overwinter sites



of tracked wood frogs was 26.6% VMC, which is typical of upland soils in the Storrs area (J. O'Connor unpublished data). While most frogs utilized unsaturated soils, seven frogs selected overwintering sites where soil moisture was > 40% VMC, which is typical of wetland edges and wetland basins. In addition, conditional logistic regression indicated moisture does not play a role in habitat selection during the early fall, but that by late fall soil moisture at wood frog locations was higher than at random available habitat locations. This seems counter-intuitive given wood frogs' preference for overwintering in upland habitat. However, considering average soil moisture was relatively low at overwintering locations, selecting microhabitats that are relatively moister than surrounding available habitat may be important to avoid desiccation during the winter.

While it has been suggested that overwintering in saturated soils may increase risk of death due to freezing or prolonged ice cover (Baldwin et al. 2006), the results from this study do not provide evidence for a strong relationship between soil moisture and survival. In addition a frog in this study survived the majority of the winter in a flooded enclosure after it filled with water following substantial rainfall. This frog was in the paired location treatment, and was removed from the survival analysis due to the unique circumstances. The frog survived underwater despite a period of ice cover that lasted for over six weeks. Although this is just a single observation, it substantiates the findings Licht (1991) who reported complete survival of 8 wood frogs experimentally submerged in water at -1.5°C. Together these findings indicate that like other ranids, wood frogs are physiologically capable of surviving prolonged submergence in ice covered water. Licht (1991) suggests that the behavioral preference for terrestrial wintering sites explains why wood frogs are successful in northern portions of their range where small fish free pools are uncommon. Overwintering in shallow terrestrial burrows may also allow wood

frogs to respond to rising air temperatures during early spring, which submerged frogs would be buffered from. This would allow wood frogs to initiate breeding activity even before water temperatures have risen.

### ***Late Fall Movement***

Movement of wood frogs between 4 October and 10 December was strongly linked to both air temperature and precipitation. Spring and summer movements are known to be associated with rainfall (Baldwin et al. 2006, Rittenhouse et al. 2009). The relationship between air temperature and movement may be stronger during the late fall than during summer, as average temperatures drop below thresholds optimal for activity. Long distance movements by wood frogs in my study corroborate the observations of Baldwin et al. (2006), who suggest that wood frogs may make long distance migrations during fall. Capture rates of adult wood frogs at drift fences adjacent to wetlands in Massachusetts peaked in both spring and fall, suggesting that wood frogs may be moving back towards breeding wetlands during the fall (Regosin et al. 2003). I observed one male frog moved 314 m uphill from a stream edge to the edge of a dry breeding wetland in one rainy night. In addition, a few frogs moved in the general direction of wetlands at both of my sites, although no other frog moved directly adjacent to a wetland. I was not able to confirm that wood frogs were indeed moving towards breeding sites because I did not track frogs following spring emergence. Moving back to breeding sites before the onset of winter may allow wood frogs to reach wetlands earlier in the spring and provide a breeding advantage (Zweifel 1989). I did not observe any difference between fall movement patterns between males and females despite evidence that males tend to overwinter closer to breeding pools than females (Regosin et al. 2003).

### ***Conclusion***

While this study demonstrates that snow loss can increase wood frog mortality by increasing exposure to colder temperatures, future snow loss is predicted to result from higher average winter temperatures. This complicates prediction of the effect of snow loss on wood frog populations in the future, and highlights the need for more research into this understudied life stage. Current climate research suggests that reduced midwinter snow accumulation will result from both an increase in the proportion of precipitation falling as rain rather than snow and also from an increase in the rate of snow melt (Räisänen 2008). While this study suggests that loss of snow has the potential to dramatically increase mortality, the true effect of snow loss resulting from winter warming will ultimately hinge on the timing of weather patterns. Increased snow melt will increase the proportion of days during the winter without snow cover. If temperature during snow free periods remains above freezing then the effect of snow loss will likely be dampened. However, wood frogs will also be vulnerable to rapid cooling events during these periods. Future research is need to assess the risk of freeze events following mid-winter snow melts.

The effect of tracking group suggests that late fall movement may increase the likelihood of surviving the winter. Tracked frogs were on average deeper than paired frogs. This could be due to differences in soil characteristics between the pre-late fall movement location and the post-late fall movement location or behavioral differences between paired and tracked frogs. In addition, while mean temperatures did not vary between tracked and paired locations, paired locations experienced colder minimum temperatures, and more freeze/thaw cycles than frogs at tracked location. Thus, some mortality at paired locations may be attributable to differences in temperature conditions between tracked and paired locations. This is consistent with the results from the habitat selection model which indicated that at a fine scale wood frogs selected warmer

sites than randomly available during late fall. This suggests that wood frogs are selecting overwintering sites that increase the likelihood of surviving the winter. Future research into what factors affect overwinter habitat selection will provide useful information for habitat managers.

Survival is a fundamental parameter governing population growth. Given that winter climate is changing in the northeastern United States, it is important to understand how organisms will respond, in order to provide useful information and guidance to conservation planners. This study is the first to provide a direct estimate of winter survival in a freeze-tolerant amphibian. This study also provides baseline data on the range of temperature and moisture conditions experienced by wood frogs during a typical winter, which should help with the design of future overwintering experiments. Future research should incorporate more refined winter climate predictions or simulations to provide a better understanding of how winter changes will affect wood frog survival. Experimental manipulations of soil temperature and moisture will help improve the precision of parameter estimates. Additionally, development of spatially explicit survival models that link survival to widely available air temperature and precipitation data would be a useful tool for conservation planners.

## **Acknowledgements**

I thank Tracy Rittenhouse for support and advice at every stage of this project. I appreciate field assistance provided by Kelly O'Connor, Michael Evans, Jan-Michael Hessenauer, Annie Stupik, Rachael Hyland, Hayley Morway, David Zebrowski, and Sarah Banker. Isaac Ortega and Mark Urban provided helpful comments on the manuscript. This project also benefited from discussion during Rittenhouse-Vokoun lab group meetings.

## References

- Anholt BR, Hotz H, Guex G, Semlitsch RD. 2003. Overwinter survival of *Rana lessonae* and its hemiclinal associate *Rana esculenta*. *Ecology* 84(2):391-7.
- Baldwin RF, Calhoun AJK, deMaynadier PG. 2006. Conservation planning for amphibian species with complex habitat requirements: A case study using movements and habitat selection of the wood frog *Rana sylvatica*. *J Herpetol* 40(4):442-53.
- Costanzo JP, Lee Jr RE, Wright MF. 1991. Effect of cooling rate on the survival of frozen wood frogs, *Rana sylvatica*. *Journal of Comparative Physiology B* 161(3):225-9.
- Costanzo JP and Lee RE. 2013. Avoidance and tolerance of freezing in ectothermic vertebrates. *J Exp Biol* 216(11):1961-7.
- Costanzo JP and Lee RE, Jr. 2008. Urea loading enhances freezing survival and postfreeze recovery in a terrestrially hibernating frog. *J Exp Biol* 211(18):2969-75.
- Costanzo JP, do Amaral MCF, Rosendale AJ, Lee RE. 2013. Hibernation physiology, freezing adaptation and extreme freeze tolerance in a northern population of the wood frog. *The Journal of Experimental Biology* 216(18):3461-73.
- Cox DR. 1972. Regression models and life-tables. *Journal of the Royal Statistical Society. Series B (Methodological)* 34(2):187-220.
- Efron B. 1977. The efficiency of cox's likelihood function for censored data. *Journal of the American Statistical Association* 72(359):557-65.
- Gandrud, C. 2014. simPH: Tools for simulating and plotting quantities of interest estimated from Cox Proportional Hazards models. R package version 1.2.1. <http://CRAN.R-project.org/package=simPH>
- Hayhoe K, Wake C, Huntington T, Luo L, Schwartz M, Sheffield J, Wood E, Anderson B, Bradbury J, DeGaetano A, et al. 2007. Past and future changes in climate and hydrological indicators in the US northeast. *Clim Dyn* 28(4):381-407.
- Heemeyer JL and Lannoo MJ. 2012. Breeding migrations in crawfish frogs (*Lithobates areolatus*): Long-distance movements, burrow philopatry, and mortality in a near-threatened species. *Copeia* 2012(3):440-50.
- James SM, Little EE, Semlitsch RD. 2004. Effects of multiple routes of cadmium exposure on the hibernation success of the american toad (*Bufo americanus*). *Arch Environ Contam Toxicol* 46(4):518-27.

- Layne JR, Costanzo JP, Lee RE. 1998. Freeze duration influences postfreeze survival in the frog *rana sylvatica*. *J Exp Zool* 280(2):197-201.
- Licht LE. 1991. Habitat selection of *rana pipiens* and *rana sylvatica* during exposure to warm and cold temperatures. *Am Midl Nat* 125(2):259.
- McCaffery RM and Maxell BA. 2010. Decreased winter severity increases viability of a montane frog population. *Proceedings of the National Academy of Sciences* 107(19):8644-9.
- Pope KL and Matthews KR. 2002. Influence of anuran prey on the condition and distribution of *rana muscosa* in the sierra nevada. *Herpetologica* 58(3):354-63.
- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <http://www.R-project.org/>.
- Räsänen J. 2008. Warmer climate: Less or more snow? *Clim Dyn* 30(2):307-19.
- Reading CJ. 2007. Linking global warming to amphibian declines through its effect on female body condition and survivorship. *Oecologia* 151:125.
- Regosin JV, Windmiller BS, Reed JM. 2003. Terrestrial habitat use and winter densities of the wood frog (*rana sylvatica*). *J Herpetol* 37(2):390-4.
- Rittenhouse TAG, Semlitsch RD, Thompson FR, III. 2009. Survival costs associated with wood frog breeding migrations: Effects of timber harvest and drought. *Ecology* 90(6):1620-30.
- Rustad L, Campbell J, Dukes JS, Huntington T, Lambert KF, Mohan J, Rodenhouse N. 2011. Changing climate, changing forests: The impacts of climate change on forests of the northeastern united states and eastern canada. Newtown Square, PA: U.S. Forest Service. Report nr NRS-99.
- Scherer RD, Muths E, Lambert BA. 2008. Effects of weather on survival in populations of boreal toads in colorado. *J Herpetol* 42(3):508-17.
- Schmid WD. 1982. Survival of frogs in low temperature. *Science* 215(4533):697-8.
- Schmidt B, Schaub M, Steinfartz S. 2007. Apparent survival of the salamander *salamandra salamandra* is low because of high migratory activity. *Frontiers in Zoology* 4(1):19.
- Sinclair BJ, Stinziano JR, Williams CM, MacMillan HA, Marshall KE, Storey KB. 2013. Real-time measurement of metabolic rate during freezing and thawing of the wood frog, *rana sylvatica*: Implications for overwinter energy use. *The Journal of Experimental Biology* 216(2):292-302.

- Storey K and Storey J. 1984. Biochemical adaption for freezing tolerance in the wood frog, *Rana sylvatica*. *Journal of Comparative Physiology B* 155(1):29-36.
- Tattersall GJ and Ultsch GR. 2008. Physiological ecology of aquatic overwintering in ranid frogs. *Biological Reviews* 83(2):119-40.
- Therneau T. 2014. A Package for Survival Analysis in S. R package version 2.37-7, <URL: <http://CRAN.R-project.org/package=survival>>.
- Venables, WN and Ripley, BD. 2002. *Modern Applied Statistics with S*. Fourth Edition. Springer, New York. ISBN 0-387-95457-0
- Zweifel RG. 1989. Calling by the frog, *Rana sylvatica*, outside the breeding season. *J Herpetol* 23(2):185-6.

## Tables

**Table 1: F statistics (P-values) from ANOVA testing effects of frog sex and site on log average daily movement distance, log maximum daily movement, and average direction moved.**

	<b>DF, Residual DF</b>	<b>Log(Average Daily Movement)</b>	<b>Log(Maximum Daily Movement)</b>	<b>Average Direction Moved</b>
Sex	1, 34	0.0601 (0.8078)	1.4158 (0.2423)	2.4390 (0.1276)
Site	1, 34	0.1025 (0.7508)	0.0226 (0.8813)	2.5246 (0.1213)
Sex:Site	1, 34	0.2353 (0.6308)	0.0009 (0.9764)	0.0239 (0.8780)



**Table 2: Parameter estimates , Odds Ratios, and Wald  $X^2$  statistics for conditional logistic regression models comparing wood frog locations with previous day location and random locations. Parameter estimates reported as  $\beta \pm \text{SE}$ . P-values reported in parentheses after Wald  $X^2$  statistic.**

	Current Location Vs. Previous Location			Current Location Vs. Random Location		
	$\beta$	Odds Ratio	Wald $X^2$	$\beta$	Odds Ratio	Wald $X^2$
<b>Soil Temperature</b>	-0.8394 $\pm$ 0.6060	0.432	-1.39 (0.170)	0.1834 $\pm$ 0.0462	0.183	-2.41 (0.016)
<b>Soil Temperature*Time</b>	0.0832 $\pm$ 0.0465	1.087	1.79 (0.073)	0.1467 $\pm$ 0.0561	1.158	2.62 (0.009)
<b>Soil Moisture</b>	---	---	---	-0.0680 $\pm$ 0.0366	0.934	-1.86 (0.063)
<b>Soil Moisture*Time</b>	---	---	---	0.0039 $\pm$ 0.0027	1.004	1.46 (0.146)

**Table 3: Averages and ranges of temperature conditions within experimental treatments.**

	Average Average Temperature	Range of Average Temperatures	Average Minimum Temperature	Range of Minimum Temperatures	Average # of Freeze Thaw Cycles	Range # of Freeze Thaw Cycles	Average Max Freeze Duration	Range of Max Freeze Durations
Paired, No Snow Tracked,	-0.52	(-2.60, 1.88)	-2.54	(-7.5,0.5)	5.9	(0, 30)	1.08	(0, 4.67)
No Snow	-0.41	(-2.54, 1.77)	-2.41	(-8.5,0)	7.8	(0, 31)	0.87	(0, 3.79)
Paired, Snow	0.16	(-2.17, 4.11)	-1.45	(-4.5,3.5)	2.9	(0,11)	0.23	(0, 2.04)
Tracked, Snow	0.11	(-1.76, 2.65)	-1.31	(-3.5,0.5)	3.8	(0,43)	0.17	(0, 2.42)

**Table 4: Cox proportional hazards model that tests survival effects of treatments: tracking group (radio-tagged versus paired) and snow group (natural snow cover versus snow removal). Snow treatment by snow indicator interaction included to account for non-proportional hazard of snow treatment over time. N = 288, number of events = 39.**

	$\hat{\beta}$	SE	Wald $X^2$	$p$	Hazard Ratio
<b>Tracking(Paired)</b>	1.00	0.33	3.03	0.0024	2.74
<b>Snow(No Snow)</b>	-0.05	0.81	-0.067	0.9465	0.94
<b>Snow*Percent Snow Cover</b>	2.74	1.39	1.97	0.0489	15.55

**Table 5: AIC Table for cox regression models. All models, including null are stratified by tracking and snow group. Models ranked by AICc value. Models are shaded by survival hypothesis. Dark grey indicates desiccation hypothesis, intermediate grey indicates acute exposure hypothesis, and light grey indicates energy exhaustion hypothesis. Null model, global model, and sex model are not shaded.**

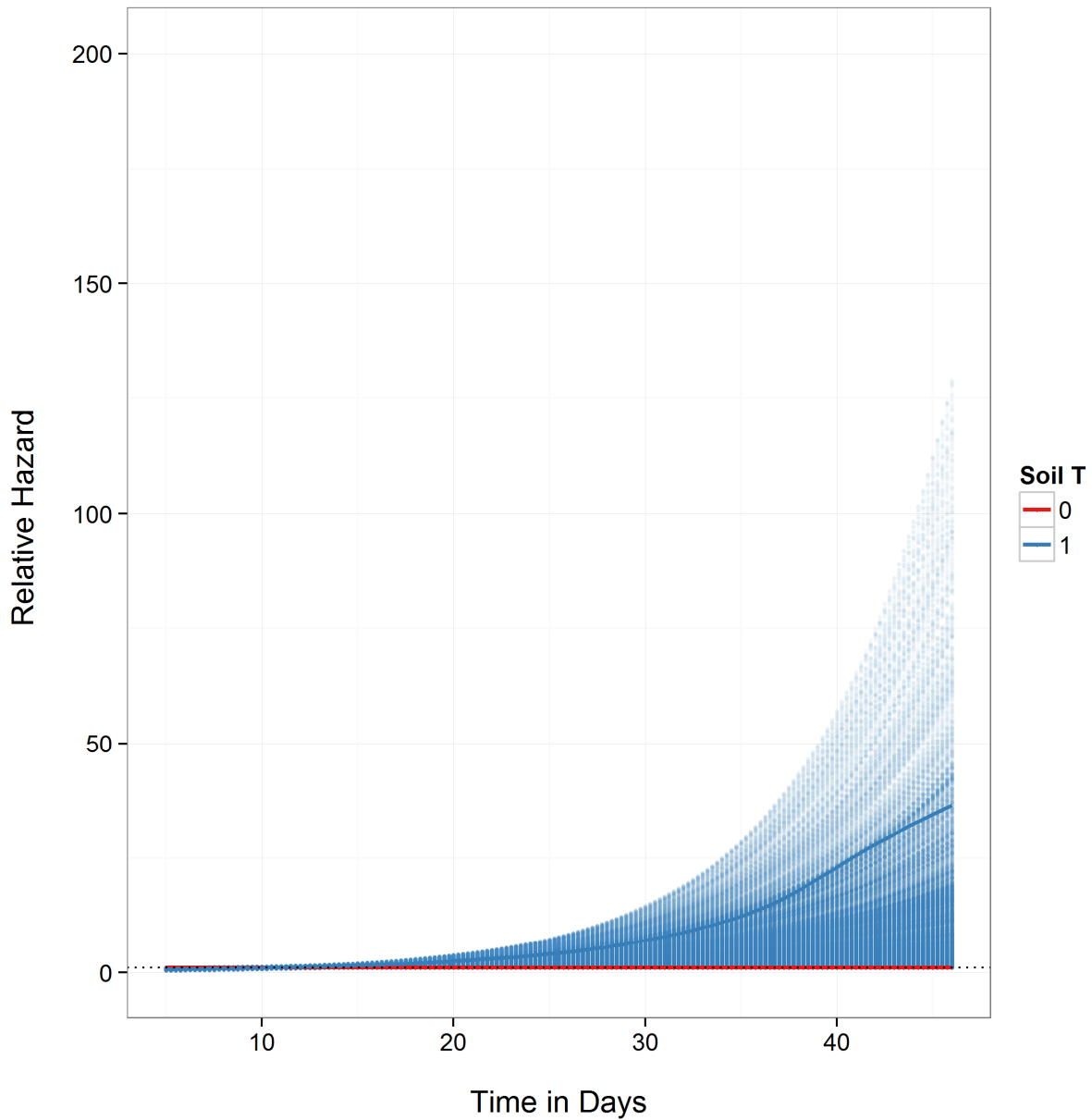
<b>Model</b>	<b>K</b>	<b>AICc</b>	<b><math>\Delta</math>AICc</b>	<b>w</b>	<b>LL</b>
Minimum Temperature + Frog Depth	2	170.874	0	0.242	-83.416
Frog Depth	1	171.163	0.289	0.210	-84.574
Minimum Temperature	1	172.925	2.051	0.087	-85.456
Maximum Freeze Duration	1	172.960	2.086	0.085	-85.473
<b>Null model</b>	0	173.141	2.267	0.078	-86.571
Max Daily Temperature Decrease	1	173.998	3.123	0.051	-85.992
Average Temperature	1	174.081	3.207	0.049	-86.034
Soil Moisture	1	174.135	3.261	0.047	-86.061
MinTemp + MaxFrzDuration + MaxDayTempDecr	2	174.669	3.795	0.036	-85.313
# Frz/Thaw Cycles	1	175.115	4.241	0.029	-86.551
Mass	1	175.140	4.265	0.029	-86.563
Sex	1	175.147	4.273	0.028	-86.567
AvgTemp + # Frz/Thaw Cycles	2	175.892	5.018	0.020	-85.925
AvgTemp + # Frz/Thaw Cycles + Mass	3	177.931	7.056	0.007	-85.923
<b>Global</b>	9	181.733	10.859	0.001	-81.543

K = number of estimable parameters in the model, AICc= Akaike information criterion corrected for small sample size,  $\Delta$ AICc = difference in AICc score between model and top ranked model, w = Akaike weight, LL = Log-likelihood

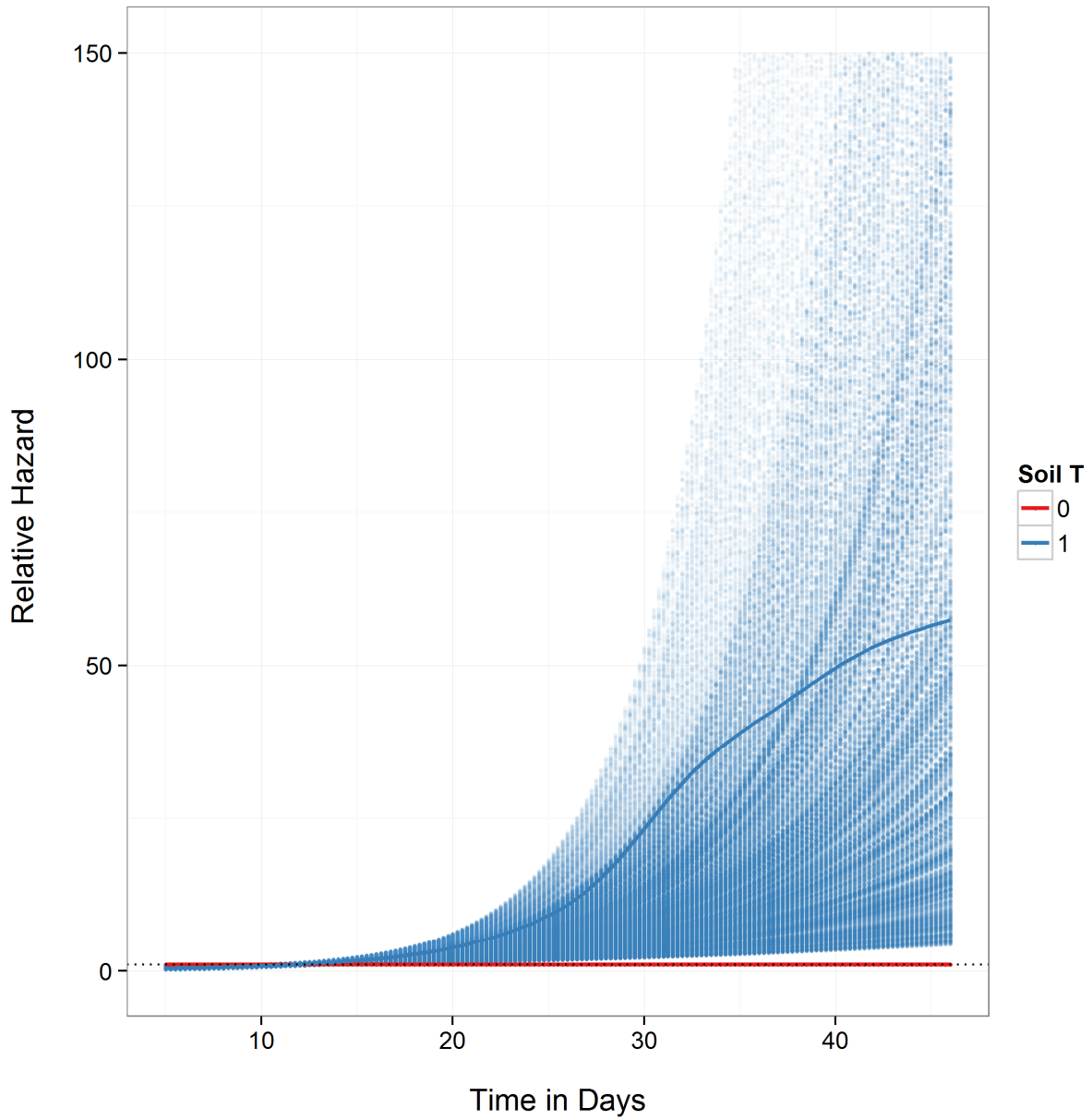
**Table 6: Model averaged parameter estimates, 95 % confidence intervals, Hazard Ratios and relative variable importance. Relative importance is calculated by summing Akaike weights. Parameter estimates reported as point estimate  $\pm$  unconditional standard error.**

Covariate	$\hat{\beta}$	95% CI	Hazard Ratio	Relative Importance
Frog Depth	-0.795 $\pm$ 0.434	(-1.672, 0.082)	0.452	0.453
Minimum Temperature	-0.198 $\pm$ 0.154	(-0.509, 0.111)	0.820	0.367
Maximum Freeze Duration	0.217 $\pm$ 0.214	(-0.215, 0.649)	1.242	0.122
Max Temp Decrease in 24hrs	-0.076 $\pm$ 0.086	(-0.250, 0.098)	0.927	0.088
Average Temperature	-0.307 $\pm$ 0.302	(-0.916, 0.304)	0.736	0.076
# of Freeze-Thaw Cycles	-0.027 $\pm$ 0.799	(-0.188, 0.135)	0.974	0.057
Mass	-0.003 $\pm$ 0.035	(-0.073, 0.067)	0.997	0.037
Moisture	0.015 $\pm$ 0.014	(-0.013, 0.041)	1.014	0.049
Sex	0.047 $\pm$ 0.367	(-0.695, 0.788)	1.048	0.030

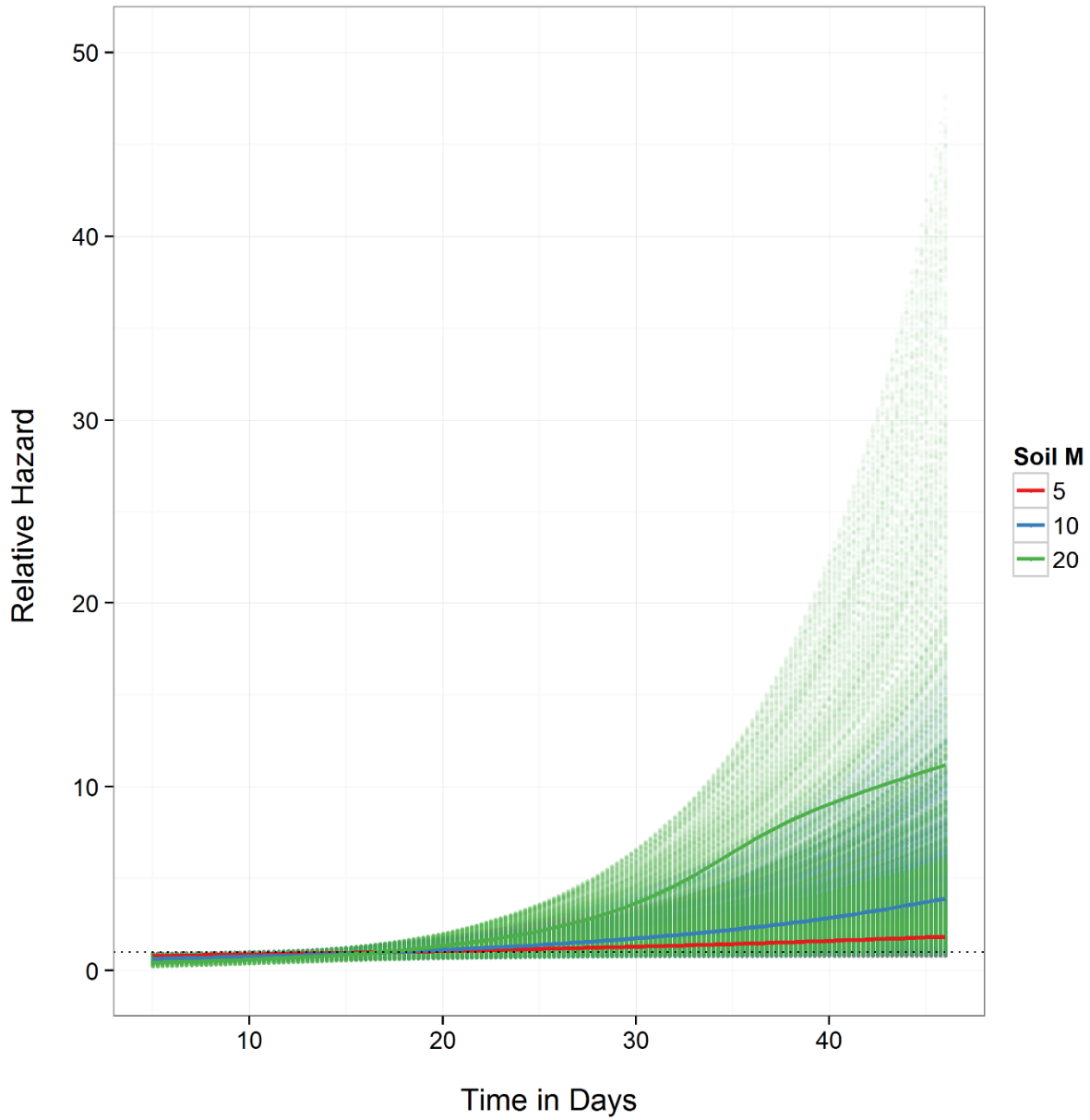
## Figures



**Figure 2: Relative odds of a wood frog using a given location if it is 1°C warmer than its previous days location plotted over the course of the tracking period. Relative hazard of 10 means that a frog is 10 times as likely to use a new location if it 1 degree warmer than the previous one.**

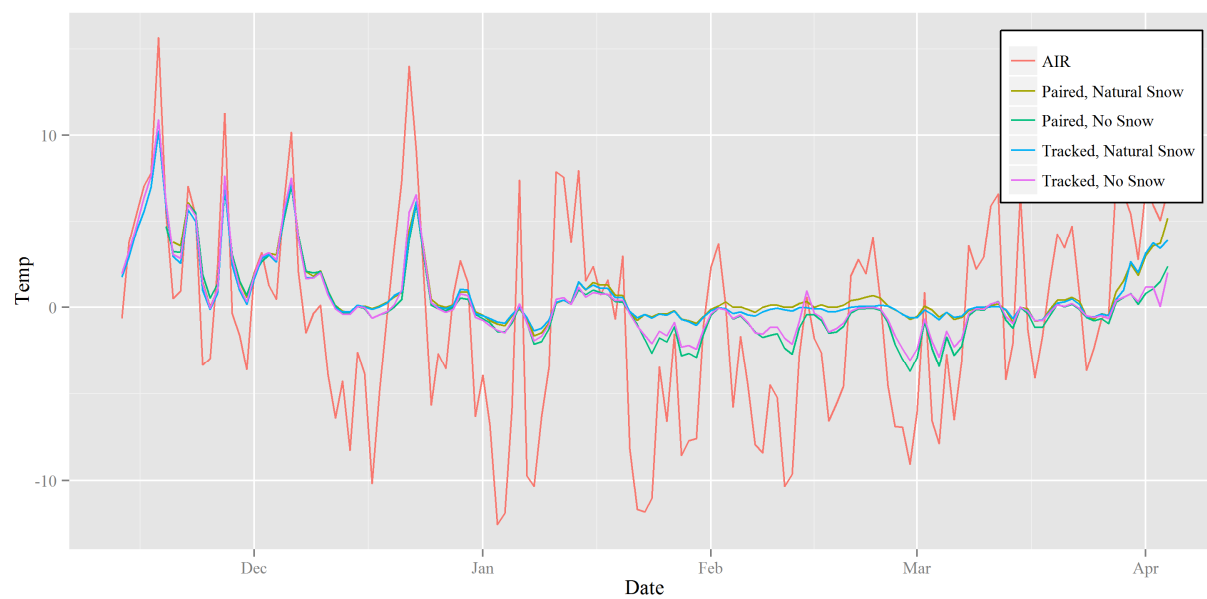


**Figure 2: Relative odds of a wood frog using a given location if it is 1°C warmer than a randomly selected available location plotted over the course of the tracking period. Relative hazard of 10 means that a frog is 10 times as likely to use a new location if it 1 degree warmer than the previous one.**



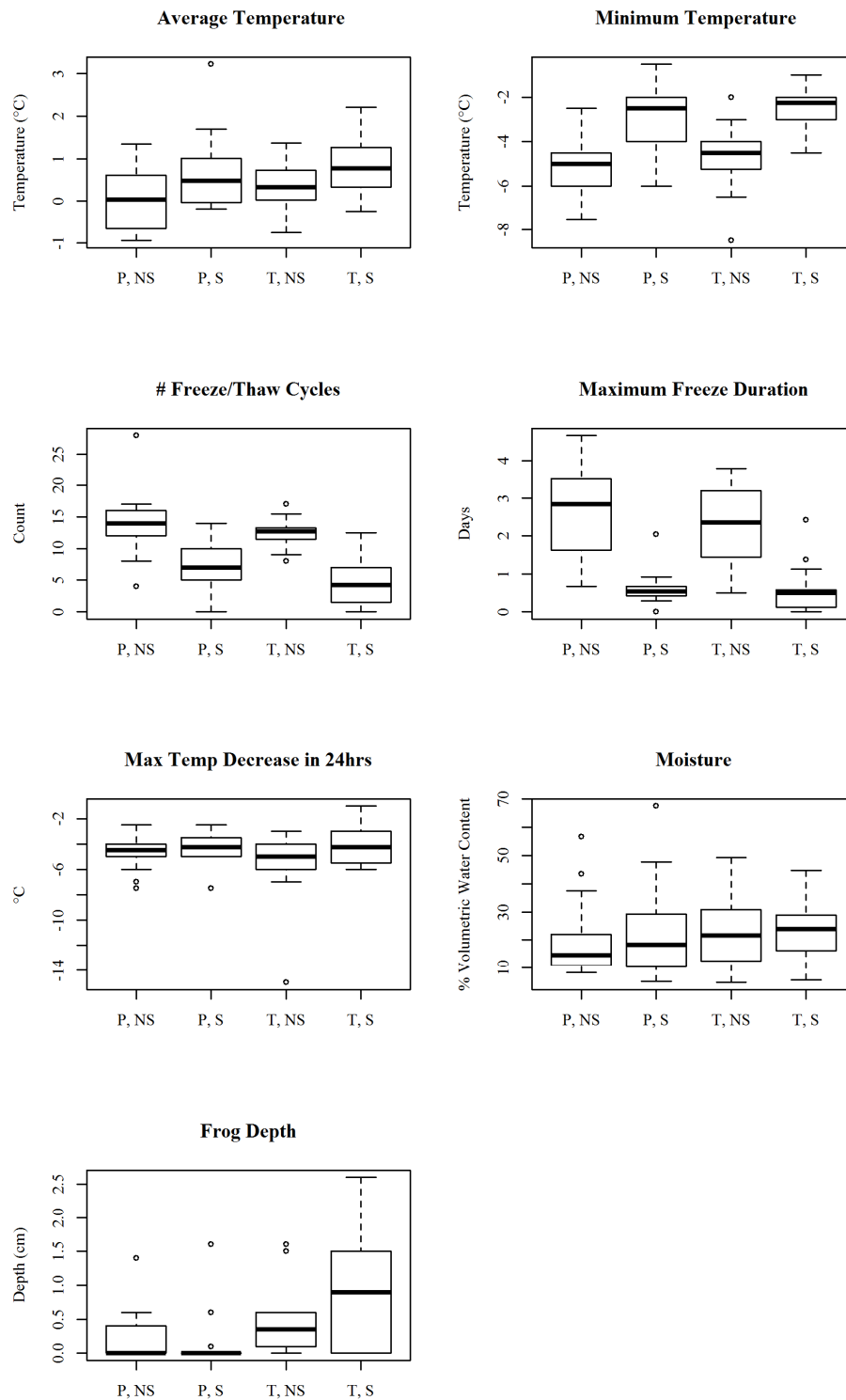
**Figure 3: Relative odds of a wood frog using a given location if it has 5%, 10%, or 20% higher volumetric moisture content than a randomly selected available location. Relative hazard of 10 means that a frog is 10 times as likely to use a new location given the change in soil moisture**



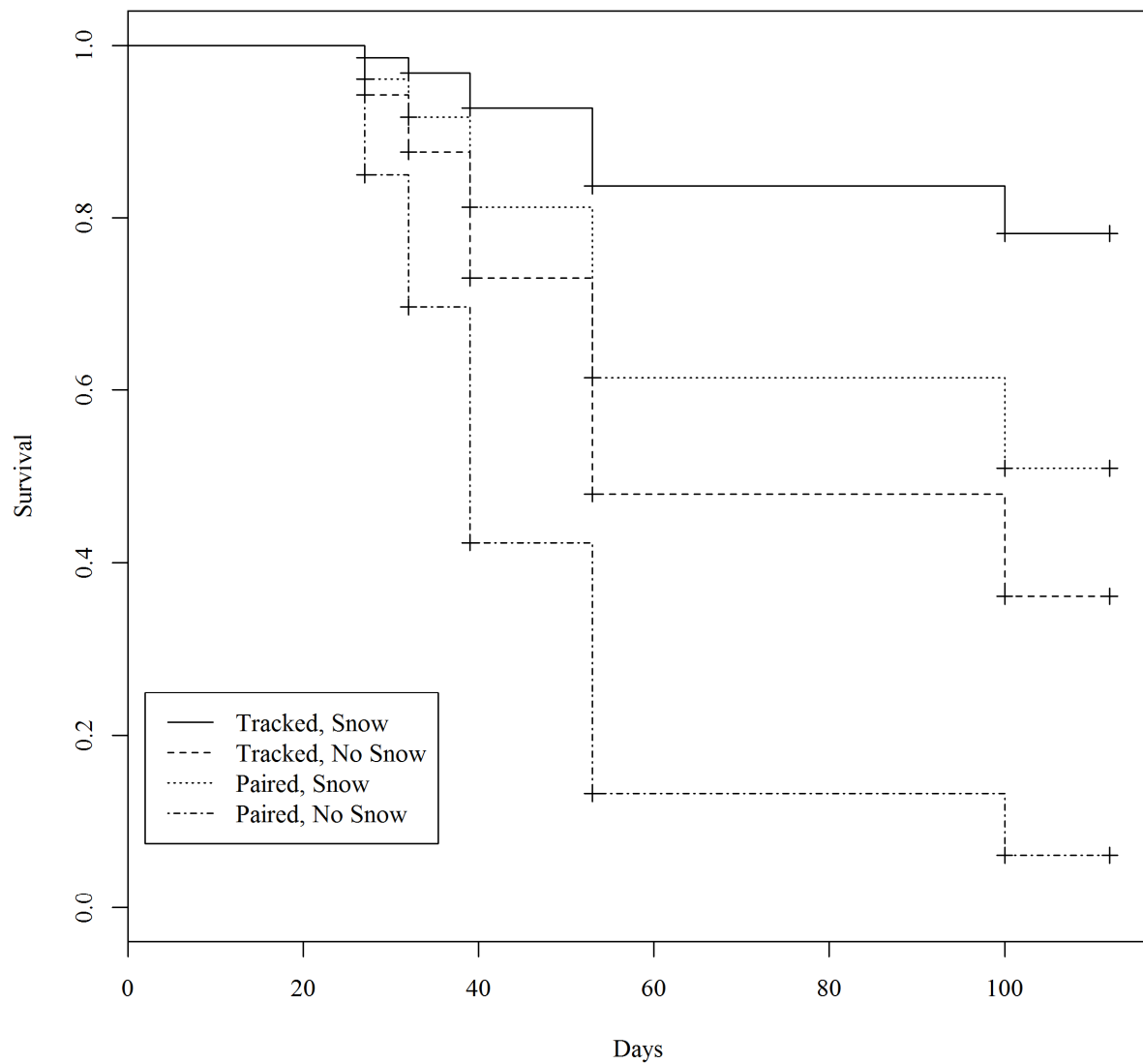


**Figure 4: Soil temperature for each treatment plotted against air temperature**

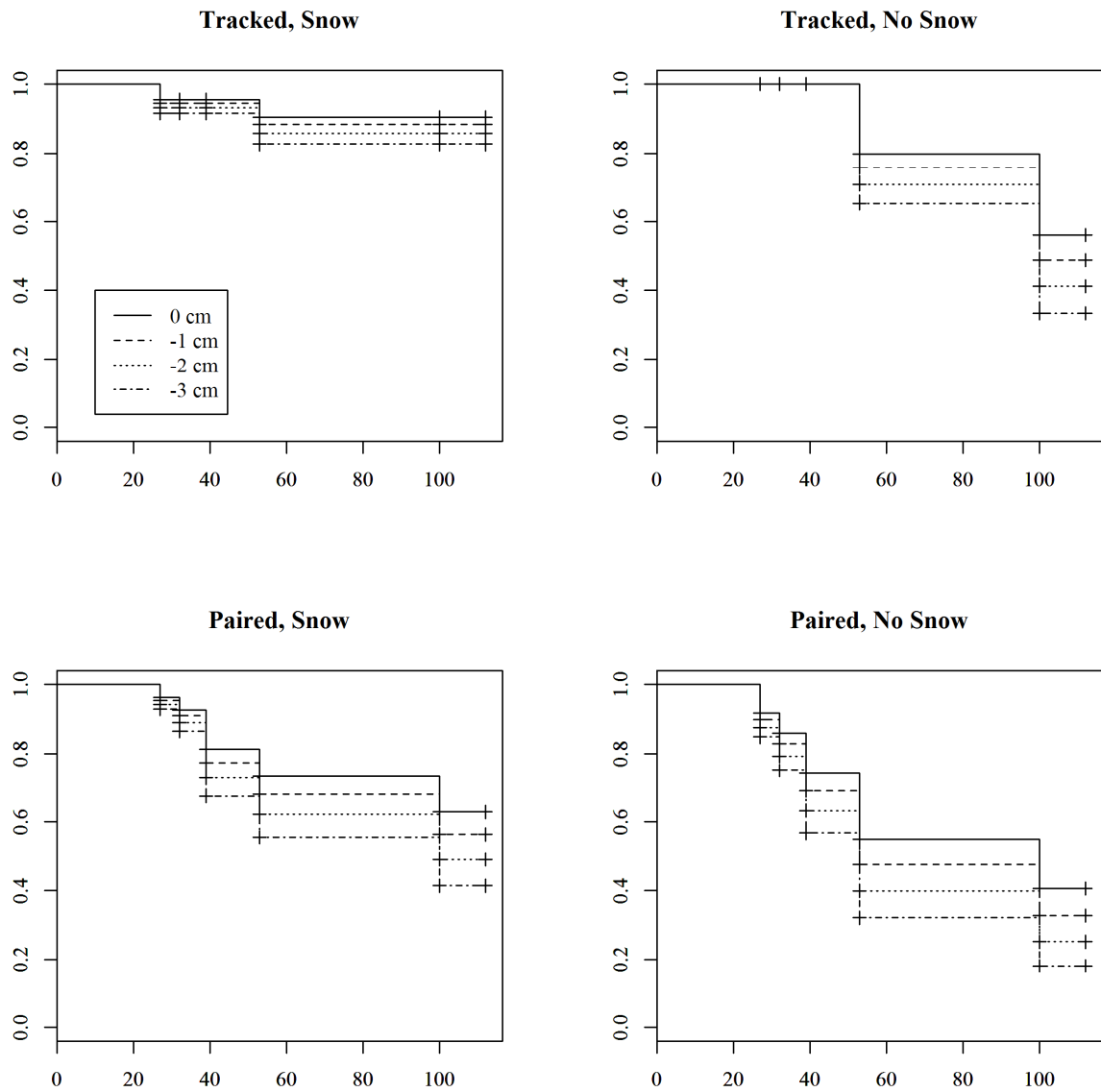
•



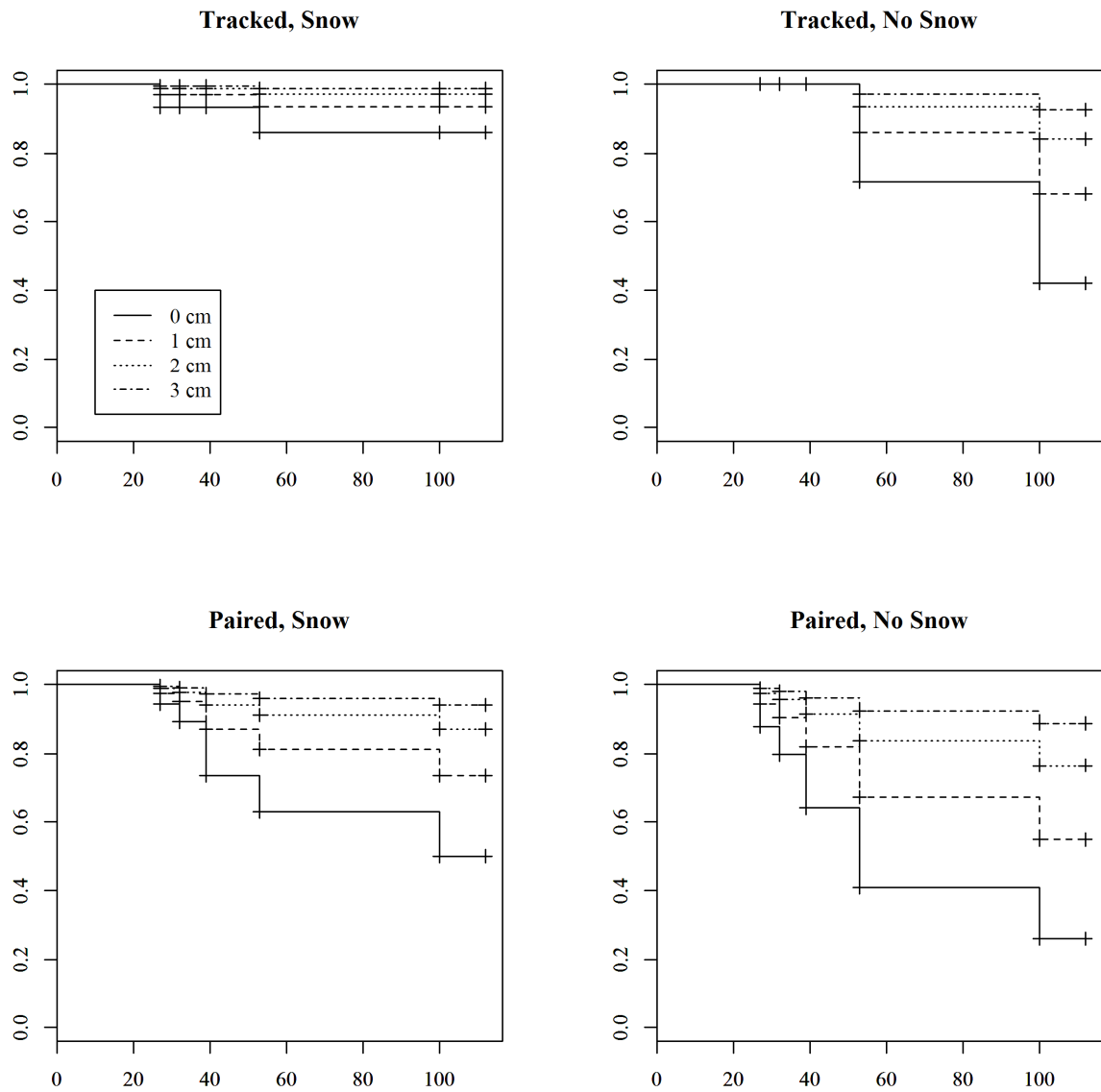
**Figure 5: Winter microhabitat conditions for Tracking and Snow cover experimental treatments. P = Paired, T= Tracked, NS = No snow cover, S = Natural snow cover.**



**Figure 6: Estimated survival curves for cox proportional hazards model including tracking treatment, snow treatment, and snow treatment by percentage of snow cover days interaction term. The percentage of days with snow cover for this estimation is set at 0.538, which is the actual percentage of days with snow cover experienced during the study.**



**Figure 7: Effect of minimum temperature on survival from most supported cox proportional hazards, including both minimum temperature and frog depth as covariates. Model stratified by tracking group and snow removal group. Strata plotted separately. Day 0 is December 10, 2013.**



**Figure 8: Effect of frog depth on survival from most supported cox proportional hazards, including both minimum temperature and frog depth as covariates. Model stratified by tracking group and snow removal group. Strata plotted separately. Day 0 is December 10, 2013.**