Movement and Habitat Use of the Long-Toed Salamander (*Ambystoma macrodactylum*) in Waterton Lakes National Park, Alberta

by

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Abstract

Population estimates for adult long-toed salamander (*Ambystoma macrodactylum*) at Linnet Lake in Waterton Lakes National Park, Alberta, showed a 60% decline from 1994 and 2008 –2009. To prevent further decline, in 2008 Parks Canada installed four under-road crossing structures (tunnels) and directional fencing along the road bordering Linnet Lake to reduce road mortality, which was known to be high. Parks later learned that predacious fish had colonized the lake, likely during natural flooding. In 2010 and 2011, Parks removed and relocated 35000 fish from Linnet Lake over 11 days of trapping. Measures intended to restore wildlife populations to historic levels often go unmonitored, and success or failure is not systematically assessed. Long-toed salamanders are small and delicate, and are difficult to monitor when they inhabit the terrestrial environment during the 10 – 11 month non-breeding season. To determine the status of the Linnet Lake long-toed salamander population and investigate terrestrial movement patterns (orientation) and habitat-use, I conducted research in 2013 and 2014 at Linnet Lake and a nearby reference site (Stable Pond, 1.2 km away). I conducted a mark-recapture study at Linnet Lake by marking salamanders with passive integrated transponder (PIT) tags, and I used radio frequency identification (RFID) antennas and cameras in tunnels to monitor use by tagged individuals and compare the two methods. I also used PIT tags to mark salamanders at Stable Pond and, using a home-made portable RFID antenna (scanner), I conducted “PIT telemetry” to locate tagged animals in the terrestrial environment at both sites and tested the scanners read range. At Linnet Lake, I found no increase in adult salamander population size from estimates made during the 2008 –2009 study and I found little evidence of recruitment when I compared demographic data to Stable Pond. Population estimates of 1380 (95% CI: 1138, 1702) in 2013 and 706 (95% CI: 575, 893) in 2014 indicate a declining population at Linnet Lake and raise concern regarding the viability of the population and urgency for conservation efforts. RFID antennas were 6.5 times more likely than cameras to detect a tagged salamander entering or exiting tunnels. Salamander orientation was non-uniform at both study sites, with movement patterns staying consistent between years at Linnet Lake and differing between age classes and as salamanders moved further from the shore at Stable Pond. Using PIT telemetry, I relocated 32 individuals in the terrestrial habitats around Linnet Lake and 80 at Stable Pond. I was able to locate and characterize nine overwintering sites and each was associated with decomposing tree roots. Tests of the portable RFID antenna’s read range in three substrates (soil, rock, water) at multiple depths showed the highest read range in water, and a non-linear effect of depth on horizontal read range. This study provides important data for monitoring the long-term effects of mitigation efforts at Linnet Lake, and demonstrates the utility of RFID and PIT tags for tracking small terrestrial vertebrates and monitoring the use of road-crossing structures.

Preface

The fourth chapter of this thesis represents collaborative research that has been submitted as a co-authored work for publication. I was primarily responsible for constructing the portable RFID antenna, experimental design, conducting field trials, data analysis, and manuscript preparation. C. Paszkowski contributed largely to manuscript preparation and provided edits and other advice. G. Scrimgeour filled an advisory role for the experimental design, analysis, and provided edits and created figures during manuscript preparation.

Animal research ethics approval was required for the research activities conducted in this study. Approval was obtained from the University of Alberta Animal Care and Use Committee for two animal-use protocols: AUP00000535 "Techniques for Marking and Tracking Amphibians" on February 26, 2013; and AUP00000501 "Ecology and Behaviour of Ambystoma Salamanders" on April 10, 2013.

I would like to dedicate this thesis to my family

To my grandfathers Franklin Scott Adams and William “Bill” Russel Atkinson,
whose names I bear and whose lives inspire

To my parents David and Ruth
for bringing me up in the wilds of Alaska
and fostering my love for nature and truth

And to my brothers Chad, Seth, and Mark
who have each chosen an unique adventure for themself

“And God made the beast of the earth after his kind, and cattle after their kind, and every thing that creepeth upon the earth after his kind: and God saw that it was good.”
-Genesis 1:25

“Not too often do you get all of this neatness in one location. That’s called nature”
-Lenny Pepperbottom

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I learned a lot in the field, but I must also thank the teachers I had at the U of A; Rich Moses, Evie Merrill, and Erin Bayne, for the insight and knowledge they passed on to me through the courses I took with them. The course aspect of this degree was just as important as the fieldwork for learning how to do science, and I appreciate the investment of the instructors.

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And God. I must thank God.

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Chapter 1: General Introduction

Globally, amphibians have received a great deal of conservation attention since reports of world-wide declines were synthesized in the early 1990s (Wake and Morowitz 1990, Wake 1991). On a large scale, declines have been attributed to habitat alteration and fragmentation, climate change, and emergent diseases, but the causes of local declines are often context-specific (Beebee and Griffiths 2005). One major challenge that amphibians face as a group is a lack of information regarding long-term population trends and life history characteristics for species (Pechmann et al. 1991, Lawler et al. 2006). Another factor that makes the conservation of amphibians challenging is their complex habitat requirements. Many temperate amphibians require semi-permanent or permanent bodies of water for breeding, egg deposition, and larval development, and nearby terrestrial habitat for foraging, overwintering, and juvenile dispersal (Pittman et al. 2014). Often, local amphibian populations are members of a larger metapopulation and require connectivity among sites for long-term persistence (Hecnar and M'Closkey 1996, Marsh and Trenham 2001, Smith et al. 2005).

Of threats to amphibians, human-caused habitat loss and alteration in its various forms (e.g. road building, logging, urban development, chemical pollution, introduction of nonnative species) have consistently been ranked at the top (Corn 1994, Hecnar and McCloskey 1996, Collins and Storfer 2003, Beebee and Griffiths 2005, Cushman 2006). In cases where habitat alteration is known to affect an amphibian population negatively, mitigation efforts are commonly undertaken to ameliorate harmful effects. These efforts vary depending on the nature of the threat. Examples range from logistically simple, such as moving amphibians across roads to reduce road mortality (van Bohemen 1998), to more intensive efforts like constructing new breeding sites to offset habitat loss (Pechmann et al. 2001), reintroducing species to counteract local extinctions (Griffiths and Pavajeau 2008), and removing nonnative species to restore habitat quality (Kanppe et al. 2007, Boone et al. 2008).

In cases where mitigation occurs, it is important to have a scientifically rigorous design to ensure effectiveness. This includes collecting baseline data pre-mitigation, and developing a monitoring plan to assess long-term effects. Unfortunately, such pre- and post-activities are seldom done, and the effects of many efforts to restore wildlife populations go unevaluated (Block et al. 2001). Semlitsch (2000) has outlined the special needs and life history characteristics that should be taken into account for conservation-management of amphibians, but the kinds of information necessary for effective management and restoration (e.g. terrestrial habitat requirements, population and community dynamics, responses to different types of disturbance), are not known for many species (Lawler et al. 2006). The natural short-term variability of amphibian population sizes heightens the difficulty in distinguishing human-caused change from natural fluctuations (Pechmann and Wilbur 1994). For example, Pechmann et al. (1991) showed that occasional abundance estimates, even if conducted long-term, can lead to incorrect interpretations of trends and can misguide conservation efforts. If management goals for amphibians include restoration efforts and/or detecting change at the population-level, then continuous, science-based monitoring rooted in an understanding of basic life-history traits is necessary.

In recent years, a new method for tracking small vertebrates has been applied to amphibians, thus allowing researchers to investigate life history features (e.g. movement patterns and microhabitat use) of species for with traditional tracking methods (e.g. radio telemetry) are not feasible. PIT telemetry, first used with legless lizards (*Anniella pulchra*) by Kuhnz 2000 (but also see Faber 1997 and Roussel 2000), involves marking animals with passive integrated transponder (PIT) tags and remotely detecting their location using a portable, radio-frequency identification (RFID) antenna. In the past, RFID systems have been commonly used to monitor passage of PIT-tagged animals through confined spaces using stationary antennae, and PIT tags by themselves have been used to uniquely mark individual animals for mark-recapture studies (Gibbons and Andrews 2004). PIT telemetry mobilizes the application of RFID technology, but has some limitations for use with terrestrial amphibians. These limitations include: the small detection range ("read range") of commercially available portable RFID antennas, the above-ground complexity of terrestrial habitats in which amphibians are often found, and tag loss by marked individuals (Hamed at al. 2008, Ousterhout and Semlitsch 2014, Ryan et al. 2014). Regardless of limitations, the adaptability of RFID technology makes PIT telemetry a promising technique for examining the terrestrial (and aquatic) habits of many small and inconspicuous amphibian species (Connette and Semlitsch 2012).

Protected natural areas such as national parks can provide an ideal location in which to learn about the basic ecology of animal species, as well as document population-level responses to natural and anthropogenic stressors. For example, in Waterton Lakes National Park (WLNP), Alberta, Canada, the breeding population of long-toed salamanders (*Ambystoma macrodactulum*, Baird 1849) at Linnet Lake has declined by 60% between 1994 and 2008–2009 (Pagnucco et al. 2011) despite relatively minor changes to habitat in and around the lake. This system provides an opportunity to examine recent mitigation efforts to halt amphibian population decline at a local scale, and the protected nature of the Park provides a relatively undisturbed setting in which to explore little-known aspects of this species' life-history.

Study Species

The long-toed salamander is one of the smallest members of its genus (~15 cm total length) and ranges from California along the west coast of North America to Alaska and east to the eastern foothills of the Rocky Mountains in Alberta, Montana, and Idaho (Stebbins 2003). It was separated into five sub-species, distinguished primarily by coloration patterns (Ferguson 1961), and supported by genetics (Lese-Yaw 2012). The eastern long-toed salamander (*A. m. krausii*) is the lineage most often encountered in Alberta and is only found along the western edge of the province in the Rocky Mountains and their foothills, which also represents the northeast edge of the species' range.

Like most other ambystomids, long-toed salamanders are primarily nocturnal and spend the majority of the year below the ground surface foraging or inactive. During the ~1-month breeding season, which varies temporally by latitude and elevation (January – July), adults will make over-land migrations from terrestrial overwintering sites (immigration) to aquatic breeding sites (usually lacking fish) to lay eggs on aquatic vegetation and other available substrates. After breeding, adults return to terrestrial areas (emigration) around the breeding site for the duration of the year. Eggs hatch after several weeks (longer at cold temperatures) and larvae grow and metamorphose usually by late summer or fall, but can take an additional year to develop and emerge at high elevations

(Kezer and Farner 1955). Upon metamorphosis, juveniles disperse into the terrestrial environment to forage and overwinter and will reach sexual maturity at 2 – 3 years of age (Russell et al. 1996).

The terrestrial habits of long-toed salamanders are largely unknown due to difficulties tracking animals their size. They are thought to be able to disperse fairly long distances (1170 m, Funk and Dunlap 1999, Smith et al. 2005) with lower relative dispersal ability among high-elevation mountain sites (Giordano et al. 2007). Fine-scale terrestrial foraging and overwintering habitat, home range size, and overwintering site characteristics are limited to observations near Canmore, Alberta, Canada (Sheppard 1977) and opportunistic observations in other parts of the species' range (e.g. Anderson 1967). Studies of coarse-scale habitat-use and movement (Beneski et al. 1986, Goldberg and Waits 2009), and studies of the effects of various stressors on local distributions (Naughton et al. 2000, Pearl et al 2005) have been conducted in parts of the species' range. Still, the long-toed salamander remains largely unstudied compared to other members of its genus.

Study System

Waterton Lakes National Park occupies the southwest corner of Alberta, Canada, bordered to the west by British Columbia, and to the south by Montana and Glacier National Park, USA. It is the smallest (505 km^2) of Canada's Rocky Mountain national parks and lies at the transition between prairie and montane cordillera ecozones (Parks Canada 2013). This study was conducted at two long-toed salamander breeding sites within WLNP: Linnet Lake and Stable Pond (Figure 1.1).

Linnet Lake (49° 04' N, 113° 54' W) is a small (3.9 ha), foot-shaped, shallow (5 m maximum depth) lake at an elevation of ~1260 m in a bowl-like catchment immediately north-west of the Prince of Wales Hotel. The lake is surrounded by moderately steep-sloping (up to 15%) hillside dominated by Douglas fir (*Pseudotsuga menziesii*) and poplar (*Populus* spp.) forest with a shrubby understory and open grassland except on the north end, which is a flat, low-lying area containing a parking lot (~30 m x 70 m), adjacent to Middle Waterton Lake. Occasionally Linnet Lake experiences high water conditions that connect it to Middle Waterton Lake at this point (observed in 2008 and 2014), which provides an avenue for colonization by fish. The size and identity of fish populations have varied through time due to winterkill eliminating colonists and historical purposeful stocking by the Park. The Park's Entrance Road parallels the west side of the lake at a straight-line distance of 13 – 110 m and the road leading to the Prince of Wales Hotel passes 50 m to the south.

Road mortality of adult long-toed salamanders during overland breeding migrations was thought to be driving the recent decline in the Linnet Lake population (Pagnucco et al. 2011). In May 2008, four salamander tunnels (ACO Technologies, Shefford, UK) spaced ~80 – 110 m apart and connected by directional fencing were installed along the Entrance Road (described by Pagnucco et al. 2012) to reduce road mortality. In 2008, sucker (*Catostomus catostomus* and *C. commersonii*) and lake chub (*Couesius plumbeus*) were found in Linnet Lake and lake chub were demonstrated experimentally to consume salamander larvae readily, with incidental evidence of egg consumption (Pagnucco et al. 2012). Consequently, during a combined 2 weeks in September/October 2010 and in May 2011, 10,698 lake chub and 25,016 suckers (primarily *C. commersonii*) were removed from Linnet Lake and released in Middle Waterton Lake (Appendix A) as a step toward improving long-toed salamander recruitment.

Mine is the third study to examine the long-toed salamander population at Linnet Lake. Previous study began in 1993 – 1994 with J. Fukumoto who characterized the Linnet Lake population and examined the species' distribution within the Park. Fukumoto estimated the population at Linnet Lake in 1994 to be 3856 individuals and found populations at 10 additional sites, the closest being Stable Pond 1.2 km away (Fukumoto and Herrero 1998). In 2008 – 2009 K. Pagnucco examined the population at Linnet Lake and assessed the utility for breeding adults of under-road tunnels as a means of crossing the Entrance Road. Pagnucco estimated the breeding population to be 1492 individuals in 2008 and 1372 in 2009 (Pagnucco et al. 2011). Pagnucco used nearby Stable Pond as a reference site for monitoring long-toed salamander breeding activity, and detected egg masses in high abundance using visual shore-line surveys.

Stable Pond (49° 04' N, 113° 53' W) is a small (0.15 ha), fishless, ephemeral pond at an elevation of 1275 m with a maximum depth of 1.6 m, which fills with snowmelt and ground water and typically dries up by late July/early August (July 22 in 2013, August 7 in 2014) to become a grass-dominated meadow. Stable Pond is surrounded on all sides by poplar forest and flat terrain. To the west the pond is bordered by a bike path and the Entrance Road, across which lies on open, dry, grassy ridge capped by a stand of Douglas fir. Western toads (*Anaxyrus boreas*) and boreal chorus frogs (*Pseudacris maculata*) also breed in Stable Pond.

Thesis Goals and Outline

In this study, I asked two primary questions: 1) What is the current status of the Linnet Lake long-toed salamander population 4 – 5 years after under-road tunnels were installed to reduce road mortality and 2 – 3 years after fish were removed to increase recruitment? and 2) How do long-toed salamanders use the terrestrial habitat around aquatic breeding sites, specifically during adult migrations, juvenile dispersal, and for overwintering? In addition to my primary questions, I also evaluated how PIT tags and RFID technology perform compared to wildlife cameras as a means for monitoring tunnel-use by long-toed salamanders, and whether PIT telemetry can be successfully used in a northern system to detect tagged long-toed salamanders in overwintering refugia up to 70 cm below ground, the deepest observed for this species (Sheppard 1977).

In Chapter 2, I determined the status of the Linnet Lake long-toed salamander population by creating population estimates for 2013 and 2014 and comparing demographic characteristics with the nearest known long-toed salamander population at Stable Pond. I compared tunnel-use patterns with those observed in 2008 and 2009, and assessed the utility of using PIT tags and RFID in conjunction with wildlife cameras to monitor tunnel-use by long-toed salamanders. I monitored road mortality and compared rates with those seen in 1994, 2008, and 2009. I sampled fish in Linnet Lake to determine their relative abundance compared to numbers removed in 2010 – 2011.

In Chapter 3, I examined how long-toed salamanders use the terrestrial environment around breeding sites by comparing orientation patterns of immigrating and emigrating adults with dispersing young-of-year at two distances from the margin of Stable Pond, and by examining orientation patterns of adults in 2013 and 2014 at Linnet

Lake. I also used a portable RFID antenna that I constructed to conduct PIT telemetry in the late fall to detect overwintering salamanders in their below-ground refugia at Linnet Lake (2013) and Stable Pond (2014) and described of overwintering sites for this species. In Chapter 4, I quantitatively assessed the performance of my portable RFID antenna by measuring the detectability of PIT tags at multiple depths within three substrates.

This study provides important follow-up data for two complementary mitigation measures employed by Parks Canada to halt the decline of a local amphibian population. It assesses the effectiveness of RFID as an alternative method to monitor use of under-road amphibian tunnels, which are an increasingly popular option for mitigating amphibian road mortality. It also examines the utility of PIT telemetry for collecting habitat-use data for small vertebrate species that have historically been difficult to track in the terrestrial environment. Finally, my research provides important life-history information for one of the most widespread and most northerly, yet relatively under-studied, amphibians in western North America. Ultimately, this study attempts to shed light on the obstinate question: "Why did the salamander cross the road?"

Figures

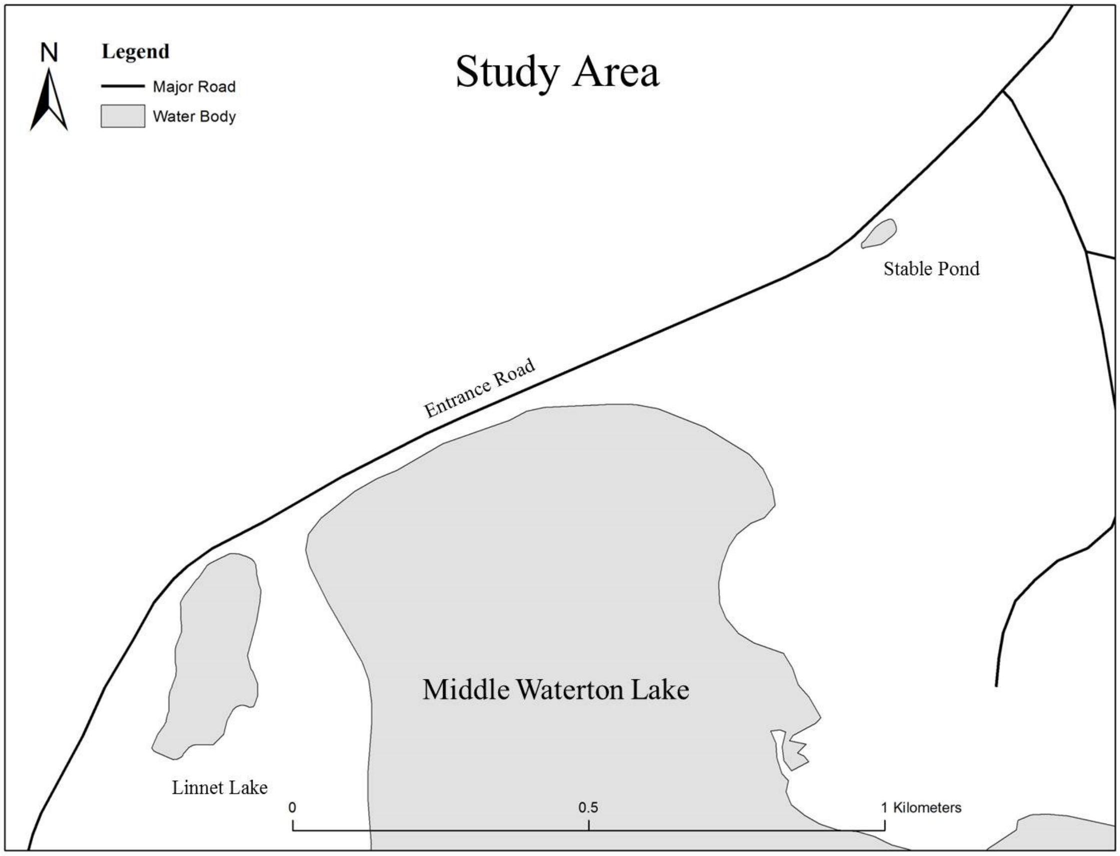

Figure 1.1 Map of Study area in Waterton Lakes National Park, Alberta, Canada. Research was conducted at Linnet Lake (2013 and 2014) and Stable Pond (2014).

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Chapter 2: Status of the Linnet Lake Long-toed Salamander Population

Introduction

Global amphibian declines have been widely documented and studied since the phenomenon was reported in the early 1990s (reviewed by Beebee and Griffiths 2005). Locally, however, amphibian populations often vary widely in size over short time-frames (Pechmann et al. 1991, Pechmann and Wilbur 1994, Corn 1994). Such variation can mask long-term trends in population size that are only detectable through long-term monitoring.

Amphibian population-fluctuations can be generally attributed to either natural or anthropogenic causes, or some combination of the two. According to Pechmann and Wilbur (1994), most fluctuations are likely natural and can be driven by local climate variability (e.g. periodic droughts), changes in predator-prey dynamics, inter- and intra-specific competition, disease, and other stochastic disturbance events. Determining the exact cause of natural fluctuations can be difficult due to the often complex life history of amphibians, as well as a time lag between successful recruitment years and the ability to detect the consequent addition of breeding individuals to the population.

Anthropogenic disturbances can cause declines at multiple scales ranging from local to global. Due to the variety of anthropogenic disturbances that may affect amphibian populations, detecting change in a population's size that is a direct result of a specific disturbance is highly dependent on the nature of the disturbance and the species in question. For example, four amphibian populations (one frog and three salamander species) using the same breeding site widely fluctuate naturally, but the magnitude and timing were different among the species (Pechman et al. 1991). Detecting the effects of climate change on a population will likely require more time than detecting the effects of something more immediately damaging, such as the in-filling of a wetland or the introduction of predatory fishes to a breeding site. In many cases, long-term monitoring of multiple sites in close proximity and containing similar species assemblages, as well as having direct evidence of specific anthropogenic disturbances, will enhance the ability to determine a relationship between a disturbance and a population decline.

Anthropogenic disturbances known to cause declines in amphibian populations are of concern largely because they are seen as preventable or repairable. Common examples of human activity that can harm populations include habitat destruction and alteration, introduction of predators, pollution, stress-induced disease susceptibility, and climate change (Corn 1994). Of these, Corn highlights habitat loss and introduction of alien predators as being the most prevalent anthropogenic cause of amphibian (frogs, specifically) population declines in western North America.

Among the ways in which humans alter or degrade habitat, road building is almost ubiquitous. Forman (2000) estimated the total area affected ecologically by the 6.2 million km of public roads in the contiguous U.S. to be 22% of the total land mass. Besides removing the vegetation and other habitat components needed by animals, the presence of a road exposes local populations to vehicle collisions as a source of mortality. Amphibians that exhibit episodic, large-scale movements, either during adult breeding migrations or juvenile dispersal, are at risk of vehicle collisions when roads intersect migratory paths. A review by Trombulak and Frissell (2000) highlighted the negative effects of roads on both terrestrial and aquatic animal communities. Hels and Buchwald

(2001) showed how road mortalities can have population-level effects on two frog species.

The introduction of alien predators that negatively affect amphibians is exemplified by the historic stocking of non-native trout (family *Salmonidae*) for recreational fishing. The stocking of non-native trout species or even native trout species in historically trout-free waters often eliminates amphibian populations. This was demonstrated in California when the mountain yellow-legged frog (*Rana muscosa*) experienced a large-scale population decline following the introduction of trout (Knapp and Matthews 2000), and then a recovery following their removal (Knapp et al. 2007). Other small predatory fish species not typically found to consume adult amphibians can still pose a threat when they are introduced to breeding sites either by humans or natural means (i.e. flooding events or recolonization after a winterkill) by reducing recruitment through direct predation on larvae and eggs and/or increasing energetic needs of larvae by harassment, which can injure larvae and incite anti-predator behavioral responses (Hecnar and M'Closkey 1997, Kats and Ferrer 2003, Eaton 2004, Pagnucco et al. 2011). In addition to predatory fish, in North America invasive American bullfrogs (*Lithobates catesbeianus*) have become another alien amphibian-predator and competitor throughout the west (Moyle 1973, Bury and Whelan 1985, Kats and Ferrer 2003).

Linnet Lake, in Waterton Lakes National Park, Alberta, is a system in which both road mortality and, more recently, the presence of predatory fishes have been hypothesized to be responsible for a large documented decline (60%) in the long-toed salamander (*Ambystoma macrodactylum*) population since the population's discovery in 1991 (Pagnucco 2010). This decline was initially attributed almost entirely to road mortality of adults during yearly breeding migrations (Fukumoto 1995, K. J. Pearson unpublished data). Parks Canada, in an attempt to reverse the decline of this population, installed four under-road crossing structures (tunnels) at Linnet Lake in spring 2008 (AT500 Amphibian Tunnels, ACO Technologies, Shefford, UK) . The tunnels were each 60 cm wide x 52 cm high and ~12 m long (Pagnucco et al. 2012) and had 1-m high silt-fence drift fencing (later replaced with permanent rigid plastic barriers) affixed to the entrances and running along the length of the road in-between tunnels to reduce road-access of salamanders and increase tunnel-use (Figure 2.1). Also, sampling of Linnet Lake in 2008 indicated that large populations of lake chub (*Couesius plumbeus*) and white and longnose sucker (*Catostomus commersonii* and *Catostomus catostomus*, respectively) were present and that predation on eggs and larvae by lake chub was likely contributing to the salamander decline (Pagnucco et al. 2011). Parks Canada netted and trapped fish from the Lake during one-week periods in fall 2010 and spring 2011, which resulted in the relocation of 10,698 lake chub and 25,016 suckers (primarily *C. commersonii*) to nearby Middle Waterton Lake (see Appendix A for detailed methods and catch data) (G. Scrimgeour, unpublished data). The size (total length) of removed lake chub had a median of 8.0 cm and ranged from 3.8 cm – 22.4 cm and sucker had a median length of 8.7 cm and ranged from 4.3 cm – 47.0 cm.

Follow-up studies on mitigation projects are rare, but yield important data for developing working strategies (Block et al. 2001). Long-term, science-based monitoring is the only way to document success or failure of mitigation projects to restore populations or communities to pre-disturbance states (Clevenger and Waltho 2004, van der Grift et al. 2013, Park 2004). Ideally, long-term monitoring should be cost effective and scientifically rigorous.

Monitoring of the Linnet Lake population of long-toed salamanders began in 1993 with a 2-year baseline study conducted by J. Fukumoto (Fukumoto 1995, Fukumoto and Herrero 1998). Fukumoto and Herrero documented migration patterns and basic demographic information for the population, and was able to produce a mark-recapture population estimate based on 1 year (1994) of capture records. They found that breeding movements (immigration) began on April 12 and the peak breeding migration (equal number of immigrants/emigrants) occurred on May 14. They also found an unusual 3:1 female:male sex ratio, mean female snout-vent length (SVL) of 67mm (n = 410, SE = 0.191), mean male SVL of 64mm (n = 132, SE = 0.287), and estimated the adult breeding population to be 3856 (95%CI: 3274, 4690). Fukumoto (1995) documented 43 road mortalities from surveys in May, August, and September 1993, and 67 adult road mortalities (1.4 - 2.0% of 1994 estimated population) between mid-April and mid-September 1994. By comparison, Pagnucco (2010) and Pagnucco et al. (2012) reported that breeding movements in 2008 and 2009 began on May 3 and 4, respectively, and peaked May 12 – 15 both years. Pagnucco et al. (2012) reported mean female SVLs of 64.5 (n = 280, SE = 0.24) and 64.6 (n = 172, SE = 0.26) in 2008 and 2009 respectively, and mean male SVLs of 61.0 (n = 244, SE = 0.22) and 61.2 (n = 138, SE = 0.34) in the same two years. They estimated the adult breeding population at 1492 (95%CI: 1243,1865) in 2008 and 1372 (95%CI: 1045,2001) in 2009. They documented 10 road mortalities in 2008, and two in 2009, representing <0.7% of the estimated breeding population in both years, a large a reduction from estimates made by Fukumoto and

Herrero (1998), presumably reflecting the presence of roadside barriers. Pagnucco et al. (2011) found little evidence of recruitment (one juvenile over 2 years) compared to Fukumoto (1995) (50 juveniles over 2 years).

Beginning in 2008, Parks Canada staff monitored the tunnels with wildlife cameras (RapidFire PC85, Reconyx, Holmen, Wisconsin, USA) to document usage by long-toed salamanders and other animal species. Pagnucco et al. (2011) examined the performance of these wildlife cameras for detecting salamanders passing through tunnels in 2008 – 2009. A goal of their study was to determine if this method could be used to monitor the salamander population size and demographic structure effectively at Linnet Lake, as well as the method's appropriateness for monitoring amphibian use of road-crossing structures in general. They used pitfall traps placed at the entrances of tunnels to capture salamanders passing through and compared trap capture records to the number of salamanders caught on camera during nightly sampling sessions. Pagnucco et al. (2011) found that cameras were effective at detecting salamanders using the tunnels, and that this method produced more information (i.e. movement speeds, predation events, and other behavioral observations) compared to using only pitfall traps placed at tunnel entrances, but they also noted the limitations of using these cameras at Linnet Lake system. Cameras worked most effectively when placed on a timed-interval schedule of 1 image per minute from 2100 h – 0600 h (81% of 58 salamander images collected from April 22 – October 14, 2009) rather than relying on motion detection (19% of salamander images). The timed interval setting produced a large number of "empty" images. Also, they discovered that pitfall traps did not capture 100% of the salamanders that passed through the tunnel. This observation makes their calculated camera efficiency of 44%

questionable and indicates that the absolute number of salamanders that crossed through tunnels could not be estimated using camera data alone. If camera efficiency was more accurately known, the estimated number of salamanders using tunnels could be coupled with the population abundance estimate made the same year. This would provide Park managers with a method of estimating relative population abundance at Linnet Lake based on only tunnel-use data recorded by cameras. Using cameras in this fashion would provide a repeatable, affordable way to document long-term population trends of the long-toed salamander at Linnet Lake, and the effectiveness of the culverts as road-crossing structures for other animal species.

Another method that can be used for monitoring traffic through a confined area is marking animals with passive integrated transponder tags (PIT tags) and using radio frequency identification (RFID) antennae to detect passage. This technology has many applications (Gibbons and Andrews 2004) and is often used in studies monitoring fish passage through streams or other confined areas (e.g. culverts, fish passes, etc.). Monitoring animal movements with PIT tags can be used in terrestrial environments just as effectively provided tagged animals pass near enough to an RFID antenna to be detected (Boarman et al. 1998). The under-road culverts at Linnet Lake provide an ideal system in which to implement this technology as an alternative to using pitfall traps for comparing tunnel-use by salamanders to camera detections. When a salamander marked with a PIT tag passes by an RFID antenna in close proximity to a camera, its unique ID is recorded along with the time of passage, and this data can be used to check camera records for images of the individual. Camera efficiency can then be calculated. This method would limit the number of images that needed to be viewed to those within a small time frame that includes the RFID detection, assuming the accuracy of RFID detection is known. It also would allow the identity of individual salamanders to be recorded, and should provide a more reliable history of tunnel passage for PIT tagged individuals compared to pitfall trapping.

My objectives were to 1) determine the current demographic characteristics (2013 and 2014) of the Linnet Lake long-toed salamander population following mitigation actions directed at road mortality and fish predation, and 2) to assess the utility of using PIT tags and RFID technology to monitor tunnel-use by long-toed salamanders in conjunction with cameras. Overall, I predicted that the effects of tunnel installation over 4 years, coupled with the effects of fish removal over 2 years, would increase survival of breeding adults by reducing road mortality of sexually mature individuals and increase recruitment by relieving pressure on eggs and larvae by predatory fishes in Linnet Lake.

Methods

Approaches and Predictions

To address my first objective – assessing the status of the long-toed salamander population at Linnet Lake – (i) I created population estimates for 2013 and 2014 using mark-recapture methods to compare with estimates made in 1994 by Fukumoto and Herrero (1998), and in 2008 and 2009 by Pagnucco (2010), who showed a decline of approximately 60% in the time between the two studies (1994 – 2008/2009). I predicted that the decline in the population would be halted and possibly reversed, and this change would be manifested by a higher estimated population size caused by more migrating individuals (sexually mature adults) available for encounter and mark-recapture. (ii) I compared the size structure of salamanders in Linnet Lake with those presented in the above-mentioned studies as well as with Stable Pond. I compared Linnet Lake to Stable Pond as a reference due to the pond's small size (see study site description), lack of fish or documented road mortality, and its historically healthy breeding long-toed salamander population (Fukumoto 1995, Pagnucco et al. 2011). I predicted that, when compared to adult, migrating salamanders captured at Stable Pond, the Linnet Lake population would show a skewed or bi-modal size distribution (Semlitsch 1983), with most being large (old) individuals, and possibly a high proportion of small individuals (young adults) indicating successful recent recruitment, and that Stable Pond would show a normal, or near normal size distribution slightly skewed toward smaller individuals (Anderson 1967, Beneski et al. 1986, Trenham et al. 2000). I also predicted that recruitment at Linnet Lake would be manifested through an increase in the proportion of small individuals captured compared to Pagnucco's (2010) finding of one juvenile over 2 years. (iii) I documented patterns of salamander tunnel-use and road mortality at Linnet Lake for comparison with those reported by Pagnucco et al. (2012) and with road mortality reported by Fukumoto and Herrero (1998). I expected to find similar tunnel-use and rates of road mortality compared to those found by Pagnucco et al. (2012) in 2009. (iv) I sampled the fish in Linnet Lake in 2013 using mark-recapture methods to assess the relative impact of removals in 2010 and 2011 on fish abundance 2 years post-removal and to look for any evidence of reduced numbers, with the goal of relating relative fish abundance to any noticeable change in salamander recruitment. I predicted that the relative abundance of lake chub and sucker in Linnet Lake would be reduced from the number encountered during fish removals.

Study Sites

Between 2013 and 2014, I conducted research at two study sites: Linnet Lake and Stable Pond. In 2013, I conducted research activities at Linnet Lake only, and in 2014 I conducted research at both sites.

Linnet Lake (49° 04' N, 113° 54' W) is a small (3.9 ha), foot-shaped, shallow (5 m maximum depth) lake at an elevation of ~1260 m in Waterton Lakes National Park in a bowl-like catchment immediately north-west of the Prince of Wales Hotel. The vegetation around the lake is dominated by stands of Douglas fir (*Pseudotsuga menziesii*) and poplar (*Populus* spp.), with an understory of small trees and shrubs, e.g. chokecherry (*Prunus virginiana*), saskatoon (*Amelanchier alnifolia*), and snowberry (*Symphoricarpos albus*), and open grasslands. The lake is surrounded by moderately steep-sloping (up to 15%) hillside except on the north end, which is a flat, low-lying area containing a parking lot (~30 m x 70 m), adjacent to Middle Waterton Lake. Occasionally high water conditions link Linnet Lake to Middle Waterton Lake over this low spot, which provides an avenue for colonization by fish. Pagnucco (pers. communication) observed fish movements during high water conditions in 2008, and I observed them in 2014. From the parking lot, a 2-m wide paved foot path encircles the lake. The Park's Entrance Road parallels the west side of the lake at a strait-line distance of 13 – 110 m and the road leading to the Prince of Wales Hotel passes by 50 m to the south. The Entrance Road is punctuated by 4 salamander tunnels spaced ~80 – 110 m apart and described in detail by Pagnucco et al. (2012). In addition to long-toed salamanders, Linnet Lake may support breeding populations of western toad (*Anaxyrus boreas*) and western tiger salamander (Ambystoma mavortium) (personal observation).

Stable Pond (49° 04' N, 113° 53' W) is a small (0.15 ha), fishless, ephemeral pond at an elevation of 1275 m with a maximum depth of 1.6 m that typically dries up by late July/early August (July 22 in 2013, August 7 in 2014) to become a grass-dominated meadow. Stable Pond is immediately surrounded on all sides by poplar forest and flat terrain. This quickly breaks into open grass/low shrub mix to the south and poplar forest with small isolated stands of Douglas fir to the east. To the west, the pond is immediately bordered by a bike path and the Entrance Road, across which lies on open, dry, grassy ridge capped by a stand of Douglas fir. Western toads and boreal chorus frogs (*Pseudacris maculata*) also breed in Stable Pond.

Salamander Capture: Linnet Lake

To capture salamanders at Linnet Lake for PIT tag implantation and demographic data collection, I installed a series of drift fences around the lake to create a temporary barrier to salamander movement during their breeding migrations. I also used permanent fencing already in place along both sides of the Entrance Road for this purpose. Drift fencing was composed of 1-m high silt fencing buried to a depth of 5 – 10 cm and stapled to wooden stakes for support. In 2013 I installed 16 30-m drift fences around Linnet Lake, spaced 15 m apart and ranging between 10 m and 25 m from the lake edge. In 2014 I installed eight of the original 16 fences around Linnet Lake. Permanent fencing was composed of 718 m of curved, corrugated, plastic culvert material buried to depths varying between 0 and 10 cm and standing approximately 45 cm above ground, with the direction of curve facing away from the road. Permanent fencing was installed at an obtuse angle to the road at each culvert entrance, creating four connected V-like formations on both sides of the road. This fencing was designed to funnel salamanders towards under-road culverts (Figure 2.1). Permanent fence length varied by tunnel (40 – 123 m) and created a semi-impermeable barrier to salamander movement along the entire length (~380 m) of the Entrance Road bordering Linnet Lake and extended beyond the first and last tunnel 74 - 85 m to the south and 31 - 40 m to the north. I did not install drift fencing at the North end of Linnet Lake due to the presence of the paved parking lot and little vegetation for salamander foraging, or suitable habitat for overwintering.

I walked along fences nightly beginning the first night an individual was encountered during preliminary surveys (April 25 in 2013, April 17 in 2014) and collected all unmarked salamanders encountered. I continued night searches until five consecutive nights passed with no salamander encounters, which occurred in late June both years. I then re-initiated night searches on the next rainy night and continued nightly until no salamanders were encountered. I completely ceased night searches after no salamanders were encountered on a rainy night (July 8 in 2013 and July 2 in 2014). I also caught salamanders encountered opportunistically while walking in between fences and on paths around Linnet Lake. All salamanders captured were placed in a small plastic container with moist paper towel for transport to and from the lab where I took measurements and marked individuals.

Salamander Capture: Stable Pond

At Stable Pond, I installed 10 drift fences in 2014 using the same materials and methods as at Linnet Lake. I installed five 30-m fences, 5 m apart around the pond within 3 m of the high water line (referred to as "inner fences"). I installed five more 30-m drift fences 50 m away from the high water line ("outer fences") and directly in line with a corresponding inner fence, except for one fence across the Entrance Road that had to be offset 20° SW due to impenetrable soil conditions caused by an exposed rock outcropping (Figure 2.2). At Stable Pond, I used 50 pitfall traps buried along the fences instead of conducting night searches. Pitfall traps were made from #10 food service cans containing a stick long enough to project out over the top of the trap as a ramp for trapped mammals and a 4 x 7-cm piece of sponge to provide moisture and cover for trapped amphibians. In addition to fences and pitfall traps, I placed 10 Gee minnow-traps (42 x 19 cm, 6.4 mm mesh, 2.5 cm openings) in the pond evenly spaced around the perimeter and at varying distances from shore depending on pond depth and I tried to ensure an air space existed in minnow traps to prevent animals from drowning. When the water table was too high for pitfall traps to be functional, they were replaced temporarily with Gee minnow-traps placed parallel with the drift fence with sticks and mud used to create a "funnel" to encourage salamanders into the trap entrances.

I focused capture effort on migrating adult salamanders in the spring (April 17 to June 14) by placing eight pitfall traps along inner fences, four on each side evenly spaced (~7.5 m apart), and placing two pitfall traps along outer fences, one at each end on the side facing the pond. I used aquatic Gee minnow-traps in the pond beginning April 12 for the same time period. In the late summer/fall, I focused capture effort on dispersing young-of-year (YOY) salamanders using pitfall traps installed only on the pond-side of all drift fences from July 16 to August 22. The rationale for pitfall trap array design is further described in Chapter 3. When traps were in place, I checked them daily, usually within 1h of sunrise. I used the same materials and methods to contain and transport captured salamanders as at Linnet Lake. Upon capture, I immediately released any other amphibians or mammals present in traps on the opposite side of the fence or directly into the pond at the trap location.

Salamander Processing: Measurements and Marking:

Once captured, salamanders were held in a labeled plastic container containing moist paper towel and transported them a short distance to an indoor laboratory for processing. Salamander processing consisted of a combination of physical and demographic measurements and marking. I conducted all measurement and marking procedures (all animals were double-marked) on individuals while they were under anesthesia.

To process juvenile and adult salamanders, an individual was first anaesthetized by immersion in 1g/L trimethane sulfonate (TMS) solution until unresponsive to prodding (typically 6-10 minutes). Once the salamander was unconscious, I measured its weight, total length (TL, measured as the distance from tip of the snout to distal end of tail), and snout-vent length (SVL, measured as the distance from tip of snout to posterior edge of vent), and determined sex and age-class (i.e. young-of-year, juvenile, or adult). Salamanders with swollen vents (able to be sexed) were considered mature adults, salamanders without swollen vents (unable to be sexed) were considered juveniles and were usually noticeably smaller than adults, and juvenile salamanders captured in July and later, that had gill-remnants behind the jaw, were considered young-of-year (YOY). If an individual was to be uniquely marked, I inserted a 12 x 2.12-mm sterile half-duplex PIT tag (Texas Instruments via Oregon RFID, Portland, Oregon, USA) into the body cavity via a 3mm incision made using a fresh #11 scalpel blade just anterior to the right hind leg and slightly toward the midline, and either clipped one toe at the second phalangeal joint on toe three of the right hind leg, or injected red (2013) or orange (2014) visual implant elastomer (VIE) (Northwest Marine Technologies, Shaw Island, Washington, USA) sub-dermally at the ventral base of the tail just posterior to the vent. I closed the PIT tag incision with Vetbond™ Tissue Adhesive (3M, St. Paul, Minnesota, USA), which I applied to the dried incision while holding the opposing edges of the incision together with forceps. During a brief period each year when I had no PIT tags, I created individual marks with VIE by making a series of three or four dots and dashes (e.g. — · —, — — —, · · · , etc.). After marking, which usually took 1 – 3 min, I placed individuals in a slanted container and immersed them in non-chlorinated water with their heads above water until they recovered from anesthesia (typically 10 – 15 min). Once recovered (awake and responsive to prodding), individuals were placed back into their original containers until release near the point of capture.

Fish Capture and Marking

I captured fish at Linnet Lake over 5 d (August 6 - August 10) in 2013. Only two species were encountered: lake chub and white sucker, although some of the juvenile fish identified as white sucker could have been the similar-looking longnose sucker. I used 68 metal Gee minnow-traps (42 x 19 cm, 3.2 – 6.4-mm mesh, 2.5 cm openings) spaced evenly around the lake (~ 13 m apart) and placed 2 m from shore at varying depths with the trap openings parallel to the shoreline. I also used seven fyke nets (five 1.2-m dia. hoops, 0.64-cm mesh) approximately evenly spaced along the lake shore with the 7.6-m leaders (1.2-m deep) anchored to shore and the opening of the net perpendicular to the shoreline. On the first day I used all seven nets, but due to long processing times and a much higher catch than expected, I alternated which of the seven nets I deployed, using three or four nets per day for the remainder of the study.

I marked fish of both species by clipping the dorsal lobe of the caudal fin. I also measured total length of a minimum of 100 randomly selected fish (mixed chub and sucker) each day (only 50 for day 1, all from minnow traps), 50 from minnow traps and 50 from one fyke net (different traps each day for both trap types), for comparison with previous fish surveys. I did not capture equal numbers of chub and sucker during daily sampling, but the total sample size for both was sufficient after 5 d of sampling for comparison with data from 2010 – 2011 (see Appendix A).

Population Estimates

I generated population estimates for fish (lake chub and sucker separately) for 2013 and long-toed salamanders for 2013 and 2014 using closed capture models in the program MARK (White and Burnham 1999). For fish species, I used a mark-resight model without individual identification that used each sampling day as an encounter occasion. For salamanders I used a closed capture model with individual encounter histories treating each day as an encounter occasion, and I assumed that probability of individual capture and recapture were equal to each other, but varied by day. Additionally, I used the Schnabel method (Krebs 1999), as did Pagnucco et al. (2011), to generate a second set of salamander-abundance estimates for 2013 and 2014 more directly comparable to 2008 and 2009 values. In all models (fish and salamander), population size was assumed to be constant for the sampling period.

Salamander Demographic Comparison

To compare the size of adult long-toed salamanders through time at Linnet Lake, I used a one-way analysis of variance (ANOVA) for each sex to compare the average SVL among each study (1994, 2008 – 2009, and 2013 – 2014) at this site. I pooled Pagnucco's lengths for 2008 – 2009 and my values for 2013 – 2014, resulting in a comparison of 3 mean SVLs for each sex. I analyzed sexes separately to account for dimorphism, as males are smaller than females. I performed a two-tailed t-test for males and females separately to test for a difference SVL between 2013 and 2014.

To compare the size distribution of adult long-toed salamanders between Linnet Lake and Stable Pond in 2014, I used a one-tailed Kolmogorov-Smirnov test for males and females separately. The test assumed a higher proportion of large (older) individuals at Linnet Lake compared to Stable Pond. I controlled for an inherent difference in mean size due to different environmental conditions or larval densities between both sites by adjusting mean SVL to zero for both sexes at each site before analysis.

Road Mortality

In 2013 I monitored road mortalities at Linnet Lake from April 26 - August 30 (daily through July 8 during the migration season, opportunistically thereafter) by walking the section of road protected by the tunnel-fence system (~380 m). I focused on one side of the road at a time, walking down one side and then back on the other. I began road mortality surveys in the early morning to avoid traffic and removal of road mortalities by scavengers. I conducted most of the road mortality surveys within 30 min of sunrise (0530 – 0630 h) and almost all surveys before 1000 h. I identified and recorded the location of all vertebrate road mortalities relative to the nearest tunnel(s). I removed all carcasses from the road surface and placed them in the vegetation alongside the road to prevent duplicate counting.

In 2014 I monitored road mortalities from April 18 - August 29 (daily through July 19, then opportunistically) at Linnet Lake in the same manner as 2013 and also included the road bordering Stable Pond (~70 m). I monitored road mortality at Stable Pond by walking along the road as at Linnet Lake, but using the pond as a reference for road mortality locations instead of tunnels. The survey extended 15 m beyond Stable Pond at both ends.

In 2013 and 2014 I found salamander tunnel-use to be highest in tunnels 3 and 4. To see if there was significantly more road mortality near these tunnels due the concentration of salamander movement, I split the road along the tunnel system into two sections: the "north section" including the length of road north of and protected by tunnels 1 and 2 (180 m), and the "south section" protected by tunnels 3 and 4 (202 m). I used a chi-square goodness of fit analysis with a Yates correction for continuity to test for differences in the number of long-toed salamanders killed between these two sections for 2013 and 2014 combined while correcting for road length.

To address my second objective – assessing the utility of using PIT tags and RFID antennas instead of cameras to monitor tunnel-use – (i) I monitored both entrances to two of the four tunnels in 2013 and 2014 to document the passage of PIT-tagged salamanders and compare RFID records with camera records. I used the comparison of these two methods to calculate an approximate camera efficiency to compare with the estimate generated by Pagnucco et al. (2011) via pitfall trapping. I predicted that by using RFID technology, I could create a more accurate estimate for tunnel camera efficiency at detecting salamanders using tunnels than the 44% efficiency generated by K. Pagnucco using pitfall traps in 2009. Pagnucco's (2011) traps were known to miss some salamanders (minimum of 26 missed out of 130 possible captures based on camera images), while the ability of RFID antennas to detect tagged individuals can be very reliable (approaching 100%) when antenna arrays are well designed (Zydlewski et al. 2006) to take into account PIT tag size, antenna read-range, the physical characteristics of the monitoring site, and the behavioral characteristics (i.e. movement speed) of the tagged animal (Boarman et al. 1998).

Tunnel-use Based on Cameras and RFID

In 2013 and 2014 I monitored all four tunnels with cameras in the same manner as Pagnucco et al. (2010). I mounted one Reconyx camera to the roof of each tunnel entrance and programmed it to take three photos at 1-sec intervals whenever motion was detected and 1-min timed-interval pictures from 2100 – 0600 h nightly. Cameras were in place from 27 April - 30 August in 2013 and 22 April - 22 August in 2014.

Both years, I also monitored four tunnel entrances using four hand-made RFID antennae and a multi-antenna HDX reader from Oregon RFID (Portland, Oregon, USA). I placed the antennae at the entrance to the tunnel interior to the cameras at the point where the tunnel's width became constant (Figure 2.3). All four antennae were "pass-over" style antennae with an approximate read range of 15 cm. Five loops of 16AWG speaker wire were taped into an oval shape 60 cm long (width of the tunnel) and ~15 cm wide. In 2013 I monitored both ends of tunnels 2 and 3 based on K. Pagnucco's observation that these two tunnels had the highest salamander use in 2009. In 2014 I monitored both ends of tunnels 3 and 4 because I found these to be the most-used tunnels in 2013. Antennas operated from May 13 – August 30, 2013 and from April 15 – August 30, 2014. Antennas scanned for PIT tags every 4 sec except from May 13 – May 19, 2013 when antennas scanned every 1 sec.

To compare these two methods of monitoring tunnel-use by long-toed salamanders, I examined images captured 30 min before and after each RFID detection. If I found an image of a long-toed salamander within this time frame, I considered that individual to be detected by both methods. I calculated detection efficiency by cameras as

$$\frac{\text{\# of images of individual salamanders}}{\text{\# of tagged salamanders detected by RFID}}$$

Data Analysis

All statistical analyses were performed using R (R Core Team 2014) unless otherwise specified. All distances and geographical measurements were conducted using ArcGIS 10.2.2 (ESRI, Redlands, California, USA).

Results

Population Estimates

In 2013 I captured 413 long-toed salamanders (404 PIT-tagged) over 73 d during night surveys at Linnet Lake and recaptured 97 at least once. I estimated the salamander population of Linnet Lake in 2013 to be 1380 (95% CI: 1138, 1702) individuals (Figure 2.4, Figure 2.5) with the Program MARK and 1135 (95% CI: 942, 1426) individuals with the Schnabel method. In 2014 I captured 247 individuals (239 tagged) over 64 d with 93 recaptured at least once. Twelve salamanders were originally tagged in the 2013 field season, eight of these were captured more than once, and I treated them as new individuals in the 2014 analysis. I estimated the salamander population of Linnet Lake in 2014 to be 706 (95% CI: 575, 893) individuals (Figure 2.4) with MARK, and 375 (95% CI: 314, 465) individuals with the Schnabel method.

I captured a total of 6550 lake chub with 4137 recaptures and 7524 sucker with 2197 recaptures in 2013. I estimated the 2013 population of lake chub in Linnet Lake to be 10463 (95% CI: 10193, 10747) and sucker to be 20554 (95% CI: 19693, 21467). Minnow traps accounted for 61% of lake chub captures and 34% of sucker. The 210 measured lake chub ranged in size (TL) from 3.5 cm – 13 cm with a median size of 8.5 mm and 250 sucker ranged from 6.5 cm – 37.5 cm with a median size of 9.0 mm (See Appendix A for more details).

Salamander Population Comparison

Average size (SVL) for both male and female salamanders was largest in 2013 – 2014 (Figure 2.5). Both sexes differed in size among years (one-way ANOVA; males: $F_{0.05, [2, 671]} = 190.5$, $p < 0.001$; females: $F_{0.05, [2, 1332]} = 309.0$, $p < 0.001$). Post hoc Tukey HSD tests showed significant ($p < 0.001$) differences among all pairs of years for each sex. Salamanders were 2.4 -3.0 mm smaller in 2008 – 2009 than in 1993 – 1994, but in 2013 – 2014 were 3.1 – 3.6 mm larger than 1993 – 1994 and 6.1mm larger than in 2008 – 2009. In 2014, males at Linnet Lake were 1.2 mm larger than 2013 ($t_{[0.05, 158]} = 2.72$, $p = .007$) and females were 1.3 mm larger ($t_{[0.05, 472]} = 3.93$, $p < .001$). Also, I found a similar 3:1 female-biased sex ratio as reported by Fukumoto and Herrero (1998), but not observed by Pagnucco (2010).

In 2014 I captured 611 long-toed salamanders at Stable Pond; 337 females and 274 males. Mean SVL was larger for each sex at Linnet Lake (2013-2014) than Stable

Pond (Female $t_{[0.05, 809]} = 5.42$, $p < 0.001$; Male $t_{[0.05, 432]} = 15.23$, $p < 0.001$), but only marginally so for females (1.36 mm) compared to males (4.27 mm). Comparison of the shape of size frequency distributions (mean size corrected to zero) for adult salamanders from Linnet Lake for 2013 – 2014 and Stable Pond for 2014 showed no significant decrease in the cumulative distribution of size frequencies (which would indicate skew toward larger individuals in Linnet Lake) for males $_{n=160, 274}$ (Kolmogorov-Smirnov $D = 0.101$, $p = 0.126$) or females $_{n= 474, 337}$ ($D = 0.036$, $p = 0.597$).

Road Mortality

In 2013 I found 20 road-killed long-toed salamanders at Linnet Lake. In 2014 I found 24 road-killed salamanders at Linnet Lake, and eight at Stable Pond. At Stable Pond, four mortalities were juveniles and seven of the eight mortalities occurred during late-season movements in August. At Linnet Lake only one long-toed salamander was killed during late-season movements in 2013 – 2014. Annual road mortality of long-toed salamanders at Linnet Lake varied appreciably from 1994 – 2014, and was highest in 1994 compared to 2008 – 2014 (Figure 2.6A). Average annual mortality in 2013 and 2014 was about three times greater than in 2008 and 2009 (Figure 2.6). Similarly, relative road mortality in 2013 and 2014 was at least 5 times greater than 2008 and 2009 (Figure 2.6B).

At Linnet Lake, analysis for equal distribution of road mortality proportional to road length in association with the tunnel system indicated a significantly higher road mortality rate along the southern portion of the road at Linnet Lake ($\chi_c^2{}_{\upsilon = 1} = 15.2$, $p < 0.001$). Road mortality in both years was higher in the southern portion of the road, beginning around tunnel 3 and extending south past the directional fencing approximately

30 m (Figure 2.7). At Stable Pond low sample size prevented statistical analysis, but more road mortalities occurred south of the pond (five) compared to north of the pond (two).

In addition to long-toed salamanders, I observed road mortalities of birds, mammals, snakes, toads, and one other species of salamander between the two sites (Table 2.1).

Movement Patterns

In 2013 the immigration period at Linnet Lake lasted 38 d (April 27 - June 3) and emigration lasted 64 d (May 6 - July 8) (Figure 2.8). In 2014 immigration lasted 42 d (April 17 - May 28) and emigration lasted 49 d (May 2 - June 19) (Figure 2.9). For both years combined, I found the highest frequency of tunnel-use, based on encounters along permanent fencing to be tunnel 3 (206), followed by tunnel 4 (184), tunnel 2 (72), and tunnel 1 (23). Road mortality and RFID detection events in tunnels reflected these temporal and spatial capture patterns both years, and movement patterns appeared to coincide with the occurrence of precipitation (Figure 2.8, Figure 2.9). In both years, the majority of individuals I captured on drift fences near Linnet Lake were along the south-west corner of the lake, an area partially overlapping with the southern extent of permanent fencing for tunnel 4 (Figure 2.1).

Camera vs. RFID Detections

In 2013 there were 169 RFID detection events (90 of 404 PIT-tagged individuals), 105 of which occurred during camera surveillance periods. In 2014 there were 126 RFID detections (67 of 643 PIT-tagged individuals), 110 during camera surveillance.

In 2013 a total of 11 images contained salamanders within the 30 min period before and after each RFID detection, producing a camera detection rate of 10.5%. The camera detection rate was 16.7% for tunnel 2 (six images corresponding to 36 RFID detections), and 7.2% (five images from 69 RFID detections) for tunnel 3. In 2014 a total of 22 images from RFID detections contained salamanders, producing a camera detection rate of 20%. The detection rate was 12.2% for tunnel 3 (nine images from 74 RFID detections) and 36.1% for tunnel 4 (13 images for 36 RFID detections). Combining all cameras and both years gives a pooled detection rate of 15.3%. (Figure 2.10). In 2013 and 2014 respectively, 80% and 99% of RFID detections occurred during timed-interval camera surveillance.

Discussion

Linnet Lake Long-Toed Salamander Population Estimates

I found no evidence that the breeding population of long-toed salamanders at Linnet Lake had increased based on 2008 and 2009 abundance estimates, or that the decline reported by Pagnucco et al. (2011) had been halted by the installation of amphibian tunnels along the Entrance Road and removal of fish from Linnet Lake. This is surprising as both reductions in fish abundance (Eaton et al. 2005, Knapp et al. 2007) and adding road-crossing structures (Beebee 2013) have had positive effects on other amphibian populations, although long-term population-level effects of road mitigation are still largely unknown (Lesbarreres and Fahrig 2012).

My analysis used the program MARK, but when I used the Schnabel method employed by Pagnucco to estimate abundance, I arrived at lower estimates than were generated in MARK: 2013 estimate = 1135 (95% CI: 942, 1426) and 2014 estimate = 375

(95% CI: 314, 465). Estimates in MARK can be considered more robust because the model takes into account individual recapture probabilities when estimating the recapture probability for the population. The Schnabel method as employed by Pagnucco does not take into account individual identity, so multiple recaptures of the same individual will inflate the average recapture probability for the population and may produce an underestimate of population size.

My estimate of abundance for 2013 was similar to values for 2008 (1492) and 2009 (1372), but my 2014 estimate was about 50% lower. This is an appreciable decline between the two years and warrants further investigation. Other amphibian breeding populations have been shown to naturally fluctuate widely from year to year due to drought (Pechmann et al. 1991), and mass mortality events have been observed in breeding adult spotted salamanders (*A. maculatum*) when temperatures suddenly drop below freezing (Brodman 1995, Madison 1997) but I did not observe either conditions at Linnet Lake in 2013 or 2014. Adult ambystomid salamanders and eastern newts (*Notophthalmus viridescens*) have been noted to occasionally skip breeding seasons (Gill 1985, Pechmann et al. 1991), but Pagnucco et al.'s (2011) Linnet Lake estimates were similar both years, so I have little reason to believe that adults foregoing breeding was responsible for the lower estimate. One possible explanation for the large decline is that the adult population is aging and, in the absence of recruitment, may decline further due to the inevitable loss of individuals due to senescence. It is also possible that the abundance estimate for 2014 is an underestimate of the true population size. One reason for this could be that I violated the assumption of equal catchability and experienced an inflated recapture rate in 2014 compared to 2013. This would drive the population estimate down by overestimating how likely a given individual in the population is to be encountered. In 2013 I released groups of salamanders captured along the permanent road-side fencing either at the tunnel entrance or across the road in the presumed direction of travel. In 2014, however, I released salamanders captured along permanent fencing near the site of capture later the same night, thus increasing my chances of capturing those individuals again if they did not continue across the road that night. To try to account for this, I eliminated recaptures that occurred within 3 days of the previous capture from my analysis, and for consistency I also applied this correction to 2013 data. This correction is reflected in the values I reported. An additional piece of evidence that the large reduction in numbers between the two years may have been artificial is that I noticed an under-representation of salamanders marked in 2013 in my hand-capture records (10% of 247 animals in 2014) compared to RFID detections at tunnel entrances (22% of 67 animals). I assume that the RFID system had less bias in detecting tagged salamanders because it operated 24 h per day, giving tagged animals an equal chance of being detected regardless of time of day or weather patterns, both factors that influenced my ability to detect individuals visually on a given night. If I had captured a similar proportion of "old" (marked in 2013) to "new" (captured for the first time in 2014) individuals by hand as the RFID system detected, the population estimate would be larger.

Factors Affecting Population Size: Mitigating Mortality

Effects of Road Mortality

Tunnels and associated permanent fencing reduced road mortality of adult long-toed salamanders during breeding migrations well below pre-tunnel levels reported by

Fukumoto and Herrero (1998), even though I found almost four times the mortality (44) reported by Pagnucco et al. (2012) (12) and captured five times more live long-toed salamanders on the road during my two years of study (25 in 2013 - 2014 compared to five in 2008 – 2009). Although the total number of salamanders killed by vehicles is reduced, the relative number of salamanders, when compared to the estimated population size, is similar to or greater than pre-tunnel levels (Figure 2.7).

The increase in road mortality from 2008 – 2009 to 2013 – 2014 may be due to a several factors. The type of fencing currently in place along the Entrance Road is more permeable than the temporary fencing used by Pagnucco et al. (2012), which consisted of 788 m of 1-m high silt fencing buried 15 cm. The permanent fencing is 718 m of ~45-cm high curved, corrugated plastic culvert material buried from 0 – 10 cm. Although it is designed to be permanent, it has not been maintained to function at its highest capacity. In several places, the junction between fence and tunnel is not continuous, and I observed salamanders crawling up the sides of the tunnel entrance onto the road (Figure 2.11). Also, the fences are easily undercut in several places by overland flow during heavy rain events or by burrowing mammals, creating a space beneath the fence for salamanders to pass. Additionally, some yearly variation in road mortality is simply a result of the timing of rain events and the corresponding migratory movements. If large migratory movements occur during a weekend, there is much more traffic and the likelihood of encountering a car increases when a salamander crosses the road surface (Hels and Buchwald 2001, Gibbs and Shriver 2005). I observed the highest road mortality rates after rainy nights coinciding with weekends in May and early June (Figure 2.8, Figure 2.9).

Gibbs and Shriver (2005) modeled the effects of road mortality on populations of a closely related salamander species (*A. maculatum*) in Massachusetts, USA, and determined that an annual risk of road mortality between 10% and 20% for breeding adults can lead to local extinction of a population. Even before tunnel installation at Linnet Lake, the percentage of breeding adults in the population killed was below this threshold (only 1.4 – 2.0 % in 1994) (Fukumoto and Herrero 1998). In another study of the effects of road mortality on anurans, Hels and Buchwald (2001) concluded that road mortality in the adult breeding population becomes an additive source of mortality when the population is limited by density-independent factors (e.g. climate variability), but when density-dependent intra-specific competition, often in the larval stage, limit a population, road mortality in adults is compensated by higher larval survival because road mortality removes breeding adults from the population, and thus lowers larval density and reduces larval competition. Although, due to the 1994 observation of < 10% breeding adult mortality, it is possible that the contribution of road mortality alone to the population decline has been overestimated since studies of the population began in 1993. Predation and interspecific competition with fish counteract the expected increase in larval survival, and therefore recruitment, resulting from a reduction in the adult population as proposed by Hels and Buchwald (2001), and road mortality is likely acting in an additive way to reduce the population.

Effects of Fish in Linnet Lake

I found no evidence that the negative effects of fish, specifically lake chub, on recruitment (Pagnucco et al. 2011) have been remediated by fish removals. Natural declines in small-bodied fish via winterkill were shown by Eaton et al. (2005) to cause increased wood frog (*Lithobates sylvaticus*) recruitment, seen by both an increase in metamorphs in the summer following winterkill (up to 8 times more), and juveniles the following year (4 times more). I expected fish removals in fall 2010 and spring 2011 at Linnet Lake to have a similar effect to winterkill, and subsequently expected to encounter more juvenile salamanders during spring trapping than were encountered in the 2008 – 2009 study (one juvenile) and a higher proportion of small adults in the migrating population when compared to Stable Pond. I only captured four juveniles over 75 d in 2013 and one over 76 d in 2014. By comparison, Fukumoto (1995) captured six juveniles in pitfall traps over 57 d in 1994 using only 60 m of drift fence and nightly searches along 375 m of the Entrance Road. I used 782 – 897 m of fence in 2013 and 542 – 656 m in 2014 as well as irregular searches along the road.

Comparisons of size frequency distributions between Linnet Lake and Stable Pond to detect the occurrence of successful recruitment at Linnet Lake (increased frequency of smaller individuals), indicated no difference for adult salamanders. Semlitsch (1983) observed a clear bimodal size distribution in an *A. tigrinum* population during 1 year of a 4-y study in South Carolina that monitored breeding adults and recruitment rates at two sites. He attributed this to an increase in small-sized juveniles entering the breeding population that originated from successful recruitment 2 years previous. In his study, successful recruitment had been absent in earlier years, and average SVL of breeding adults had slowly increased each year. I found a similar pattern at Linnet Lake where the average SVL for adults in 2013 – 2014 was 6.1 mm larger than in 2008 –2009 and increased by 1.2 – 1.3 mm from 2013 to 2014. Increased adult size through time is likely a result of an aging population with little successful recruitment.

One week of fish sampling in 2013 resulted in the capture and removal of 6550 lake chub, 61% the number (10698) removed 2 years previous, and an estimated catchable population (10463) at 98% the number removed. Although it appears there are less fish in Linnet Lake, it does not appear that enough fish were removed to release larval salamanders from either predation or interspecific competition. The size range of fish present in Linnet Lake did not appear different from that observed during fish removals (G. Scrimgeour, unpublished data). If large fish had been preferentially removed so that only small fish remained in Linnet Lake (e.g. due to mesh-size limitations of gear), salamander eggs and larvae may have been able to survive in higher numbers, at least for some period of time (Kloskowski 2009, Pagnucco et al. 2011). Also, flooding between Linnet Lake and Middle Waterton Lake appears to occur regularly, perhaps on a decadal cycle, although the frequency has not been formally documented. Flooding in 2014 provided a new opportunity for fish movement between the two water bodies, potentially eliminating the possibility of monitoring the effect of 2010/2011 fish removals into the future.

Movement Patterns

Breeding salamanders at Linnet Lake showed similar temporal movement patterns to those observed by Pagnucco in 2008 – 2009, but movement began earlier and lasted longer in 2013 – 2014. I observed peak immigration in early May, and peak emigration in late May both years. Air temperature can be the primary factor influencing when migratory movements occur during ambystomid salamander breeding seasons, closely followed by precipitation (Semlitsch 1983, Beneski et al. 1986, Sexton et al. 1990). Sexton et al. (1990) also suggested that soil temperature is an additional cue, and in northern and high elevation areas, timing of snow melt likely plays a role. Fukumoto (1998) observed the migration at Linnet Lake beginning by April 12 in 1994 when the region was experiencing drought conditions. I did not quantify the effects of temperature or precipitation on movement, but I observed immigration begin as soon as night time temperatures were above freezing and there had been sufficient precipitation or snow melt to create moist conditions. I would occasionally encounter salamanders on dry, windy nights, but large movements were limited to nights that coincided with or followed precipitation that occurred in the afternoon or evening of that day (Figure 2.8, Figure 2.9).

Migrating adult salamanders also maintained similar, but slightly shifted, spatial movement patterns regarding tunnel-use between 2009 and 2013 – 2014. In one of the few studies observing migratory movement patterns for multiple years, Jenkins et al. (2006) found adult spotted salamander movement patterns shift slightly over 5 years among nine breeding ponds, but maintain the same approximate pattern. Likewise, road mortality patterns can shift from year-to-year at a landscape scale (S. Boyle unpublished data), but generally remain spatially consistent. In 2008 Pagnucco captured most salamanders by hand along fences associated with tunnels 2 and 3, and in 2009 tunnels 3 and 4. However, in 2009 cameras and pitfall traps documented the most use for tunnels 2 and 3. Fukumoto (1994) also captured the majority of salamanders along the section of road that now contains tunnels 2 – 4, with the highest frequency near tunnel 2. I had the highest capture rates along fences associated with tunnels 3 and 4 both years and the most RFID detections in tunnel 3 both years. Road mortality patterns for long-toed salamanders reflected the concentration of migration traffic here along the tunnel-fence system.

Road Mortality at Stable Pond

To my knowledge, I documented the first-ever record of long-toed salamander road mortality at Stable Pond. I expect that road mortality rates at this site would have been higher if not for the drift fence (fence 05) I had installed between the pond and the Entrance Road (Figure 2.2). Seven of eight mortalities at this site occurred in the fall rather than spring migration, and 50% of mortalities were juvenile or YOY salamanders. It may be that adult mortality in the spring is low because not many adults forage or overwinter on the other side of the road due to poor habitat (i.e. paved road surface and open grassland) (Gibbs 1998, Rittenhouse and Semlitsch 2006), and road mortality may continually eliminate many of those adult migrants from the population. Furthermore, I observed few adults immigrating, emigrating, or breeding along the road side (NW) of the pond and few YOY dispersing toward the road from Stable Pond (Chapter 3).

The relatively high number of juvenile road mortalities in the fall at Stable Pond likely reflects the relative recruitment success at this site, and may be indirect evidence supporting largely failed recruitment at Linnet Lake. In the 2 years of road mortality surveys at Linnet Lake, I found one late-season road mortality on July 26, 2013, which was an adult. Less young-of-year road mortality may be expected at Linnet Lake because the road is generally further from the shoreline (13 – 110 m) that at Stable Pond (10 – 15 m), and the fence-tunnel system would prevent many juveniles from crossing the road surface. However, if recruitment was successful, there would be more young-of-year than adults leaving the water body both years and some road mortality would have likely occurred. Juvenile ambystomid salamanders will sometimes disperse as far or further from the breeding site as adults (Gambel et al. 2006), but this is not always the case (Semlitsch 1981). At Linnet Lake, Fukumoto (1995) captured 38 juveniles crossing the road in August and September 1993, but did not capture any in 1994, which she attributed to dry conditions.

Using RFID and Cameras to Monitor Tunnel-Use

RFID antennas worked well to document tunnel-use by PIT-tagged salamanders. To my knowledge, this is the first time RFID has been used to monitor tunnel-use by an amphibian, although Charnay et al. (2009) used a similar system to monitor ambystomid salamander movement in a terrestrial setting. I assumed that the RFID detection rate of tagged animals within range was near 100% based on the antenna design and scan frequency, but I did not test this assumption. Continuous video monitoring has been used in some studies to validate RFID detections (e.g. Scheibler et al. 2013, Guimond 2014), but this was not feasible in the Linnet Lake system. Other studies have validated RFID systems using experiments that involve releasing tagged animals or dummies in known locations along an RFID array and comparing recapture locations to RFID detections (Nunnallee et al. 1998, Charney et al. 2009, Burnett et al. 2013) and have reported detection rates from 55 – 100%. Instead of conducting experimental trials, I balanced informed design and logistical constraints, as suggested by Boarman (1998) and Zydlewski et al. (2006), to create an RFID system idealized for the scenario at Linnet Lake to monitor long-toed salamanders. As a result, monitoring two of four tunnels in 2013 and 2014 allowed me to detect 22% and 10% (respectively) of the tagged individuals in the population (ignoring unknown rates of mortality and tag loss).

Pagnucco et al. (2011) estimated that cameras captured 44% of tunnel crossings compared to pitfall traps when cameras were set to take a picture every minute at night (2100 h – 0600 h) and when motion was detected. I found that, on average, cameras captured far less salamander passages when compared to RFID detections, and that the ability of cameras to detect salamanders varied from 5% – 40% among the six cameras used and up to 9% by year for the two camera locations monitored both years. There are several reasons for this variability.

Stochastic sources of variability affecting how well a camera detected a salamander entering or exiting a tunnel included: timing of the photograph and the relative position of the salamander in the entrance. Cameras were set to take one photo every minute at night. If, by chance, one camera had more salamanders pass beneath at the same time a photo was taken, that camera's detection rate would be higher. Cameras were positioned so that the field of view did not cover the entire tunnel floor. Cameras could not photograph salamanders moving along the edge of the tunnel entrance, which I observed to be a common behavior. There is also a chance that higher detection rates were actually due to "false" camera detections resulting from high un-tagged salamander traffic where the salamander in an image associated with an RFID detection is not actually the one detected by the RFID array.

Non-random sources of variability included different physical characteristics of tunnel entrances and different image clarity among cameras and years. Tunnel entrances on the west side of the road collect sediment from runoff during heavy rain events, which affected how salamanders entered tunnels. For example, tunnel entrances were clear in 2013 until a rain storm on June 19 caused three of the tunnel entrances to be filled to varying degrees with sediment. At tunnel 4, I removed sediment with a shovel to create space for salamanders to pass beneath the camera, but I only removed sediment directly beneath the camera, creating a small corridor that effectively funneled salamanders through the cameras field of view. This camera had the highest recorded efficiency in 2014 at 40%. The presence of sediment also raises the ground's surface so that the camera's field of view was smaller and the subject could become out of focus. Each camera had deteriorating image clarity since their purchase in 2008. In 2013, images from cameras with the poorest clarity were difficult to analyze for salamander presence because a much larger portion of a salamander needed to be in the field of view to yield a detection. For 2014, cameras used in conjunction with RFID antennas were refocused by the manufacturer to a 35.6 cm focal length, which dramatically improved image clarity for the that year (Figure 2.12) and may have contributed to the two-fold increase in camera efficiency.

Salamander Tunnel-Use: Insights from RFID

Using RFID antennas at both ends of tunnels allowed me to view movement behavior differently, and sometimes more precisely, than if I had used cameras alone. The chance of detecting the same individual at both ends of a tunnel increased when using RFID because the antennas covered the entire tunnel floor and scanned every 4 seconds instead every 1 minute. For each individual detected at both ends of a tunnel, I could determine direction of movement, the amount of time spent in the tunnel, detect multiple crossing events for the same individual, and detect salamanders using tunnels outside of the camera "timed interval period" (66 detections). I detected 62 tunnel passages (14 immigrants, 48 emigrants) in 2013 and 46 (11 immigrants, 35 emigrants) in

2014 (108 total). Figure 2.13 shows the temporal pattern of initial RFID detections compared to when cameras were operating on a timed interval mode. In 2013, five of the 62 individuals detected passing through tunnels were detected at the initial tunnel entrance, then appeared to leave and wait until the next evening to move through. I documented "hesitation", similar to Pagnucco (2010), where an individual would remain in or near the read range of an RFID antenna at a tunnel's entrance in 14 of the remaining 57 crossings. Hesitation lasted from 1 min to 1 h 57 min (median = 2.5 min). In 2014, 36 of the 46 crossings occurred with no hesitation. When hesitation occurred, it ranged from 2 min to 1 h 36 min (median = 6.5 min). Once salamanders entered the tunnels, it took a median time of 10 min (range 4 min to 23 h 48 min) to pass through based on both years. This is three times slower than Pagnucco's (2010) reported average crossing speed of 2.9 min (n = 4) in 2009 using camera images. Of the 108 salamanders that crossed through tunnels, five spent at least 1 day in the culvert before continuing movements the following night. To my knowledge, this is the first time an amphibian has been documented using a crossing structure as a daytime refuge, although this has been documented in mammals (Hewitt et al 1998). I also detected three individuals that successfully passed through tunnels both years.

Although RFID can be a useful tool for monitoring tunnel-use, cameras have the ability to detect unmarked individuals and non-target species, as well as inter-specific interactions such as predation. Pagnucco (2010) saw at least 12 mammal species and three other reptiles and amphibians beside long-toed salamander, and one instance of predation. These data are "invisible" to RFID. Using these two methods in concert, however, allowed the performance of cameras to be analyzed for monitoring the population of long-toed salamanders at Linnet Lake long-term. My calibration suggests approximately 15% of salamanders that pass through tunnels are captured by timed-interval photographs. This information can be used to infer the relative abundance of salamanders using tunnels and can track trends through time at Linnet Lake.

Conclusions

The long-toed salamander population at Linnet Lake has not recovered relative to levels seen in the 1990s. Migrating salamanders use tunnels such that road mortality is reduced, but road mortality appears to be below the threshold level suggested by Gibbs and Shriver (2005) to cause population decline. Road mortality is likely acting as an additive source of mortality, but may not be the primary driver of the population decline at Linnet Lake. This is supported by the fact that over the last 20 years, large numbers of breeding salamanders have continued to move over (and later under) the Entrance Road during migrations, an unexpected behavior to persist at the population level if most road-crossing salamanders are killed by vehicles. The more likely driver of the decline is predation and competition of larval salamanders with fish that arrive from Middle Waterton Lake during periodic floods, which effectively eliminates recruitment of new adults into the population for several consecutive years. Furthermore, removing fish in 2010 and 2011 does not appear to have increased salamander recruitment in this population and fish remain prevalent in Linnet Lake. Amphibian populations are naturally quite variable through time (Pechman et al. 1991), and when coexisting with predatory fish, natural reductions in fish populations can give amphibian populations the opportunity needed to recruit a cohort of individuals to replace dying adults (Eaton et al. 2005). Many amphibian populations are regulated by metapopulation dynamics (Smith and Green 2005) and the long-toed salamander can recolonize breeding sites after extirpation caused by fish predation (Funk and Dunlap 1999). It is likely that Linnet Lake is no exception, as fish have been present at this site intermittently for the last century (Fukumoto 1995). However, it is almost impossible to attribute the current population size of breeding salamanders at Linnet Lake to only one driver of decline. Block et al. (2001) emphasizes the importance of assessing mitigation efforts for wildlife, and Lesbarreres and Fahrig (2012) and van der Grift et al. (2013) highlight the need for long-term, rigorous monitoring of road-crossing structures specifically to understand their effectiveness and improve methods. Monitoring the Linnet Lake population with camera surveillance of under-road tunnels can be a useful method for monitoring the fluctuations in this population in the future (Pagnucco et al. 2011). As predicted by Gibbons and Andrews (2004), PIT tags continue to be a useful tool for answering many wildlife research questions. I have demonstrated that using RFID with amphibians is an effective option for monitoring use of road-crossing structures and provides higher resolution for movement data than wildlife cameras.

Tables and Figures

Table 2.1 Summary of road mortality for Linnet Lake and Stable Pond for 2013 and 2014. Road mortality surveys were conducted by foot in the mornings from April 18 (2013) and April 26 (2014) – August 29.

			Linnet Lake		Stable Pond
	Common name	**Scientific Name**	**2013**	**2014**	**2014**
Amphibians and Reptiles	long-toed salamander	*Ambystoma macrodactylum*	24	20	8
	western tiger salamander	*Ambystoma mavortium*	1	0	0
	western toad	*Anaxyrus boreas*	11	12	23
	wandering garter snake	*Thamnophis elegans vagrans*	1	5	1
Birds	unknown songbird*		2	1	2
	white-crowned sparrow	*Zonotrichia leucophrys*	1	0	0
	American robin	*Turdus migratorius*	1	0	2
	ruffed grouse	*Bonasa umbellus*	0	0	1
	cedar waxwing	*Bombycilla cedrorum*	0	0	1
	duckling	*Anas* sp.	0	0	1
	hummingbird	*Stellula calliope* or *Selasphorus rufus*	0	0	1
Mammals	unknown*		2	2	2
	mouse/vole	*Zapus princeps* or *Peromyscus maniculatus; Microtus* spp.	3	1	2
	Columbian ground squirrel	*Spermophilus columbianus*	2	0	1
	shrew	*Sorex* spp.	4	1	2
	red squirrel	*Tamiasciurus hudsonicus*	0	1	0
	chipmunk	*Tamias minimus* or *T. ruficaudus*	0	1	1
	striped skunk	*Mephitis mephitis*			

* blood on pavement accompanied by feathers/fur OR unidentifiable due to condition

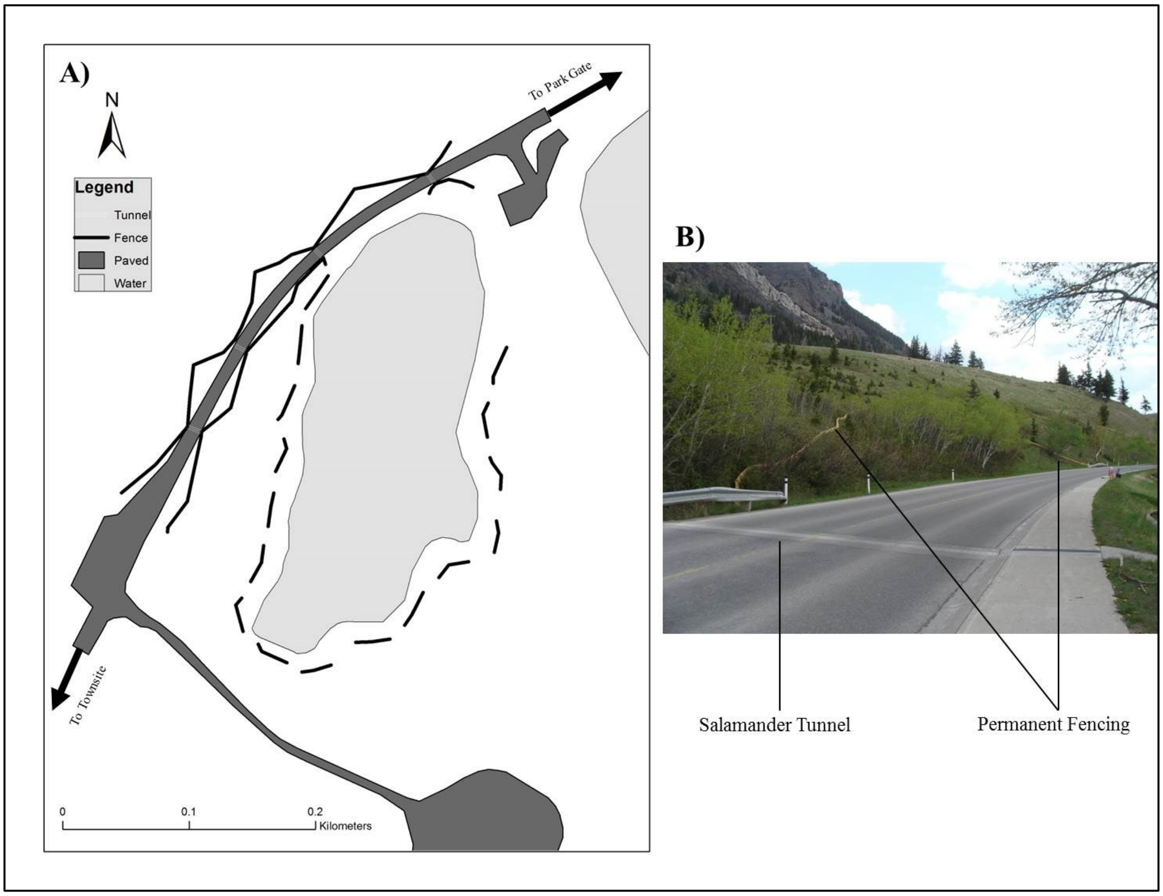

Figure 2.1 Linnet Lake fences and tunnel system. **A)** Linnet Lake with 16 30-m temporary fences around shoreline and "W-shaped" permanent fencing to funnel salamanders toward tunnels. **B)** Picture of tunnel 3 (numbered from north [1] to south [4]) showing permanent fencing (yellow structure) on hillside.

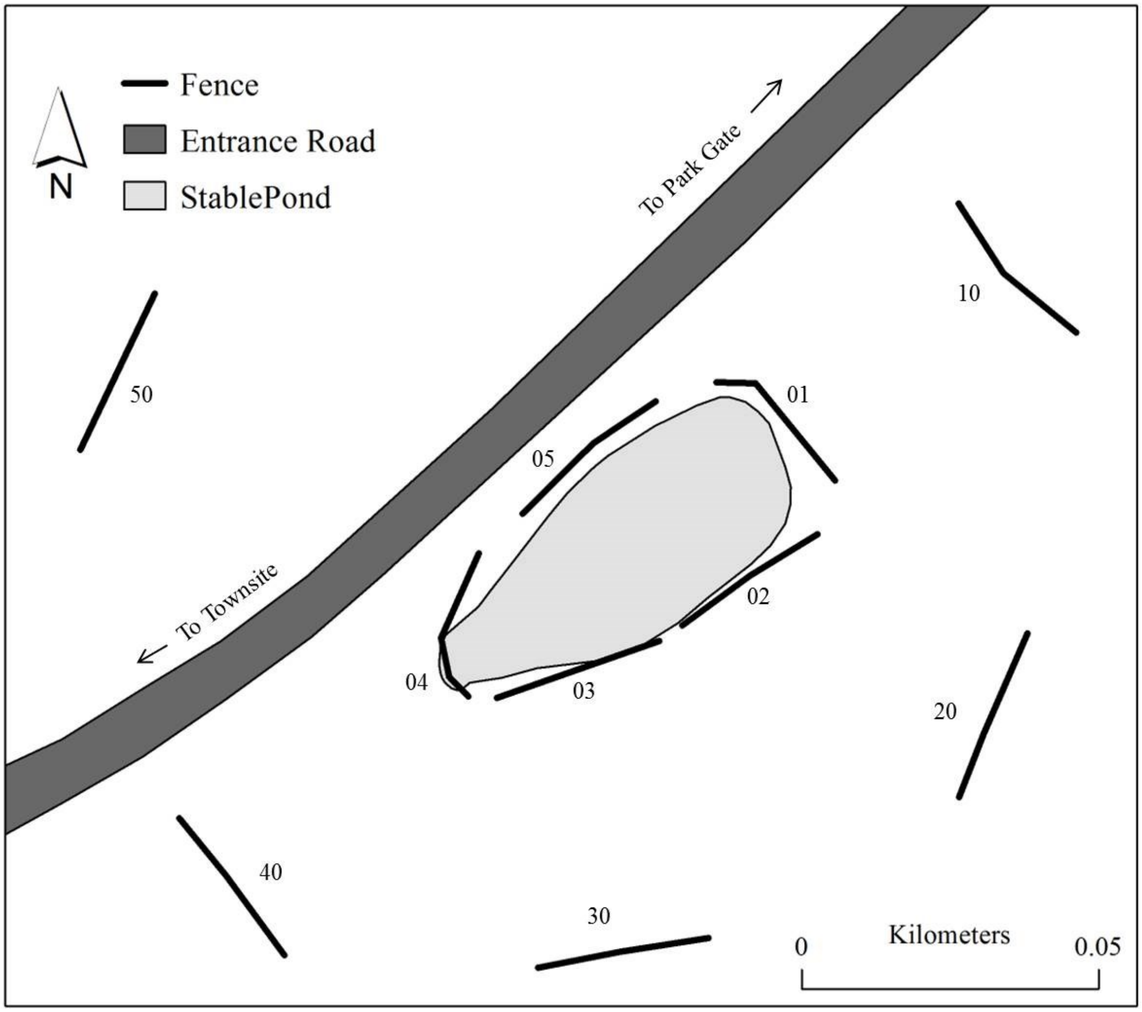

Figure 2.2 Stable Pond fence system. Shoreline “inner fences” were within 3 m of high water line. “Outer fences” were 50 m from high water line. Fences are numbered by relative position (bearing from pond centroid) where inner fence numbers (01, 02...) correspond to the nearest outer fence (10, 20…).

Figure 2.3 Photograph of an RFID antenna and camera in its protective box at the entrance of a tunnel. The red dashed line shows where the antenna is buried beneath the substrate. Cameras operated on a motion-detection setting 24 h each day and on a timed interval setting from 0600 h – 2100 h. Antennas scanned every 4 s, 24 h each day.

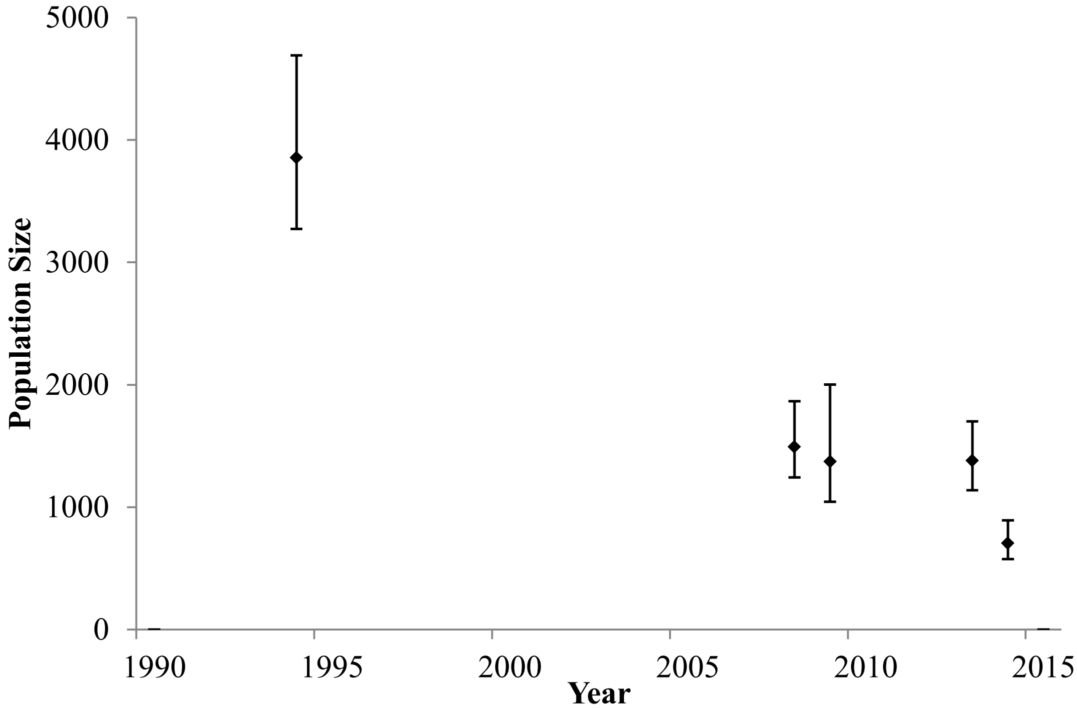

Figure 2.4 Population estimates through time for breeding long-toed salamanders at Linnet Lake (1994 estimate from Fukumoto and Herero 1998, 2008-09 estimates from Pagnucco et al. 2011). Error bars indicate 95% confidence intervals.

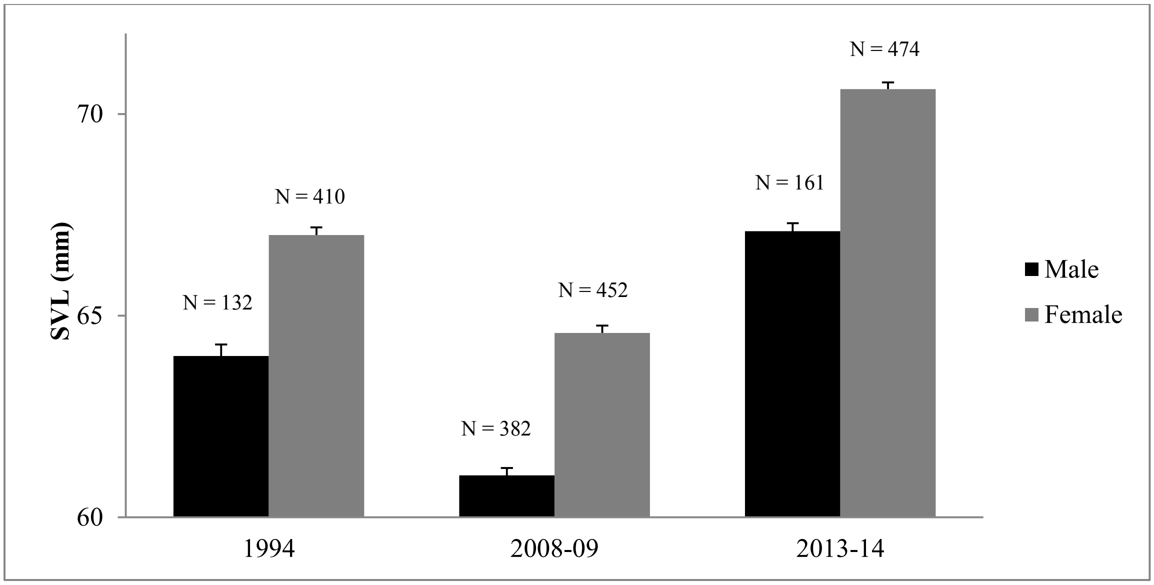

Figure 2.5 Mean (± 1 SE) snout-vent length (SVL) of long-toed salamanders captured at Linnet Lake, Alberta, Canada in 1994 (J. Fukumoto), 2008–2009 (K. Pagnucco), and 2013–2014 (this thesis). Sizes are significantly different among years for both sexes (males: $F_{0.05, [2, 671]} = 190.5$, $p < 0.001$; females: $F_{0.05, [2, 1332]} = 309.0$, $p < 0.001$). SVL increased from 2008–2009 to 2013–2014.

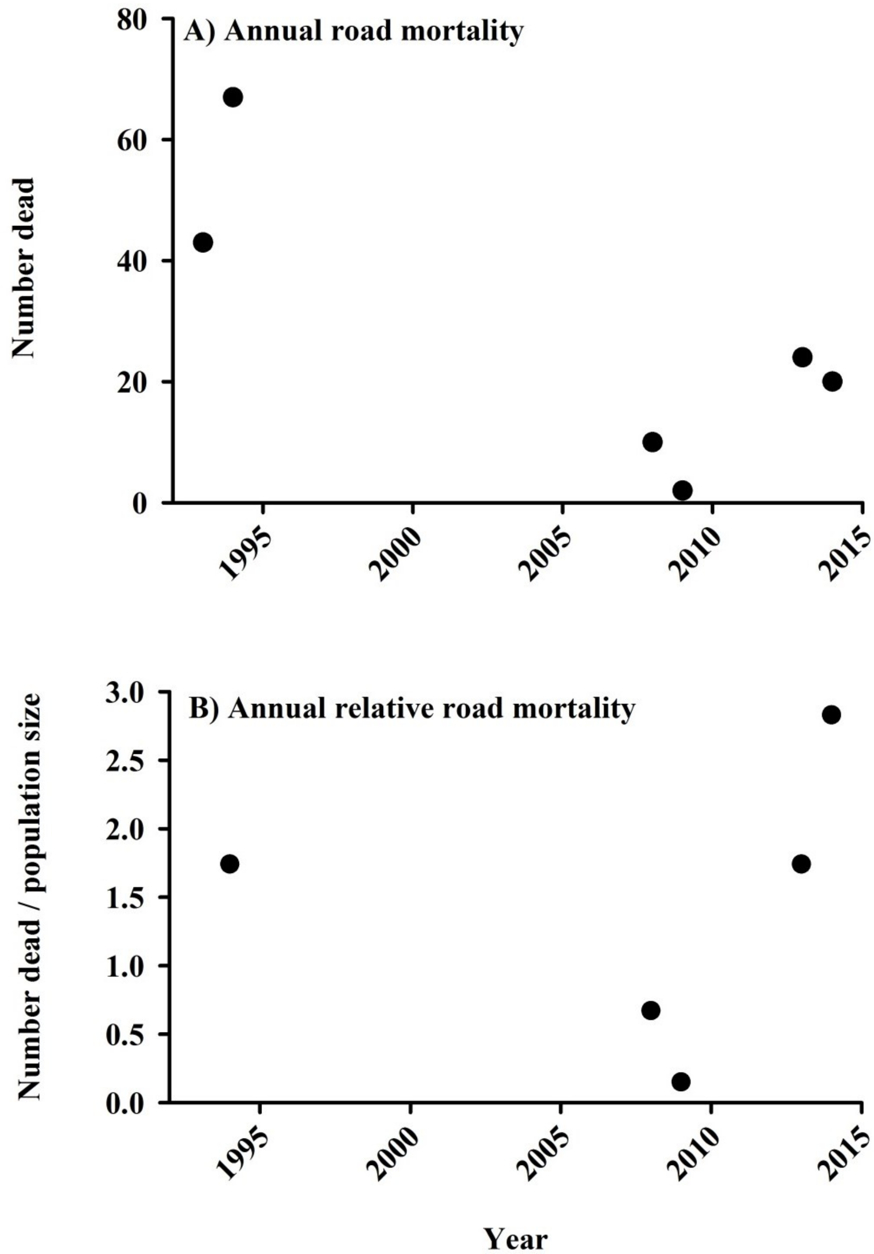

Figure 2.6 Temporal patterns in annual road mortality (absolute number observed each year) (A) and relative road mortality (number observed each year / annual population size) (B) of long-toed salamanders along the Entrance Road adjacent to Linnet Lake, Waterton Lakes National Park. Data for 1994 obtained from Fukumoto (1995) and Fukumoto and Herrero (1998), data for 2008 and 2009 from Pagnucco et al. 2011, and data for 2013 and 2014 from this thesis.

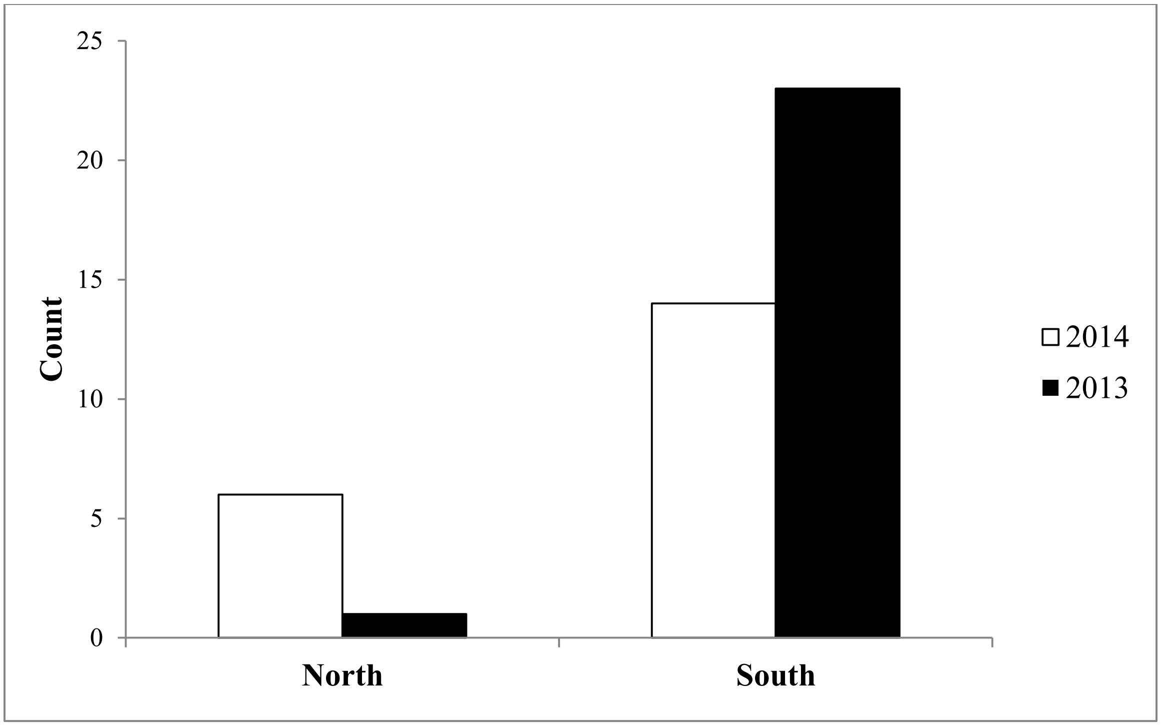

Figure 2.7 Road mortality of long-toed salamanders at Linnet Lake between north and south sections of Entrance Road in 2013 and 2014. The south section includes the location of tunnels 3 and 4, which experienced the highest salamander traffic. A chi-square goodness-of-fit test indicated unequal road mortality between the two sections ($\chi_{c}{}^{2}{}_{\upsilon = 1} = 15.2$, $p < 0.001$)

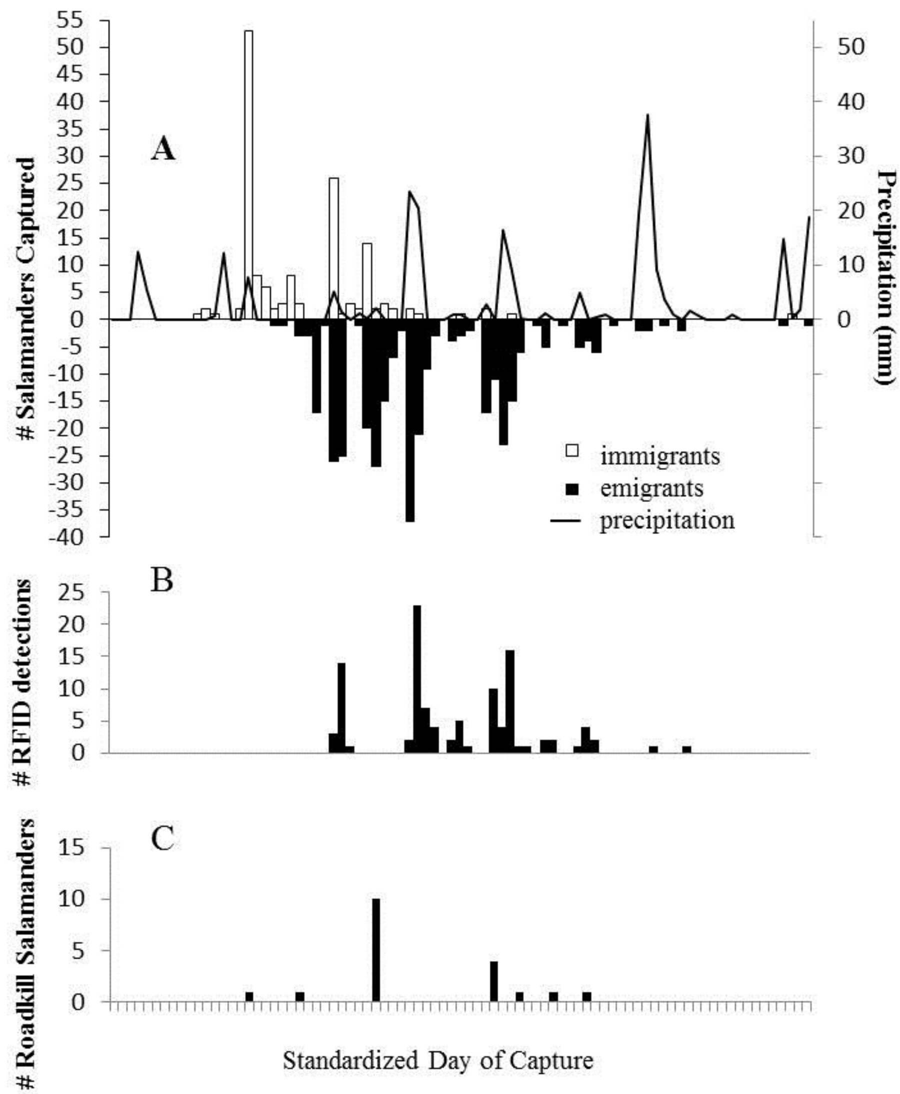

Figure 2.8 Movement patterns of long-toed salamanders at Linnet Lake in 2013 related to precipitation. (A) Immigrants and emigrants captured at Linnet Lake during night searches of 16 30-m drift fences and 718 m of permanent roadside fencing for April 25 – July 8. Precipitation data from Waterton Park gate weather station. (B) RFID detections from antennae monitoring tunnels 2 and 3 for May 13 – August 30. (C) Number of long-toed salamander road mortalities found during daily road surveys for April 26 – August 30. Standardized day of capture begins and ends on the earliest and last date, respectively, a salamander was captured in 2013 –2014.

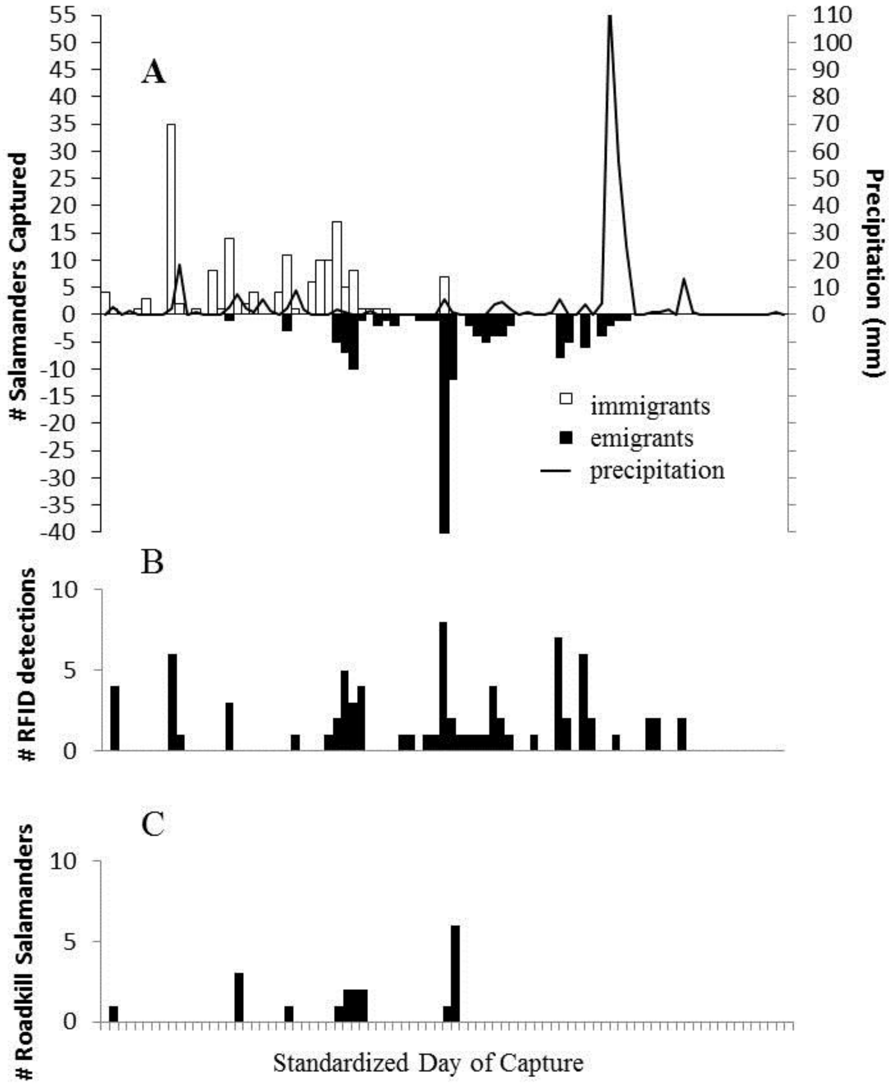

Figure 2.9 Movement patterns of long-toed salamanders at Linnet Lake in 2014 related to precipitation (A) Immigrants and emigrants captured at Linnet Lake during night searches of 8 30-m drift fences and 718 m of permanent roadside fencing for April 17 – July 2. Precipitation data from Waterton Park gate weather station. (B) RFID detections from antennae monitoring tunnels 3 and 4 for May 22 – August 22. (C) Number of long-toed salamander road mortalities found during daily road surveys for April 18 – August 29. Standardized day of capture begins and ends on the earliest and last date, respectively, a salamander was captured in 2013 –2014.

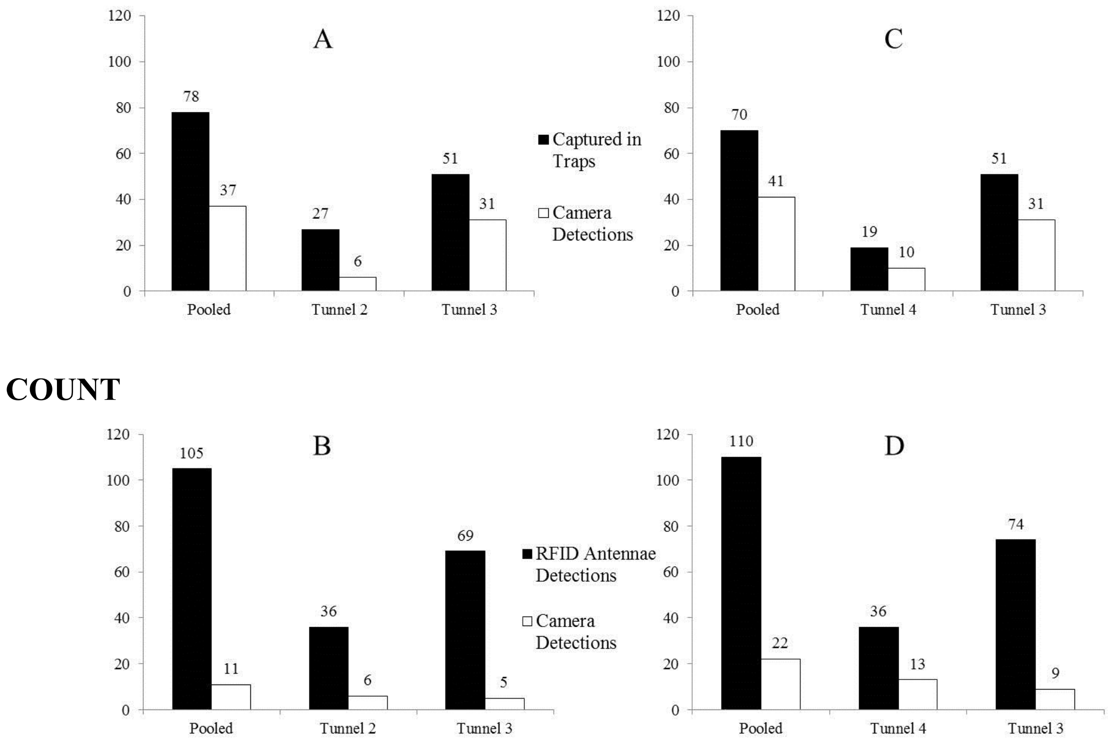

Figure 2.10 Comparison of methods for monitoring under-road tunnels at Linnet Lake. Bars labeled with number of salamanders detected. (A) and (C) K. Pagnucco's 2009 pitfall trap data with camera data paired by tunnel. (B) and (D) RFID detections with camera detections paired by tunnel for 2013 (B) and 2014 (D).

Figure 2.11 Examples of locations where salamanders could bypass the fence-tunnel system at junctions of fences and tunnels. Red arrows show possible “escape routes” and yellow shows a well-designed junction.

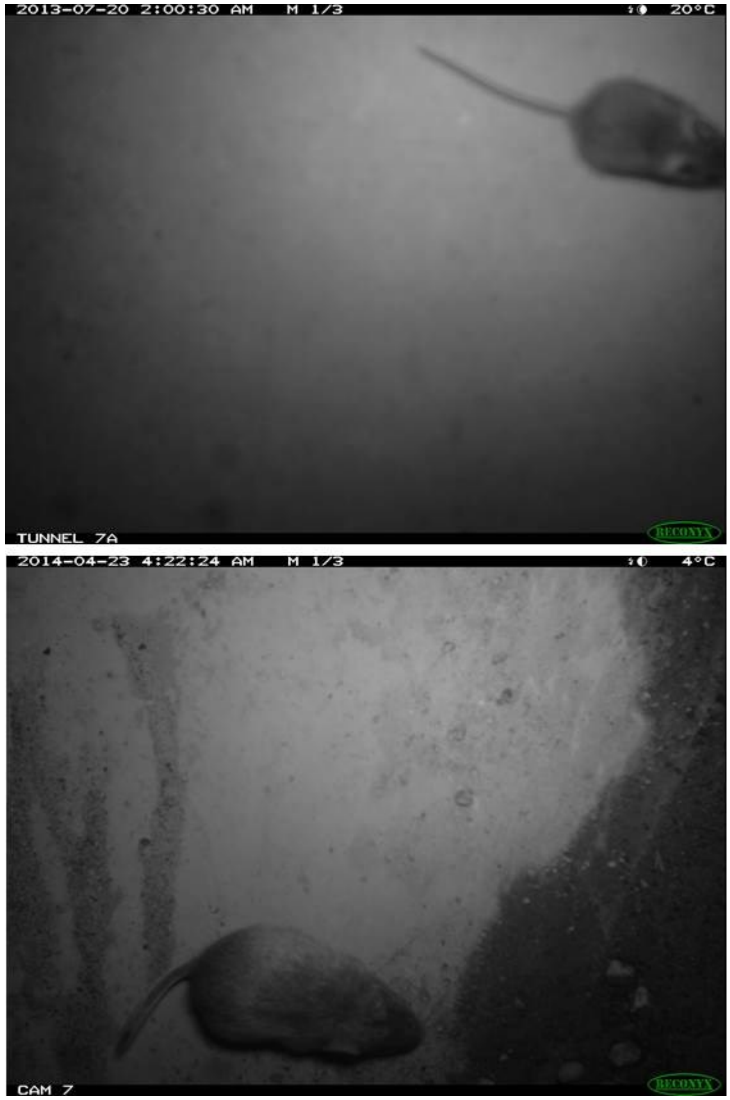

Figure 2.12 Comparison of tunnel camera images before and after refocusing. Both photos were taken with the same camera (7) in tunnel 4. The top photo was taken in 2013, 5 y after the camera was purchased. The bottom photo was taken in 2014 after the camera was refocused to 14” by the manufacturer (Reconyx, Holmen, Wisconsin, USA). The animal in the top photo is a deer mouse (*Peromyscus maniculatus*), and the bottom is a vole (*Microtus* or *Myodes* spp.).

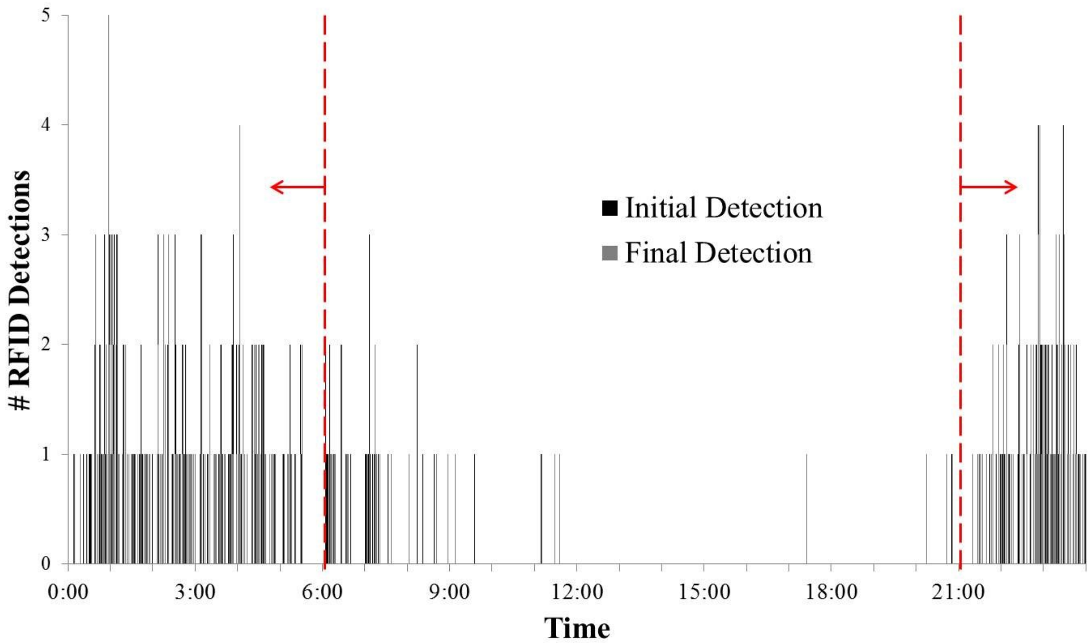

Figure 2.13 RFID detections of long-toed salamanders in under-road tunnels at Linnet Lake pooled for 2013 (110 d) and 2014 (138 d). Black bars indicate times when tagged animals first entered an RFID detection field, and grey bars indicate when animals left a detection field. The dashed lines with arrows indicate when cameras were set on a timed interval (one image per min for 2100 – 0600 h). RFID detection between the two dashed lines show tagged animals using the tunnels when cameras are on a motion detection setting only and least likely to photograph salamanders.

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Chapter 3: Patterns of Movement and Orientation During Migration and Dispersal, and Overwintering Habitat Use

Introduction

Habitat loss and alteration are among the biggest threats to the persistence of animal and plant populations in North America (Wilcove et al. 1998). Agriculture, water resource development, urbanization, recreational and extractive land uses, as well as infrastructure (including road-building) associated with all these activities can represent threats to the habitats of native species. Amphibian populations are no exception. In a summary of known declines in western North America, Corn (1994) also concluded that anthropogenic habitat destruction and alteration are the leading threats to amphibian populations. Much effort has been directed toward amphibian conservation since global declines were recognized in the early 1990s (e.g. Wake 1991).

Planning ahead to mitigate impacts of development on local animal populations, or for restoration after impacts occur, requires a fundamental understanding of the biology of the system being affected (Landres et al 1999). Depending on the nature of the disturbance, both abiotic and biotic processes may need to be conserved or restored (Hobbs et al. 1996). Unfortunately, fundamental life history characteristics are often unknown for species that currently generate little conservation concern, or have no obvious economic value (Tyler et al. 2012). Knowledge gaps can make it difficult to reduce the impacts of anthropogenic activities and/or implement conservation planning (Cayuela et al. 2009). Amphibians are among those taxa remaining relatively understudied compared to other organisms facing human threats that negatively affect life history processes by destroying or degrading habitat (Cushman 2006, Lawler et al. 2006).

For many temperate pond-breeding amphibians, a healthy population needs unobstructed movement between a suitable aquatic breeding site and terrestrial foraging and overwintering habitat (Pittman et al. 2014). Suitable breeding habitats are usually, permanent or semi-permanent water bodies lacking fish, but containing aquatic vegetation or other substrates for egg deposition. Required terrestrial foraging or overwintering habitat is often defined by a circular buffer surrounding a breeding site, which is estimated to encompass a specified percentage of the breeding population (Semlitsch 1998, Semlitsch and Bodie 2003). A buffer of 500 m has been estimated to include 95% of the breeding individuals for some amphibian species (Scott et al. 2013). The movement path individuals take between terrestrial home ranges and breeding sites may not be the shortest, straight-line distance for some species (Shoop 1968, Semlitsch 1981, Lee-Yaw 2015). Evidence suggests amphibians in some contexts will follow "movement corridors" presumably because the micro or macro habitat within corridors is more conducive to movement. However, using defined movement corridors is a trait that may be site-specific, not species-specific (Douglas and Monroe 1981, Jenkins et al. 2006).

Fine-scale use of terrestrial habitat surrounding breeding sites is often difficult to document for amphibians due to the technical limitations associated with affixing tracking devices for such small-bodied vertebrates either internally or externally. In many cases, habitat use is inferred from data that are fairly easy to collect for many pond-breeding amphibians by using drift fences to intersect movement and capture marked or unmarked individuals as they make seasonal overland migrations *en mass*e between terrestrial and aquatic habitats. Studies using coarse movement data to investigate habitat use range from complex designs that isolate specific landscape features (e.g. Gibbs 1998, Regosin et al. 2005) to studies using orientation of migrating adults and dispersing juveniles to infer habitat preferences (e.g. Douglas and Monroe 1981, Homan et al. 2008, Walston and Mullin 2008), and occasionally laboratory experiments (e.g. Lee-Yaw et al. 2015). In instances where tracking devices are used (e.g. Sheppard 1977, Madison 1997, Trenham 2001, Baldwin et al. 2003, Faccio 2003) movement patterns, foraging habitat, terrestrial refugia (including overwintering sites), and sources of mortality can be investigated with more precision. This type of information is invaluable for conservation planning (Trenham and Shaffer 2005).

Recent developments in radio-frequency identification (RFID) and passive integrated transponder (PIT) technologies have provided an alternative method for investigating habitat use and movement patterns of many small-bodied vertebrates, including amphibians (Cucherousset et al. 2008, Hamed et al. 2008, Connette and Semlitsch 2012, Ryan et al 2014). Using PIT tags and "PIT telemetry" can liberate investigators from the size constraints and battery-life limitations of conventional radio telemetry. In the past, some investigators used radioactive tags to overcome these limitations (Sheppard 1977, Semlitsch 1981). This method has become outdated because of environmental concerns, but PIT telemetry offers a flexible alternative that allows similar data to be collected for small and delicate amphibian species and life stages (Connette and Semlitsch 2012, Ousterhout and Semlitsch 2014, Ryan et al. 2014).

Ambystomatid salamanders ("mole" salamanders, Family: Ambystomatidae, Genus: Ambystoma) are physically robust, highly terrestrial and mobile pond-breeding amphibians (Stebbins 2003). Species tend to orient non-randomly when entering and exiting breeding sites (Shoop 1968, Simlitch 1981, Douglas and Monroe 1981, Jenkins et al. 2006), and use terrestrial habitats selectively during migratory movements (Semlitsch 1981, Douglas and Monroe 1981, Gibbs 1998, Homan et al. 2008) and for foraging and overwintering (Semlitsch 1981, Madison 1997, Trenham 2001, Faccio 2003). Currently, studies investigating these behaviors are mainly limited to eastern species.

The long-toed salamander (*Ambystoma macrodactylum*) ranges throughout the Pacific Northwest from California to Alaska, and as far east as the foothills and plains of the Rocky Mountains in Montana (Stebbins 2003). This salamander species consists of five recognized subspecies (Ferguson 1961, Lee-Yaw and Irwin 2012) and occupies a wide range of habitats throughout its range varying from high alpine lakes in the Sierra Mountains of California and the Rocky Mountains from Idaho through Canada, arid sagebrush (*Artemesia* spp.) habitats of the western USA, and temperate rainforests of the northwest coast in Canada and the USA. Although found in a variety of environments, little is known about specific micro-habitat requirements for this species, especially at the northern and high elevation limits of its range. Where winter conditions are harsh, it is logical to assume that population persistence at specific breeding sites is likely limited in-part by the availability of below-ground refugia for surviving cold winter months. Anderson (1967) compared the life-history of a high-alpine (2450 m) population in the Sierra Nevadas with a low elevation coastal population, but was unable to observe the migration patterns or overwintering habitat use of adults at the high altitude site. Anderson assumed at high altitude, salamanders moved to the surrounding forest to retreat below ground or within rotten logs for refuge, although at low elevations he observed salamanders on or near the ground surface in small mammal runways in pond vegetation or in partially buried woody debris until rain events made above-ground movement possible, during which salamanders seemed to selectively move towards wooded areas. In Idaho at a mid-elevation site (850 m) dominated by pine and fir (*Pinus and Abies* spp.), Beneski et al. (1986) found no orientation pattern for migrating adults, or apparent habitat selection during overland movement among field, wooded, or earthen dam habitats, or between dry and moist soil conditions.

Fine-scale tracking studies for long-toed salamander are limited to Sheppard (1977), who used radioactive tags to track individuals at a site in western Alberta, Canada dominated by spruce (*Picea* spp.) and pine interspersed with poplar (*Populus* spp.) and birch (*Betula* spp.) at an elevation of 1300 m. Sheppard found that tracked salamanders exclusively used above-ground objects (logs, rocks, and boards) for summer refuge. He never detected an individual in litter or a mammal burrow. Sheppard found salamanders beneath surface objects to 1 m below ground when the substrate was gravel with tree roots present. He also located three overwintering refugia. Each was occupied by multiple individuals (8 – 14), was next to a spruce tree, and was in coarse gravel substrate.

Non-random orientation during migratory movement (Beneski et al. 1986) and no observed use of mammal burrows at overwintering sites (Sheppard 1977) for long-toed salamander contrast with results from orientation and overwintering habitat-use studies for other northern ambystomatids in eastern North America (i.e. *A. maculatum*, *A. jeffersonianum*, and *A. opacum*) and Anderson's (1967) observations for long-toed salamander. In these studies, non-random orientation during breeding migrations and juvenile dispersal appear to be the norm, and is hypothesized to reflect the utility of habitat for movement (Douglas and Monroe 1981, Rothermel 2004, Jenkins at al. 2006).

Additionally, overwintering refugia have been found to consist of relatively deep networks of vertical mammal burrows with associated rock rubble recesses or near large tree trunks (Madison 1997, Faccio 2003).

In Waterton Lakes National Park, under-road crossing structures (tunnels) were installed in 2008 along the Entrance Road to reduce vehicle mortality for long-toed salamander during spring breeding migrations between Linnet Lake and foraging and overwintering sites across the road. Before tunnel installation, information about movement patterns for this population was limited to Fukumoto and Herrero (1998) and some unpublished observations by Waterton biologists (Parks Canada Agency unpublished data). Three assumptions that existed concerning this population before tunnel installation for this population were that 1) the majority of the adult population crossed the road during breeding migrations and were consequently subject to road mortality each spring, 2) the majority of juveniles crossed the road when dispersing from Linnet Lake following metamorphosis in the fall, and 3) salamanders crossed the road because foraging or overwintering habitat was more favorable or extensive across the road rather than immediately around the lake. Fukumoto (1995) noted that although she trapped exclusively on the west side of the lake along the road, captured almost 90% of salamanders on the road, and found eggs "predominantly" along the west shore, that some egg masses were seen along the east shore of Linnet Lake (away from any roads) and she captured three adults in the forest on the east shore. She further stated that it was possible that long-toed salamanders at this site use terrestrial habitats surrounding the entire lake year-round. After studying the population in 2008 –2009, Pagnucco (2010) found evidence that breeding adults at Linnet Lake do overwinter between the lake and the road, and suggested that future tunnel installations should be preceded by robust preliminary studies to document movement patterns and thus make installations cost-effective and useful for the conservation of populations.

Another breeding site for the long-toed salamander in Waterton Park lies 1.2 km north of Linnet Lake and is less than 10 m from the Entrance Road. This site, Stable Pond, is much smaller than Linnet Lake (1/25th the size), and has had no documented road mortality or decline in its long-toed salamander population. The pond is surrounded immediately on all sides by poplar forest and relatively flat terrain. Its size and simple terrestrial environment make it a good candidate for further investigations of long-toed salamander habitat use and movement patterns. It is also a good candidate for using PIT telemetry compared to Linnet Lake because salamanders may be more spatially focused on the landscape due to a relatively larger population and smaller overall terrestrial area occupied, which may increase encounter rates for tagged salamanders.

Observations by Fukumoto (1995) and Pagnucco (2010) indicate that long-toed salamanders move through the terrestrial habitat around Linnet Lake (i.e. "orient") non-uniformly, which is consistent to what has been seen reported for most other ambystomid species, but contrary to results from the only orientation study conducted on the long-toed salamander (Beneski et al. 1986), which found movement of individuals to and from a breeding site uniformly distributed around its perimeter. Movement patterns (uniform or non-uniform orientation) during breeding migrations may reflect the location of habitat best suited for overland movement (e.g. moisture gradients), but may also reflect the spatial arrangement of suitable foraging, and/or overwintering areas on the landscape

(Madison 1997), the exact nature of which are largely unknown for the long-toed salamander.

My overall goal in this chapter is to explore two separate aspects of long-toed salamander life history related to terrestrial movement and habitat use with the aim of reducing knowledge gaps regarding orientation during migration and dispersal, and fine-scale winter habitat. The results of this study will be useful for informing conservation planning in the northern part of this species' range, and for building a foundation for future research examining the link between movement patterns and habitat characteristics. By using PIT telemetry to relocate tagged individuals in the terrestrial environment, this study also adds to a growing body of literature supporting the use of PIT telemetry as an adaptable means of addressing questions related to microhabitat use for small and delicate vertebrate species.

To accomplish these goals, I asked two questions. 1) Do adult and newly metamorphosed long-toed salamanders orient non-randomly during breeding migrations and dispersal? and 2) What are the characteristics of overwintering refugia for this species at two sites in Waterton Lakes National Park? Based on my own preliminary observations and results from studies in the eastern USA (e.g. Jenkins et al 2006), I predicted that adult and newly metamorphosed salamanders would orient non-randomly during immigration, emigration, and dispersal, and that patterns of orientation would differ for the two age classes as young-of-year are naïve to the terrestrial environment and would not have previous knowledge of the landscape to inform movement decisions as would adults (Madison 1997). I also expected that PIT telemetry could be adapted to detect long-toed salamanders in deep overwintering refugia, and that these refugia would be associated with above-ground features (e.g. trees and decaying logs and stumps) or burrows that facilitate entry below ground.

Methods

Study Sites

I conducted research at two study sites in Waterton Lakes National Park: Linnet Lake and Stable Pond. In 2013, I conducted research activities at Linnet Lake only, and in 2014 I conducted research at both sites.

Linnet Lake (49° 04' N, 113° 54' W) is a small (3.9 ha), foot-shaped, shallow (5 m maximum depth) lake at an elevation of ~1260 m in a bowl-like catchment. The vegetation around the lake is dominated by stands of Douglas fir and poplar, with an understory of small trees and shrubs, e.g., chokecherry (*Prunus virginiana*), saskatoon (*Amelanchier alnifolia*), and snowberry (*Symphoricarpos albus*), and open grasslands. The lake is surrounded by moderately steep-sloping (up to 15%) hillside except on the north end, which is a flat, low-lying area containing a parking lot (~30 x 70 m), adjacent to Middle Waterton Lake. From the parking lot, a 1.5-m wide paved foot path encircles the lake. The Park's Entrance Road parallels the west side of the lake at a straight-line distance of 13 – 110 m and the road leading to the Prince of Wales Hotel passes 50 m south. Entrance Road is punctuated by four salamander tunnels spaced ~80 – 110 m apart and described in detail by Pagnucco et al. (2012). Linnet Lake is inhabited by three fish species (*Catostomus commersonii*, *Catostomus catostomus*, and *Couesius plumbeus*) and western toads (*Anaxyrus boreas*) and tiger salamanders (*Ambystoma mavortium*) are also found at this site.

Stable Pond (49° 04' N, 113° 53' W) is a small (0.15 ha), fishless, ephemeral pond at an elevation of 1275 m with a maximum depth of 1.6 m that typically dries by late July/early August (July 22 in 2013, August 7 in 2014) to become a grass-dominated meadow. Stable Pond is surrounded on all sides by poplar (*Populus* spp.) forest and flat terrain. This quickly breaks into open grass/low shrub mix to the south and poplar forest with small isolated stands of Douglas fir (*Pseudotsuga menziesii*) to the east. To the west the pond is immediately bordered by a bike path and the Entrance Road, across which lies an open, dry, grassy ridge capped by a stand of Douglas fir. Western toads and boreal chorus frogs (*Pseudacris maculata*) also breed in Stable Pond.

Salamander Capture: Stable Pond

To capture salamanders at Stable Pond for marking, demographic data collection, and orientation investigation, I installed 10 1-m high drift fences in 2014 (April 17 – August 22). Drift fencing was composed of 1-m high silt fencing buried to a depth of 5 – 10 cm and stapled to wooden stakes for support. I installed five 30-m fences, 5 – 8 m apart around the pond within 3 m of the high water line in late April (referred to as "inner fences"). I installed five more 30-m drift fences 50 m away from the high water line ("outer fences") and approximately in line with a corresponding inner fence, except for one fence on the other side of the Entrance Road that was offset 20° SW due to impenetrable soil conditions caused by an exposed rock outcropping (Figure 2.2). At Stable Pond, I used 50 pitfall traps buried along the fences to capture salamanders terrestrially in lieu of conducting night searches. Pitfall traps were made from #10 food service cans containing a stick that served as a ramp for mammals incidentally trapped and a small piece of sponge (4 x 7cm), wetted as needed with pond water, to provide moisture and cover for trapped amphibians. In addition to fences and pitfall traps, I placed 10 Gee minnow-traps (42 x 19 cm, 6.4 mm mesh, 2.5 cm openings) in the pond evenly spaced around the perimeter and at varying distances from shore depending on pond depth to trap breeding adults. I tried to ensure an air space existed in minnow traps to prevent animals from drowning. When the water table was too high for pitfall traps to be functional, they were replaced temporarily with minnow traps placed against and parallel to the drift fence with sticks and mud used to create a “funnel” to encourage salamanders into the trap. In mid-June I doubled-over the fencing material (fence reduced to a height of 0.5 m) at the 50-m distance to reduce wind damage and decrease visibility to easily-startled horses being ridden on nearby trails. This should not have affected the functionality of the fence.

I focused capture effort on migrating adult salamanders from April 17 to June 14, 2014 by placing eight pitfall traps along inner fences, four on each side evenly spaced (~7.5m apart), and placing two pitfall traps along outer fences, one at each end on the side facing the pond (catching emigrating salamanders only). I used minnow traps in the pond from April 12 to June 14. As the pond dried, I removed some minnow traps along the NW margin to keep traps evenly spaced. From July 16 to August 22, I focused capture effort on dispersing young-of-year (YOY) salamanders using pitfall traps installed only on the pond-side of all drift fences.

When traps were in place, I checked them daily, usually within 1 h of sunrise. All captured salamanders were placed in a small plastic container with moist paper towel for transport to and from the lab where I took measurements and marked individuals. I immediately released any other animals present in traps on the opposite side of the fence or directly into the pond at the trap location.

Salamander Capture: Linnet Lake

To capture salamanders at Linnet Lake, I installed a series of drift fences around the lake (April 29 – August 30, 2013; April 23 – July 11, 2014) to create a temporary barrier to salamander movement during breeding migrations using the same materials and methods as at Stable Pond. I also used permanent fencing already in place along both sides of the Entrance Road for this purpose. In 2013 I installed 16 30-m drift fences around Linnet Lake spaced 15 m apart and ranging between 10 m and 25 m from the lake edge. In 2014 I installed eight of the original 16 fences around Linnet Lake. Permanent fencing was composed of 718 m of curved, corrugated, plastic culvert material buried to depths varying from 0 – 10 cm and standing approximately 45 cm above ground, with the direction of curve facing away from the road. Permanent fencing was installed at an obtuse angle to the road at each culvert entrance, creating four connected, V-like formations on both sides of the road. This fencing was designed to funnel salamanders towards under-road culverts (Figure 2.1). Permanent fence length varied by tunnel (40 – 123 m) and created a semi-impermeable barrier to salamander movement along the entire length (~380 m) of the Entrance Road bordering Linnet Lake and extended beyond the first and last tunnel, 74 – 85 m to the south and 31 – 40 m to the north. I did not install drift fencing at the north end of Linnet Lake due to the presence of the paved parking lot and little vegetation to support salamander activity.

I walked along fences nightly beginning the first night a salamander was encountered during preliminary surveys (April 25 in 2013, April 17 in 2014) and caught all unmarked salamanders I encountered. I continued night searches until five consecutive nights passed with no salamander encounters. This happened in late June both years. After this occurred, I re-initiated night searches on the next rainy night and continued nightly until no salamanders were encountered (July 8 in 2013 and July 2 in 2014). I also caught salamanders opportunistically while walking in between fences on paths around Linnet Lake and on the Entrance Road. I used the same materials and methods to contain and transport captured salamanders as at Stable Pond.

Salamander Processing: Measurements and Marking:

Once captured, salamanders from both sites were held in a labeled plastic container containing moist paper towel and transported a short distance to an indoor laboratory for processing. I conducted all measurement and marking (all animals were double-marked) while individuals were anesthetized.

Juvenile and adult salamanders were anaesthetized by immersion in 1 g / L trimethane sulfonate (TMS) solution until unresponsive to prodding (typically 6 – 10 min). I then recorded weight, total length (TL, measured as the distance from tip of snout to distal end of tail), and snout-vent length (SVL, measured as the distance from tip of snout to posterior edge of vent), and determined sex and age-class (i.e. young-of-year, juvenile, or adult). Salamanders with swollen vents (able to be sexed) were considered mature adults and smaller salamanders without swollen vents (unable to be sexed) were considered juveniles. Juvenile salamanders captured at Stable Pond in July and later that had gill-remnants behind the jaw were considered YOY; no YOY were seen at Linnet Lake. If an individual was to be uniquely marked, I inserted a 12 x 2.12 mm sterile half-duplex PIT tag (Texas Instruments purchased through Oregon RFID, Portland, Oregon, USA) into the body cavity via a 3 mm incision made using a fresh #11 scalpel blade just anterior to the right hind leg and slightly toward the midline, and either clipped one toe at the second phalangeal joint on toe three of the right hind leg, or injected red (2013) or orange (2014) visual implant elastomer (VIE) (Northwest Marine Technologies, Shaw Island, Washington, USA) sub-dermally at the ventral base of the tail just posterior to the vent. I closed the PIT tag incision with Vetbond™ Tissue Adhesive (3M, St. Paul, Minnesota, USA), which I applied to the dried incision while holding the opposing edges of the incision together with forceps. During a brief period each year when I had no PIT tags, I created individual marks with VIE by making a series of three or four dots and dashes (e.g. – · –, – – –, · · ·). After marking, which usually took 1 – 3 min, I placed individuals in a slanted container and immersed them in non-chlorinated water with their heads above water until they recovered from anesthesia (typically 10 – 15 min). Once recovered (awake and responsive to prodding), individuals were placed in original containers until release near the point of capture.

Orientation Data Analysis

I used chi-squared goodness-of-fit (GOF) tests, similar to Douglas and Monroe (1981) and Jenkins et al. (2006), to test for a non-random orientation of movement for immigrating and emigrating adults (both sites) and dispersing YOY (Stable Pond only), and to test for non-random capture among aquatic traps (Stable Pond only). I used chi-squared contingency tests to examine differences in capture patterns between adult migration phases (immigration vs emigration, both sites), between life stages (adult emigration vs YOY dispersal, Stable Pond only), with distance from the water body (inner fences vs outer fences, Stable Pond only), and between years (Linnet Lake only).

At Linnet Lake, I grouped captures by fence location because I did not use terrestrial traps, and at Stable Pond I grouped captures by fence location for analysis rather than by terrestrial trap to control for variability in capture rates among traps at each fence.

I also tested the terrestrial movement path fidelity of recaptured individuals (YOY and adults separately) by calculating the difference in angular degrees between the trap of first capture (set to 0°) with the trap(s) of subsequent capture. I used trap rather than fence location because it gave finer angular resolution. If an individual was captured more than two times, I added the angular differences between subsequent capture events to get a total change in bearing as a measure of angular departure from the original movement path.

Using PIT Telemetry to Locate Salamanders Terrestrially

To find overwintering sites, I used PIT telemetry, following Kuhnz (2000) (hereafter also referred to as “scanning”), to search the terrestrial environment systematically around both study sites for PIT-tagged animals. I did this by passing a hand-made RFID scanner as close to ground surface as possible in a lateral “sweeping” motion while moving in straight-line transects. The scanner was a wand-like, portable RFID antenna used in conjunction with a tuning capacitor and HDX backpack reader purchased through Oregon RFID (Portland, Oregon, USA) set to scan five times per sec. The portable RFID antenna (also referred to as a “scanner”) consisted of a 61-cm diameter antenna loop encased in sturdy plastic tubing attached to a length of PVC tubing (length = ~2 m, width = 3.2 cm). Preliminary testing indicated a maximum open-air read range of ~75 cm using a 12 mm half duplex (HDX) PIT tag, which is comparable to the depths at which Sheppard (1977) found overwintering long-toed salamanders. For detailed performance of the scanner for various substrates, see Chapter 4.

I used markers to delineate "scanned" areas from "unscanned" areas, and effort was made to ensure some overlap between transects. Obstacles that prevented the scanner from contacting true ground surface included large logs, rocks, trees, and dense patches of shrubs. When encountered, obstacles were scanned as thoroughly as possible, but I encountered several large objects (rocks and logs) and thickets that were impossible to scan effectively due to limitations of the antenna's shape and read range (Chapter 4).

At both sites, I began scanning the terrestrial environment after peak migrations had occurred (June 4 2013 at Linnet Lake, June 9 2014 at Stable Pond). Both years, scanning was initiated near the shoreline, working outward in a series of rectangular transects during the summer season, ending on August 29 in both years.

Based on Sheppard's (1977) research, I assumed that individuals would have summer home ranges averaging around 150 m^2, and that overwintering sites would be located within or near individuals' home ranges. Due to low success detecting overwintering sites in October 2013 at Linnet Lake, I scanned at Stable Pond in in November 2014. To search for overwintering sites (October 3 – 15, 2013 at Linnet Lake and November 15 – 23, 2014 at Stable Pond), I returned to the location of the most recently detected individuals from summer scanning efforts and scanned a 30 x 30 m (900 m^2) plot oriented N-S and centered on the relocation site using the same transect-scanning methods as the summer. I chose locations to scan by working in reverse chronological order from the most recently detected individual in the summer (August 28, 2013 and August 26, 2014). I did this to increase the chance that the individual was still in relatively close proximity to its most recent detection. In addition to scanning at known previous locations, I sampled an unscanned area above (west of) the Entrance Road at Linnet Lake in 2013 and the dried bottom of Stable Pond in 2014.

Overwintering Site Characterization

When I detected a salamander during overwintering site searches, I marked its location and then returned later for processing once the 30 x 30 m plot was scanned completely. In the event that multiple salamanders were detected within the same plot, I processed overwintering sites in the order I found them.

To characterize overwintering sites both years, I measured above-ground features (dominant vegetation, light level, % cover, and number and type of above ground objects), then attempted to excavate the individual to describe the underground hibernaculum and determine if a group was present. I measured light levels as % transmittance (foot-candles) using a light meter (Model 217, General Electric, USA) , estimated percent cover (leaf litter, grass/forb, wood vegetation, small (< 10 cm diam) woody debris, large (≥ 10 cm diam) woody debris, rock, moss, and bare ground) within a 1-m diameter circular plot centered on the relocation site, and documented the number and type of above-ground objects (small (1.5 – 10 cm diam) and large (≥ 10 cm dia.) trees, wood (bark, logs, or stumps), rocks ≥ 10 cm wide, and mammal burrows) within a 2-m radius of the relocation site. After above-ground objects were characterized, I began digging gently with a spade shovel until either the individual was located with a hand-held HDX proximity reader (Oregon RFID, Portland, Oregon, USA) (maximum read range ~ 12 cm) or I determined that further digging was too destructive to repair. In such cases, I inferred the probable type of hibernaculum.

Results

Salamander Orientation

I captured a total of 660 adult long-toed salamanders at Linnet Lake (413 in 2013 and 247 in 2014), and a total of 619 migrating adult long-toed salamanders and 1460 young-of-year at Stable Pond (see Table 3.1 for captures used in the orientation analysis).

At Stable Pond, adult salamanders trapped April 17 – June 14 exhibited non-random orientation among the five fences during both immigration ($\chi^2 = 67.36$, df = 4, $p < 0.001$, inner fence only) and emigration (inner fence: $\chi^2 = 109.51$, df = 4, $p < 0.001$; outer fence: $\chi^2 = 158.50$, df = 4, $p < 0.001$). Only inner fences were used to capture immigrants because the peak immigration had occurred before outer fences were constructed. More immigrating and emigrating adult salamanders were captured arriving from the three eastern inner fences (2 – 4) than expected and more emigrating adults than expected were captured at the southern outer fence (fence 30).

The orientation pattern did not differ between the two modes of migration at inner fences ($\chi^2 = 5.85$, df = 4, $p = 0.211$). The concentration of emigrating salamanders shifted slightly to the north compared to immigrants, but movements were still focused to the east. During emigration, patterns differed between inner and outer fences ($\chi^2 = 66.05$, df = 4, $p < 0.001$) with salamander captures heavily concentrated toward the south at outer fences. I also found, by comparing minnow trap captures among 10 traps April 12 – June 14, adults in the water were not distributed evenly along the perimeter of the pond ($\chi^2 = 280.71$, df = 9, $p < 0.001$). More adults were captured along the southeastern pond edge than expected.

YOY trapped July 16 – August 22 exhibited non-uniform patterns of dispersal at both inner and outer fences (inner fence: $\chi^2 = 337.09$, df = 4, $p < 0.001$; outer fence: $\chi^2 = 82.35$, df = 4, $p < 0.001$), and patterns differed between the two fence types ($\chi^2 = 53.18$, df = 4, $p < 0.001$). At inner fences YOY captures were concentrated toward the northeast with more captured at fences 1 – 3 than expected. At outer fences, YOY captures were concentrated to the south. Patterns of YOY dispersal differed from adult emigration at both inner and outer fences, with differences being more pronounced at outer fences (inner fence: $\chi^2 = 13.26$, df = 4, $p = 0.010$; outer fence: $\chi^2 = 31.98$, df = 4, $p < 0.001$). At inner fences, YOY captures were shifted northward compared to adults, and less focused to the south at outer fences compared to adults (Figure 3.1).

At Linnet Lake, patterns of immigration and emigration did not differ (2013: $\chi^2 = 23.21$, df = 15, $p = 0.080$; 2014: $\chi^2 = 9.37$, df = 7, $p = 0.228$) with captures being similar at each fence for immigrants and emigrants, thus I pooled captures between the two modes of migration at each fence to test for uniformity of movement patterns for each year. I found that the orientation of movement differed from random each year (2013: $\chi^2 = 246.44$, df = 15, $p < 0.001$; 2014: $\chi^2 = 53.85$, df = 7, $p < 0.001$), and that orientation patterns did not change between 2013 and 2014 ($\chi^2 = 11.60$, df = 7, $p = 0.114$). In all cases, captures were concentrated at fences at the southwest corner of the lake (Figure 3.2).

Path Fidelity for Individual Movements

Recaptured YOY departed from original dispersal directions from 0° – 177° (n = 89). The angular departure values followed a lognormal distribution with a median of 30°. For initial captures at inner fences versus recaptures at outer fences, angular departure also ranged from 0° – 177° (n = 61) and followed a lognormal distribution with a median of 21°. For adults, values of angular departure also followed lognormal distributions and cumulative angular departure ranged from 0° – 164° (n = 45) with a median of 29°. For individuals first caught immigrating, then emigrating at inner fences only, angular departure ranged from 0° – 94° (n =19) with a median of 38°. When I pooled immigrant and emigrant captures of adults at inner fences and compared them with capture locations at outer fences, angular departure ranged from 0° – 164° (n =18) with a median of 29°. Adult emigrants captured at inner fences then subsequently captured at outer fences ranged in angular departure from 0° – 76° (n = 11) with a median of 10°.

PIT Telemetry Overview

PIT telemetry worked well as a method to relocate long-toed salamanders terrestrially post-migration. In 2013 I scanned ~98850 m^2 of the area around Linnet Lake (area includes paved surfaces that were not scanned) (Figure 3.3). I detected salamanders 36 times during 81 d of summer scanning (32 of 404 tagged individuals), and in October detected six salamanders and one western toad after scanning 14 30 x 30 m plots based on old locations and 12500 m^2 of new area above the Entrance Road over 13 d. In 2014 I scanned ~51450 m^2 of the area around Stable Pond (area includes paved surfaces that were not scanned) (Figure 3.3). In the summer I detected salamanders 96 times (82 of 629 tagged individuals, two of them dead) over 83 d of scanning, and in November detected seven salamanders in six locations after scanning eight 30 x 30 m plots over 9 d.

During October and November, I never detected an individual based on focused scanning centered on 30 x 30 m “last location” plots, even though I did detect other individuals there.

Overwintering Site Descriptions

In 2013, warm weather conditions in October, as well as an equipment malfunction, made it difficult to find or characterize overwintering sites. Of the six salamanders I detected in October, three were on the ground < 5 cm deep in the leaf litter. For the three other detections, I was unable to excavate the individuals to determine actual depth, refuge type, or presence of other occupants. All three were associated with (i.e. within 2 m) old rotten stumps of coniferous or deciduous trees and were at estimated depths > 25 cm. Two appeared to be in the rotten wood matrix of the roots associated with the stump and one appeared to be within a network of red squirrel (*Tamiasciurus hudsonicus*) tunnels in the slope immediately below the stump. The dominant vegetation differed for each of the three sites and was either a mix of coniferous and deciduous trees, shrubs, or grass. By October, herbaceous plants had died back and the leaves had fallen from deciduous shrubs and trees, increasing light transmittance and reducing estimated percent cover proportions for foliage. Characteristics are presented in detail in Table 3.2. Salamanders I detected in October that had been previously located (n = 3) were 15, 19, and 134 m from previous locations. Salamanders detected in October were 25 – 167 m from the edge of Linnet Lake and 83 –283 m E or 87 m W of the Entrance Road.

Of the three potentially overwintering salamanders located on October 4 – 13, 2013, none were detected on a follow up visit on October 15 after a week of warm weather reaching up to 12°. However, a different individual was detected at one of these locations on April 12 (under 10 – 20 cm snow) and April 19 (no snow), 2014, and again on November 16, 2014 after presumably leaving the site for the summer (it was not detected when the site was scanned in May).

On October 4, 2013, I also detected and excavated one of five western toads PIT-tagged at Linnet Lake from its overwintering hibernaculum, which was composed of several large (25 – 30 cm wide), flat rocks stacked beneath the ground surface with space and soil in between them. The toad was beneath at least three of these rocks 30 cm below the ground surface. The site was < 50 cm from a fallen aspen (*Populus tremuloides*) and the entry point appeared to be a hole in the ground supported by roots and rocks, perhaps created by a mammal. It was located 56 m W of the Entrance Road and 153 m from Linnet Lake. When I checked the site again on October 15, the toad was still there. I did not detect it the following spring (April 12, 2014), but it was detected crossing through one of the salamander tunnels on April 24, 2014 by RFID antennae and a wildlife camera (Chapter 2).

In 2014, I searched for overwintering sites November 15 – 23 when weather was colder (-26° – 8°) than during scanning in October 2013. There was ~12 cm of snow on the ground in the search area, so meaningful percent cover estimates were difficult to make. I was also unable to locate rocks and mammal burrows within 2 m unless they were conspicuous. Unlike at Linnet Lake, of the six overwintering sites I detected at Stable Pond, only one was associated with a stump, and I detected two PIT tagged individuals there. I was unable to excavate them to determine co-occupancy without destroying the stump, so I stopped digging before successful visual contact. These salamanders appeared to be moving through spaces within the rotten wood matrix of the stump and its roots at a depth of ~36 cm. None of the other five overwintering sites at Stable Pond had an obvious association with a specific above-ground object, but logs and/or large deciduous trees were present within 2 m of all of them. At these remaining five sites I was able to excavate the PIT tagged individual and also found a second non-tagged individual at two of them. These salamanders were 28 – 38 cm below the ground surface in rotten roots 1.5 – 3 cm in diameter (likely *Populus* spp.) and in advanced stages of decomposition. In all cases, the bark of the roots held the shape even when no wood remained within the structure, which caused the roots to resemble tunnels. One of the relocated individuals was one of 44 PIT-tagged YOY. Detailed characteristics for the six detections can be found in (Table 3.2). Only three salamanders had been detected in the summer, and their overwintering locations were 10, 20, and 168 m from previous locations. The six overwintering sites ranged from 3 – 118 m from the edge of Stable Pond's high water extent.

Discussion

Orientation: Adult Migrations and Young-of-Year Dispersal

As expected, long-toed salamanders oriented non-randomly at both study sites in Waterton Lakes National Park and the adult orientation pattern was consistent between years at Linnet Lake. These findings are consistent with informal observations for the species (Anderson 1967), and patterns seen in other eastern ambystomid species. For example, Jenkins et al. (2006) found that adult and YOY marbled salamanders (*A. opacum*) oriented non-randomly at nine breeding ponds in Massachusetts and adults oriented differently from YOY at 52% of sites. In their study, adults maintained a similar pattern of orientation from year to year at each pond. In my study, capture patterns at a fence 30 m away from the pond edge indicated that orientation shifted as adults and YOY moved away from the breeding site. At Stable Pond, I found adults immigrated then emigrated in the same direction at inner fences, but during emigration they shifted their direction from east to south at outer fences, 50 m from the pond edge. YOY oriented differently than adults at both distances but also shifted their direction southward at outer fences. Although these findings are comparable to studies of other ambystomid species, they differed from Beneski et al.'s (1986) long-toed salamander study in Idaho that found adult salamanders orienting randomly for both immigration and emigration.

Immigration vs Emigration

At Stable Pond, directionality was maintained at the individual level for immigrant and emigrant adults at inner fences. Other studies have documented individual adult ambystomids entering and exiting breeding sites from the same locations along drift fences (Shoop 1968, Shoop and Doty 1972, Semlitsch 1981, Douglas and Monroe 1981). I found that adults encountered at inner fences during immigration changed direction a median of 38° upon exiting the pond. On the ground, this is a small directional change. Traps were on average 19° apart, and following the logic of Shoop (1965), if an immigrating salamander encountered a fence 1° to the right of a trap then turned right along the fence before falling into the next trap (18° away), and upon emigration changed its bearing by 2° from its initial immigration route and turned right again upon encountering the fence, it would have appeared to deviate from its immigration bearing by 38° even though in reality it only deviated by 2°. Therefore, like Shoop (1965), I would consider an individual captured entering and exiting within a three-trap section of fence (38° angular width on average) to be entering and exiting from the same location

Emigration vs Dispersal

Individual adults (n = 11) and YOY (n = 61) that were captured leaving the pond at inner fences, then at outer fences, changed direction by a median of 10° and 21°, respectively. These results suggest that as salamanders move further from the pond, adults have a stronger directional focus in their movements than YOY (Jenkins et al. 2006). This does not reflect the trend seen at the population level from results of contingency table analyses, which showed a larger difference in orientation patterns between inner and outer fences for adults compared to YOY. The discrepancy may be driven in part by the fact that the chi-square result reflects both a change in frequency among fences (general orientation pattern) and concentration of movement at individual fences. Another factor that may have contributed to the difference between angular comparisons and contingency tests is that fences were not perfectly symmetrical around Stable Pond, which would influence angular comparisons but not contingency tests. Although the fences were laid out in an approximately symmetric manner when referenced to Stable Pond's centroid, outer fences still ranged from 18 – 25° in angular coverage with 28 – 41° separating fences. The coarseness of the fence array's angular resolution probably allowed many individuals to pass into the terrestrial environment without encountering a drift fence; salamanders could deviate between 36° and 57° from their original bearing without encountering an outer fence. This, along with the low (~4%) recapture rate of both adults and YOY at outer fences, makes the contingency tests more robust comparisons of orientation patterns for this study.

Orientation Mechanisms

The mechanisms driving non-random orientation and a difference from inner to outer fences are not known, but may be related to salamanders' perception of habitat characteristics and landscape features along movement paths (Rittenhouse and Semlitsch, 2006), or by clumped resources on the landscape (i.e. below ground refugia) (Trenham 2001, Regosin et al. 2003), and may be different for adults and YOY (Homan et al. 2008). Some evidence suggests salamanders will move along "movement corridors" or "conduits" (Shoop 1968, Gibbs 1998), and that certain habitat edges can act as either barriers or conduits to salamanders and other amphibians, varying by species. For example, in Connecticut, USA Gibbs (1998) found that pickerel frogs (*Rana palustris*) preferred to move along stream beds, marbled salamanders moved along stream beds and forest-residential edges, and eastern newts (*Notophthalmus viridescens*) moved through forest interior habitats and avoided forest edges, but forest-road edges acted as strong barriers to these and three other migrating amphibian species.

All inner fences were located in continuous-canopy deciduous forest, but three of the five outer fences were separated from inner fences by ~ 17 – 67 m of open grassland, one of these requiring animals to cross a 12-m wide paved road before encountering the fence (fence 50). Only one adult (no YOY) was captured at fence 50, but the majority of individuals at both life stages traversed through open habitat (up to 33 m) and were captured at one of these three fences (fence 30) rather than moving through continuous forest habitat connecting Stable Pond to outer fences 10 and 20. Ambystomid salamanders will readily cross open habitat during migratory movements (Anderson 1967, Shoop 1968, Ryan and Calhoun 2015) even though open habitat typically poses the largest risk of desiccation for amphibians, especially young-of-year (Semlitsch 1981, Rothermel and Semlitsch 2002). In a laboratory experiment with the long-toed salamander, Lee Yaw et al. (2015) found that salamanders chose substrates, such as moss and grass, which balanced movement efficiency with desiccation risk. Migratory movements typically occur at night, reducing desiccation and possibly predation risk, and concurrent movement of many individuals (mass movement) is often linked to precipitation, which reduces the threat of desiccation in habitats normally posing high risk of dehydration.

Amphibians may treat roads similarly to open-grass habitats. Gibbs (1998) and deMaynadier and Hunter (2000) found that roads act as strong barriers to movement. It is possible that the combination of a paved road and open habitat impeded most passage from Stable Pond to fence 50. At Linnet Lake 1.2 km away, however, a large portion of the adult population has historically migrated over a paved road bisecting the terrestrial habitat on one side of the lake, and most road-crossing at this site occurs along the segment of road with the highest continuous forest and shrub cover on both sides (Pagnucco et al. 2012, Chapter 2). At Stable Pond, the lowest inner-fence captures for both age classes occurred at the fence bordering the road (2.3% of adult captures; 3.5% YOY captures) and I only encountered one vehicle-killed adult during the breeding migrations and four juveniles (one confirmed YOY) in August. Two adults were captured across the road at fence 50 during 2014, and I received one report by Park staff of an adult salamander seen crossing the road near fence 50 (Figure 2.2) in May, 2014. It appears some adults successfully cross the road at Stable Pond and YOY will attempt to, but the road and neighboring grassland may be perceived as a barrier by the majority of adult and YOY long-toed salamanders.

Two other components that may contribute to adult orientation patterns are previous knowledge of the landscape (memory of favorable movement paths) for individuals, and differential survival within the population caused by availability of resources or threats/risks on the landscape (Regosin et al. 2003, Homan et al. 2008). In homing studies, displaced ambystomid salamanders usually attempt to return to breeding sites, olfaction being the homing mechanism with the most support (McGregor and Teska 1989), but other sensory cues, such as physical landmarks, may play a role (Sinsch 2006). Once in the terrestrial environment, survival of individuals is linked in part to the quality of resources available. Ambystomid salamanders spend most of their time underground or under cover when in the terrestrial environment. Generally, mammal burrows provide access to sub-surface foraging and refuge from predators and desiccation. Some ambystomids can actively burrow (Semlitsch 1983a), and from June –October I encountered several foraging long-toed salamanders < 5 cm deep in leaf litter, under moss, or in rotten logs and stumps (personal observation). Sub-surface refugia can be limited at the landscape level and/or confined to specific areas (Trenham 2001), and there is some evidence that individual ambystomids will compete for these resources (Smyers et al. 2002), a trait well known in strictly terrestrial forest salamanders in the genus *Plethodon* (Jeager et al. 1982). In a northern population of long-toed salamander, Sheppard (1977) found up to 14 individuals occupying the same overwintering refuge, and when conditions were hot and dry in California, Anderson (1967) also observed the long-toed salamander sharing refuges and forming tight “balls” to reduce desiccation. In northern populations, if access to overwintering refuges of sufficient depth (below the frost line) is limited at a breeding site, differential survival of dispersing YOY may consequently drive the orientation patterns seen in adults. This would explain why the two age classes often exhibit different orientation patterns, YOY being more variable. If orientation is linked to individual survival, the 10 – 12-year lifespan of the long-toed salamander (Russel et al. 1996) provides plenty of time to reinforce orientation patterns at the population level (Homan et al. 2008).

The similarity between YOY and adult orientation patterns at inner fences may be due to favorable terrestrial habitat characteristics for movement adjacent to the pond, but may also reflect favorable aquatic characteristics along the east side of the pond. While I did not survey specifically for egg masses or larvae, I captured breeding adults in the pond much more often in traps along the east shore (130 individuals) than along the west shore (10 individuals). Little is known about how larval salamanders are spatially distributed within a water body, but larval ambystomid salamanders compete with one another for food and space (Johnson et al. 2003), and large larvae cannibalize smaller larvae (Pagnucco et al. 2011, Anderson 1967). Anderson (1967) noted that once larval long-toed salamanders reached a certain size, they dispersed somewhat uniformly around the water body to avoid each other, but would grow tolerant of each other and congregate in favorable locations as they neared metamorphosis. It is reasonable to hypothesize that if aquatic resources (e.g. prey availability or shelter from predators) are better on the east side of the Stable Pond, it could create YOY emergence “hotspots” in the more favorable areas where larger larvae may be concentrated before metamorphosis. At Linnet Lake, both Fukumoto (1998) and Pagnucco (2011) observed most egg masses and larvae along the west shore of Linnet Lake, specifically where woody debris and emergent vegetation were prevalent. I captured the majority of migrating adults along the west side of the lake, especially the southwest corner, which contains a large accumulation of woody debris that would provide both egg-laying substrates for adults and shelter for larvae from predacious lake chub. Anderson (1967) observed a similar preference of oviposition on woody debris at a high altitude site in California, and preference for this cover when larvae were in smaller, more vulnerable stages of development. The spatial distribution of larval salamanders in water bodies, mechanisms driving it, and its influence on YOY emergence patterns is an area that requires further research.

Defining Overwintering Habitats

I found long-toed salamanders overwintering exclusively in refugia associated with decomposing root systems of trees. Of the nine overwintering sites found, I was only able to estimate the point of entry to below-ground refugia for the four associated with decomposing stumps. At three of the stumps, the tagged salamander was beneath the main trunk of the stump and there were one or more tunnels entering the stump at the interface of soil and wood. The origin of the tunnels under these three stumps was unknown, but they may have been made by mammals or invertebrates. The fourth stump had several tunnels directly at its base and within 2 m most likely created by red squirrels based on their size and fragments of Douglas fir cones found around them. For the three stumps I excavated, the bark around the stump and its roots held its shape while the inner wood rotted away, creating ample space for long-toed salamanders to move vertically. The roots were often more decomposed than the body of the stump, and the wood within them was soft and spongy, dry and flakey, or gone completely. The remaining overwintering salamanders were found 27 – 38 cm below the surface in isolated rotten roots from unknown above-ground sources. These sites were in aspen-dominated deciduous forest, and the bark characteristics of the rotten roots were consistent with those of aspen, although only one was immediately next to an aspen tree. I never documented more than two salamanders sharing a refuge, but this is probably because I ceased digging once the tagged individual was recovered to reduce damage to the site. These observations have little in common with Sheppard's (1977) observations for this species at a site ~250 km northwest of Waterton. Like my sites, Sheppard also found long-toed salamanders overwintering communally and in refugia associated with the roots of trees, but at his site it appeared that salamanders were able to move below ground through the loose gravel substrate rather than by using tunnels or decomposing root systems, and he found salamanders deeper. At Sheppard's sites, the trees and roots associated with overwintering sites were living spruce, and he makes no mention of mammal burrows or any other hypothesized point of entry to below-ground areas. Also, his sites were in relatively low areas of the moraine-like topography, which had high soil moisture levels. All the sites I found but one were substantially uphill from the aquatic breeding site. Interestingly, Sheppard found three juvenile salamanders overwintering with adults and I found one YOY overwintering with a 1 – 2 yr-old juvenile.

Performance of PIT Telemetry

PIT telemetry worked well as a method to detect long-toed salamanders terrestrially, but it was not without its limitations. I never detected a salamander more than 40 cm beneath the ground surface, but I cannot rule out that this maximum depth simply reflected the read range of my RFID antenna. During preliminary tests, I was able to detect a 12-mm PIT tag in open air from 50 – 75 cm away from the antenna depending on the tag's orientation, but several ambystomid species, including the long-toed salamander, can occupy depths up to 1 m below ground (Sheppard 1977, Douglas and Monroe 1981, Trenham 2001). If the majority of overwintering sites were actually deeper than the ones I detected (i.e. Sheppard 1977), then my detections and refuge characterizations would be biased toward shallower refugia.

Another problem that I documented was the loss of PIT tags. I noticed recaptured individuals at both sites would heal within 6 – 7 d after surgery, but I also recaptured nine individuals that were missing their PIT tags and had open incisions where the Vetbond had come loose (see methods). At Linnet Lake (2013) and Stable Pond (2014) I detected nine and 55 PIT tags, respectively that were not retained by salamanders, representing 2% and 9% of the total number of PIT-tagged salamanders available for detection (404 and 616) and 22% and 41% of PIT tags detected (within and without salamanders combined) for each site, respectively. Most of these were found in close proximity to the point of release and near the ground's surface, presumably because the Vetbond did not sufficiently close the site of tag insertion after surgery. I also detected and recovered the carcasses of two dead PIT tagged salamanders at Stable Pond, thus I cannot say with certainty whether mortality or surgical procedure is the more common mode of tag-loss, although I suspect surgical technique based on the proximity of lost tags to fences. Ryan et al. (2014) experienced high tag loss rates (estimated 44%) with a smaller ambystomid salamander (*A. laterale*), and also noticed most lost tags were near release points. Lab studies testing PIT tag retention in salamanders show retention up to 100% (Ousterhout and Semlitsch 2014), but one lab experiment with poor retention (50 – 80%) attributed it to unrefined surgical technique (Ott and Scott 1999). Because I thoroughly scanned the area around breeding sites in my study, I would estimate my tag loss rate being closer to the lower 2 – 9 % than the higher 22 – 41% estimate.

Conclusions

Both adult and YOY long-toed salamanders oriented non-randomly when arriving (adults) and leaving (adults and YOY) breeding sites in Waterton Lakes National Park. Orientation patterns were similar between the two age classes at Stable Pond and between years at Linnet Lake. In both cases, orientation patterns near breeding sites likely reflect a combination of suitable terrestrial habitat for movement and aquatic habitat for adult breeding and larval development. Patterns further from breeding sites likely reflect a combination of suitable terrestrial habitat for movement and the location and availability of "quality" summer and winter refugia and foraging resources. Adapting PIT telemetry to a northern system, I was able to increase our understanding of long-toed salamander overwintering ecology and add to the growing body of literature characterizing "high quality" habitat for northern amphibian populations that must avoid freezing temperatures in winter months. Knowledge of how the long-toed salamander uses the terrestrial habitat surrounding aquatic breeding sites allows managers to make more informed decisions regarding protection of movement corridors and critical overwintering sites when development threatens upland areas around known breeding locations.

Tables and Figures

Table 3.1 Salamander captures used for orientation analysis for Linnet Lake (2013 – 2014), and Stable Pond (2014). At Linnet Lake, only eight of the 16 fences from 2013 were also used in 2014. At Stable Pond, fences 1 – 5 were within 3 m of the shoreline at high water and fences 10 – 50 were 50 m from the shoreline at high water. Adult immigrants and emigrants were captured April 25 – July 8, 2013 and April 17 – July 2, 2014 at Linnet Lake, and April 17 – June 14, 2014 at Stable Pond. Young-of year were captured July 16 – August 22, 2014 at Stable Pond.

	2013		**2014**		
Fence	Immigrants	Emigrants	Immigrants	Emigrants	Young-of-Year
Linnet Lake					
1	2	2	0	3	—
2	3	4	—	—	—
3	3	3	9	4	—
4	3	1	—	—	—
5	4	2	2	1	—
6	2	6	—	—	—
7	7	42	12	10	—
8	8	9	—	—	—
9	3	4	5	1	—
10	1	2	—	—	—
11	2	1	2	2	—
12	2	0	—	—	—
13	2	3	1	1	—
14	1	3	—	—	—
15	1	1	0	2	—
16	1	1	—	—	—
Stable Pond					
1	—	—	35	82	356
2	—	—	52	114	456
3	—	—	54	78	258
4	—	—	14	34	246
5	—	—	2	9	48
10	—	—	—	14	28
20	—	—	—	17	49
30	—	—	—	70	76
40	—	—	—	1	36
50	—	—	—	1	0

Table 3.2 Detailed descriptions of overwintering sites found while scanning at Linnet Lake October 3 – 15, 2013 and Stable Pond November 15 – 23, 2014.

Site	Date	PIT tag Number	Refuge Type	Depth* cm	Dominant Vegetation	Light %	Slope %	Aspect °	Total Leaves %	Coniferous Leaves %	Deciduous Leaves %	Dead Grass %	Woody Vegetation %	Grass / Forbes %
Linnet Lake	10/04/13	90264[ǂ]	burrow / rock rubble	30	deciduous	50	9	110	30-39	0	30-39	0	20-29	1-09
	10/08/13	135912	stump	36-40	shrub	58	11	116	20-29	10-19	10-19	1-09	1-09	1-09
	10/12/13	501602 90267	stump	36-40	grass	50	4	5	70-79	0	1-9	60-69	10-19	1-09
	10/13/13	135798	stump / burrow system	61-65	coniferous deciduous	22	5	56	30-39	10-19	10-19	1-09	1-09	1-09
Stable Pond	11/20/14	501702 517467	stump	36-40	deciduous	38	6	225	1-09	0	1-09	1-09	10-19	1-09
	11/20/14	501608[□]	root	28	deciduous	38	3	243	1-09	0	1-09	1-09	10-19	1-09
	11/21/14	522687	root	28	deciduous	47	6	216	1-09	0	1-09	1-09	10-19	1-09
	11/21/14	517363[□]	root	27	deciduous	31	5	208	1-09	0	1-09	1-09	1-09	1-09
	11/21/14	501711	root	30	deciduous	46	6	216	—	—	—	—	—	—
	11/23/14	511365	root	38	deciduous	47	3	225	1-09	0	1-09	1-09	1-09	1-09

PIT Tag Number	LWD* %	SWD* %	Rock %	Bare Ground %	Moss %	Snow %	Deciduous >10 cm diam	Deciduous <10 cm diam	Coniferous >10 cm diam	Coniferous <10 cm diam	Wood	Rocks	Burrows
90264[ǂ]	1-09	20-29	0	0	0	0	0	30	0	0	7	3	4
135912	10-19	1-09	1-09	20-29	0	0	0	18	0	3	4	2	1
501602 90267	0	1-09	0	0	1-09	0	0	6	0	0	4	0	1
135798	1-09	10-19	0	10-19	10-19	0	2	6	1	0	5	0	8
501702 517467	10-19	0	0	0	0	80-79	1	26	0	0	2	0	0
501608[□]	0	0	0	0	0	80-89	5	22	0	0	1	—	—
522687	0	0	0	0	0	70-80	3	21	0	0	2	0	0
517363[□]	0	1-09	0	0	0	80-90	2	5	0	0	0	0	1
501711	—	—	—	—	—	—	2	22	0	0	0	0	—
511365	20-29	1-09	0	0	0	60-70	1	0	0	0	2	—	—

*Estimated depths give as a range; LWD (large woody debris); SWD (small woody debris) [ǂ]Western toad [□]Found with at least one other individual

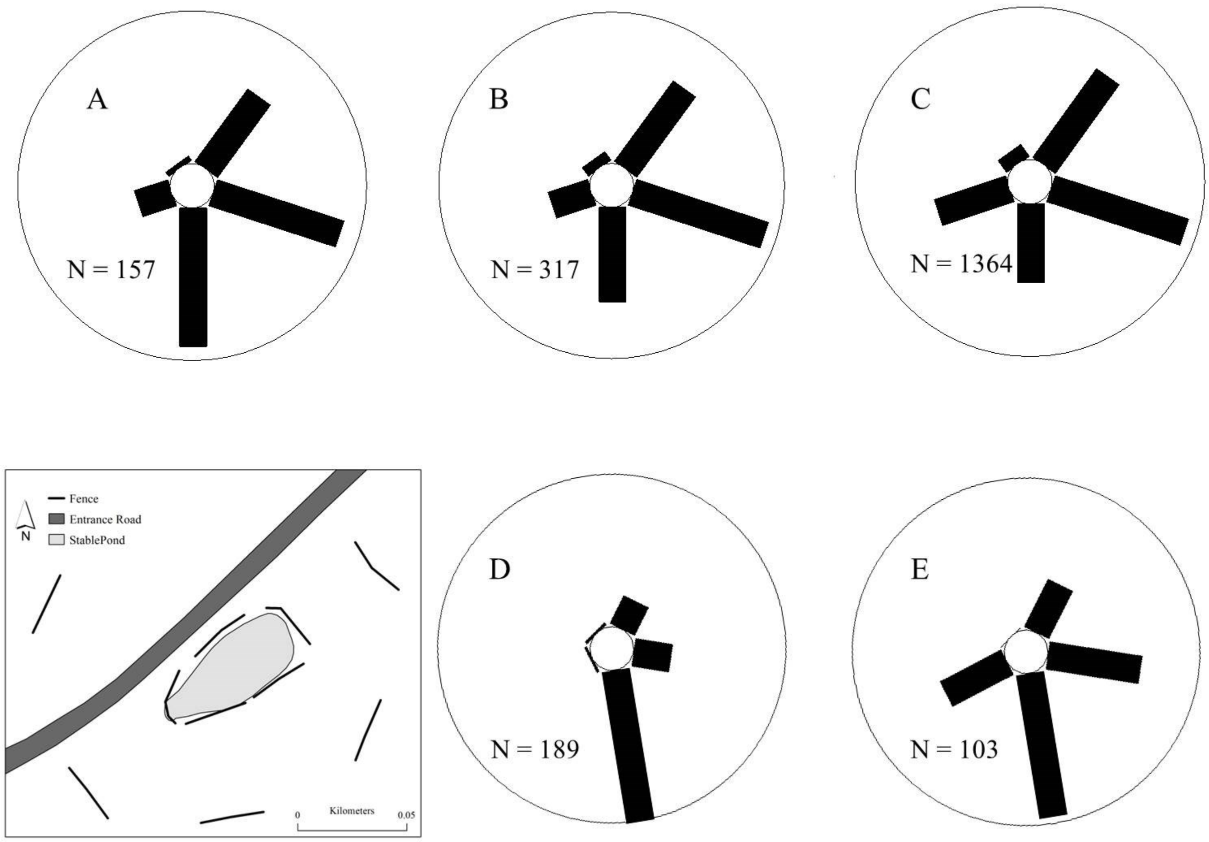

Figure 3.1 Circular histograms of pitfall trap captures at Stable Pond (bottom left) showing orientation patterns for (A) immigrating and (B) emigrating adults at inner fences, (C) dispersing young-of-year (YOY) at inner fences, and emigrating adults (D) and dispersing YOY (E) at outer fences.

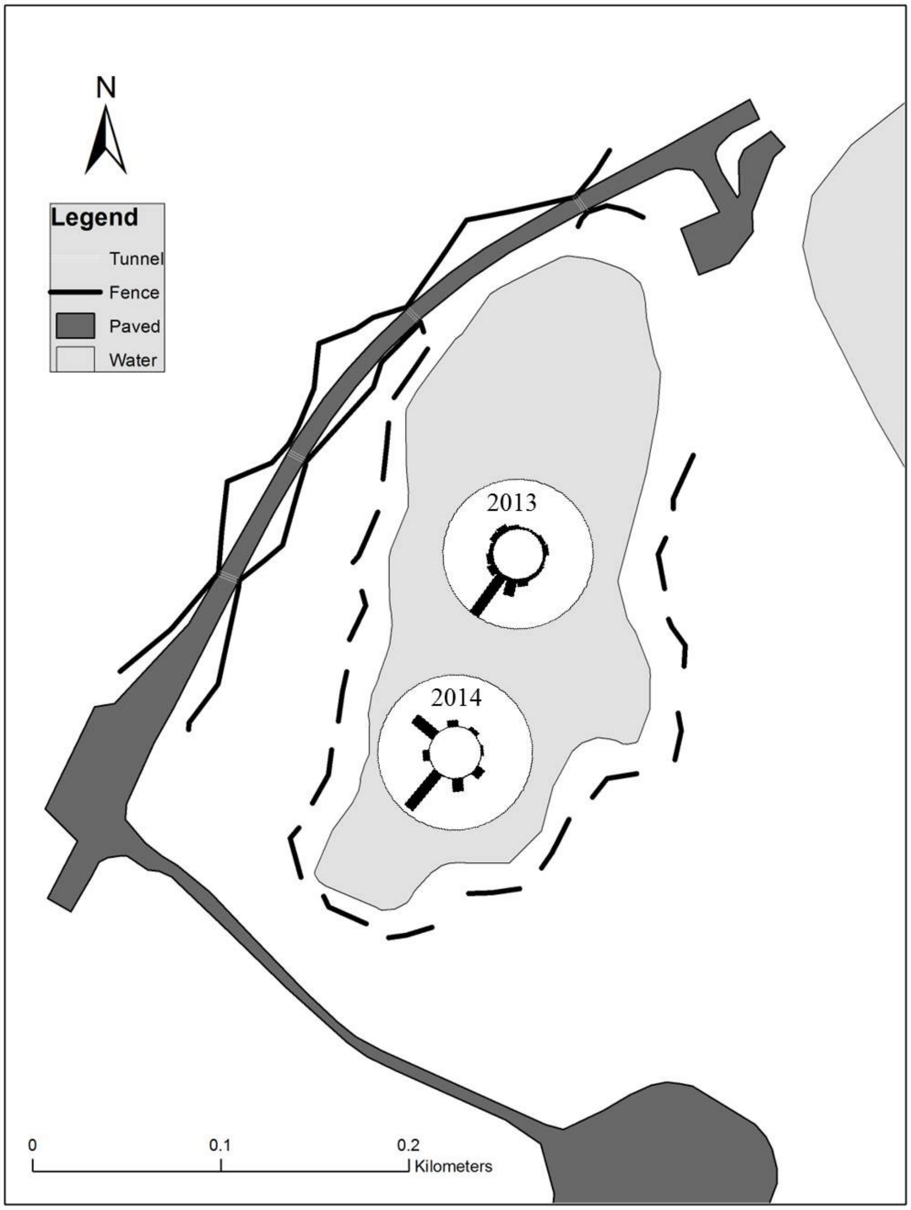

Figure 3.2 Linnet Lake with circular histograms showing hand captures by fence for 2013 (N = 129, 16 fences) and 2014 (N = 54, eight fences).

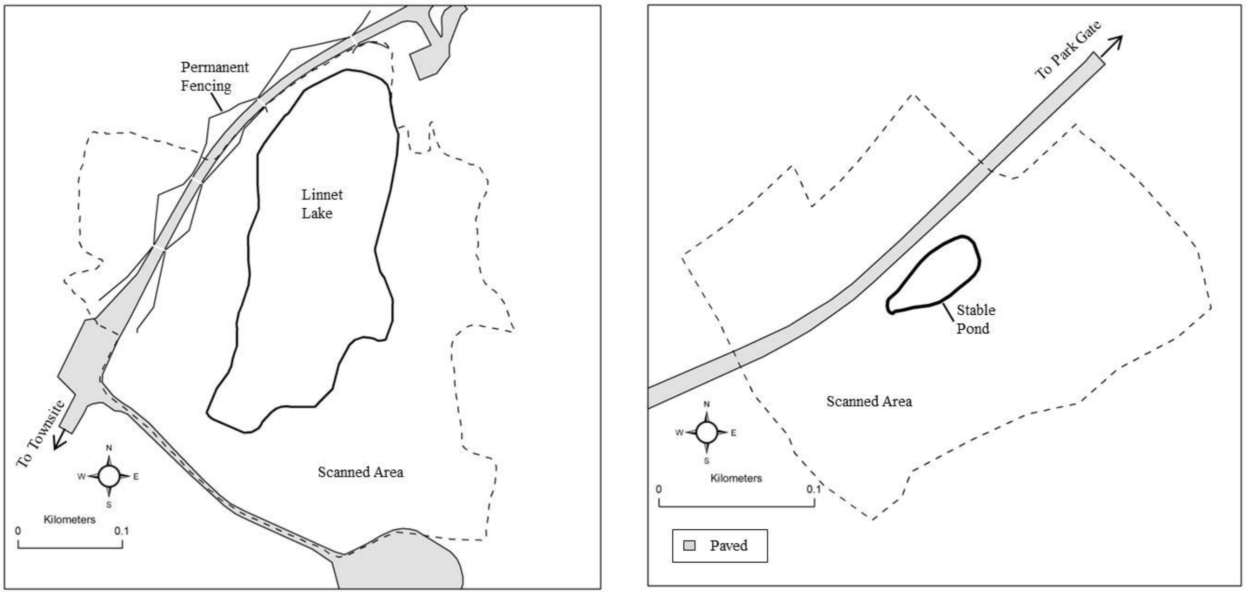

Figure 3.3 Area scanned at both Linnet Lake (98850 m^2 in 2013) and Stable Pond (51450 m^2 in 2014). Thirty two tagged individuals were detected during 81 d of scanning at Linnet Lake, and 82 individuals were detected over 83 d of scanning at Stable Pond.

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Chapter 4: Effects of Substrate on PIT Tag Detection with a Self-Made Portable RFID Antenna: Implications for PIT Telemetry

Introduction

Recent developments in radio-frequency identification (RFID) and passive integrated transponder (PIT) technologies have provided an alternative method to quantify habitat use and movement patterns of many small-bodied vertebrates including amphibians and fishes (e.g., Bubb et al. 2002; Connette and Semlitsch 2012; Cucherousset et al. 2008; Hamed 2008; Roussel et al. 2000). The ability to detect a PIT tag implanted in an animal is influenced by a variety of factors including tag type and size, its orientation relative to the antenna used, the read range of the antenna, and type and complexity of the habitat being surveyed (Hill et al. 2006; Linnansaari et al. 2007; Ousterhaut and Semlitsch 2014; Rousel et al. 2000; Ryan et al. 2014). Limitations can be exacerbated by the behavior of the study species. For example, if individuals of the species inhabit deep sub-surface refugia or physically complex above-ground habitats, they become more difficult to detect. Whereas the use of PIT tags has increased substantially over the last 5 years, uncertainties remain regarding their use and performance, including operational constraints that influence detectability (Burnett et al. 2013; Cooke et al. 2013; Cucherousset et al. 2010; Cucherousset et al. 2005; Hill et al. 2006; Ousterhout and Semlitsch 2014).

I have been evaluating the use of PIT telemetry to investigate movement patterns and fine-scale habitat use by long-toed salamanders (*Ambystoma macrodactylum*) in Waterton Lakes National Park, Alberta, Canada since 2013. The long-toed salamander is one of the smallest *Ambystoma*, and spends 10-11 months of the year in the terrestrial environment primarily below the ground surface in a variety of substrates, and like most ambystomatids is only predictably found above ground during brief seasonal migrations to and from aquatic breeding sites (Fukumoto and Herrero 1998; Pagnucco et al. 2012; Sheppard 1977). Our study area poses several challenges to the use of PIT tags because the presence of boulders, large woody debris, and dense shrubs limits opportunities to place the antenna directly at the soil surface. Additionally, terrestrial habitats are underlain by variable substrates including: medium-textured Chernozemic soils developed on stony calcareous till, gravelly coarse-textured Chernozemic soils, and weathered limestone, dolomite, and marble in small localized talus deposits. I constructed a portable RFID antenna that was capable of detecting small half-duplex PIT tags (length x width = 12 x 2.12 mm, mass = 0.1 g) up to 75 cm beneath the soil surface, but an assessment of the utility of this antenna required us to understand the effect of different substrate conditions (e.g., rock versus soil), as well as tag depth, on tag detectability.

Here I evaluate the ability of a self-made, portable RFID antenna to detect small PIT tags positioned at 5-50 cm depths in soil (medium-textured Chernozemic soils), rock (accumulations of talus) and in lake water, which represent locations where I found long-toed salamanders. I evaluate the effects of substrate type and deployment depth on the greatest horizontal and vertical distances at which tags were detected. I predicted that there would be differences in both horizontal and vertical detectability among substrate types, that horizontal and vertical detectability would decrease with increasing tag depth, and that rock would cause the greatest reduction in detection distance. Ferrous metal is the only substance listed by RFID equipment manufacturers that will cause interference with RFID antennae (Oregon RFID 2015; Biomark 2015). I expected that the reduction in detectability would be highest in a rock substrate and lowest in water due to potential differences in metal content.

Methods

I conducted trials using a hand-made, wand-like, portable radio frequency identification (RFID) antenna, a PIT tag reader (HDX backpack reader) and a 12 mm x 2.12 mm half-duplex (HDX) PIT tag purchased through Oregon RFID (Portland, Oregon, USA) (Figure 4.1). The PIT tag antenna (hereafter also referred to as a scanner) consisted of a 61-cm diameter antenna loop encased in sturdy plastic tubing attached to a length of PVC tubing (length = ~2 m, width = 3.2 cm). I tested detectability under soil and rock adjacent to Linnet Lake (49.0617°N, 113.9053°W), Waterton Lakes National Park, Alberta, Canada and under water at Astotin Lake (53.6821°N, 112.8553°W), Elk Island National Park, Alberta (Figure 4.1).

I attached a PIT tag to the end of a wooden dowel (length = 1 m, diameter = 1 cm), parallel with the dowel's long axis, with electrical tape and manipulated tag depth by inserting the dowel at five depth intervals (5 cm, 10 cm, 20 cm, 35 cm, and 50 cm) below the soil, rock, or water surface. For the soil treatment, I inserted the tag into five 1.75-cm diameter holes created with a round metal stake pounded into the soil. For the rock treatment, I constructed five rock piles of naturally occurring sedimentary and metamorphic rocks (10-50 cm diameter) to an approximate height and diameter of 75 cm and 1 m, respectively. I constructed each pile so that I could insert and manipulate the wooden dowel into its center without damaging or losing the PIT tag. For the water treatment, I chose five locations along the shoreline of Astotin Lake and conducted the five depth trials at each location. Maximum visibility was 66 cm where I ran trials.

I quantified horizontal and vertical detection distances for each depth and substrate combination by placing the PIT tag at the specified depth and then moving the antenna away (horizontally and vertically) from the dowel's point of insertion until the tag could no longer be detected. This gave us two measures of maximum detection distance for each trial. I made detection distance measurements using a tape measure (±1 cm) and measured the distance from the point of dowel insertion to the edge of the antenna loop for horizontal detection distance, and to the center of the antenna loop for vertical measurements (Figure 4.2). To obtain the vertical detection distance value used in analysis, I added the depth of the PIT tag to the antenna's distance above the substrate, i.e., the total distance between the center of the antenna and the tag. I also quantified the maximum detection depth for each substrate by inserting the tag into the substrate until it could no longer be detected while holding the antenna flat against the surface directly above the tag (Figure 4.2).

I conducted five trials for each substrate*depth combination across five locations unique to each substrate and measured both horizontal and vertical detection distances for each. I evaluated the effects of substrate type, depth, and the interaction of these terms on maximum horizontal and vertical detection distances using a two-way split-plot Analysis of Variance (ANOVA). I used a one way ANOVA to test for differences in maximum detection depths among the three substrate types. When interaction terms and main factors were statistically significant ($p \leq 0.05$) I evaluated differences among treatments and depths using pairwise tests (Tukey Honestly Significant Difference [HSD]).

Results/Discussion

I found that both substrate type and PIT tag depth affect the distance at which a tag can be detected horizontally ($F_{0.05\,[2,12]} = 4.98$, $P = 0.027$ and $F_{0.05\,[4,48]} = 2470.12$, $P < 0.001$, respectively). Substrate did not affect vertical detection distance ($F_{0.05\,[2,12]} = 0.74$, $P = 0.500$) nor did depth ($F_{0.05\,[4,48]} = 2.207$, $P = 0.082$), except for maximum detection depth among substrates.

I recorded a small (2.8-3.8 cm) but significant ($F_{0.05\ [2,12]} = 31.19$, $P < 0.001$) increase in maximum detection depth in water when compared to soil or rock.

I expected horizontal detection distance to decrease uniformly as the tag was buried incrementally deeper beneath the substrate surface. Detection distance did decrease with increasing depth, but the pattern was not a gradual reduction (Figure 4.3). I recorded only small (1.9-3.0 cm), but significant ($P < 0.001$) changes in horizontal detection distance as I increased tag depth except for a dramatic (> 25 cm) reduction in detection distance when the tag was moved from 10 to 20 cm. I also recorded a slight increase (2.7 cm) in detection distance when the tag was moved from 20 to 35 cm, which may simply reflect an asymmetry in the electromagnetic field produced by the scanner and its interaction with the tag's orientation. Horizontal detection distances at 20 cm and 50 cm depths were not different from each other ($P = 0.97$). These results suggest a threshold exists at which horizontal detection distance changes greatly, although the precise nature of this threshold may vary among individual RFID antennae.

I expected that as a PIT tag is moved deeper beneath a substrate's surface, the vertical detection distance would decrease due to increased interference from the substrate. Whereas a Levene's test for homogeneity of variance found that vertical detection distance was more variable in the rock treatment at any given depth ($F_{0.05\ [14,60]} = 2.1$, $P = 0.028$), our results did not support either of our predictions except when comparing maximum depth of detection among substrate types, where substrate did have an effect ($F_{0.05\ [2,12]} = 9.1$, $P = 0.004$) (Figure 4.4). Water had a deeper maximum detection distance when compared to rock (3.8 cm deeper, $P = 0.004$) or soil (2.8 cm deeper, $P = 0.026$), but rock and soil did not significantly differ from each other ($P = 0.542$) (Figure 4.4B).

Location of an animal with PIT telemetry requires more effort than does location with traditional radio telemetry. The antenna operator must systematically scan the habitat where target animals are expected to occur. Because a scanner can detect tags that are both adjacent and beneath its antenna loop, the operator does not need to pass the scanner over 100% of the substrate surface to detect a tagged animal. However, if an abrupt reduction in horizontal detection distance with depth, as I documented, occurs for other scanners, this implies that the depth of an animal can dramatically affect PIT tag detectability if scanning techniques are not adjusted appropriately. For example, if long-toed salamanders occupy depths < 10 cm, I would detect them if I passed the edge of our scanner within 70 cm of a previously surveyed area. If, however, long-toed salamanders typically occupy depths > 20 cm, I would need to pass the scanner ≤ 17 cm of a previously surveyed area, taking more time and effort. If scanning 100% of a study area is possible due to a lack of above ground objects to impede scanning efforts (e.g., in a grassland or shallow water body), our results indicate that there would be little reduction in detectability for moderately deep tags, even if the survey area contained multiple substrates. Researchers using PIT telemetry may, however, experience variation in detection rates among substrate types if the study organism occupies depths at the limits of the antenna's range. With our self-made antenna, I found a 3.7-5.0% reduction in maximum read range between water and rock/soil, despite negligible differences at shallower depths.

PIT telemetry is becoming a viable option for researchers to track the movements and investigate the life histories of small animals. Here I provide insight into how soil, rock, and water may affect a portable RFID antenna's performance. If an organism occupies a wide range of depths and/or substrates, or the environment it lives in is difficult to scan thoroughly due to the nature of above-ground structure (e.g., dense woody stems, boulders, or downed wood), more planning and testing will be required to use this technique effectively. In 2013 at our study site at Linnet Lake, which is quite rugged, I tagged 413 *A. macrodactylum* and ~300 hours of scanning through mid-June, July, and August 2013 resulted in 36 detections.

The suitability of PIT telemetry for a given study can be limited by the performance of commercially available portable antennae, which are available starting at $445 USD, and are currently quite limited in read range (< 29 cm maximum using 12-mm HDX PIT tags). I overcame the high cost and read range restriction of a commercial model by building our own. This scanner cost approximately $150 - $200 USD including materials and labor, and had a maximum read range of 75 cm, almost three times that of commercial models. I improved our scanner's durability as the study progressed and could repair it safely and quickly in the field avoiding delays in data collection. In conclusion, I propose that PIT telemetry offers an effective method for investigating movement patterns and habitat use for reptiles and amphibians if researchers are willing to gain a fundamental understanding of RFID technology and to assess how species specific behavior and site specific environmental heterogeneity affect tag detectability.

Figures

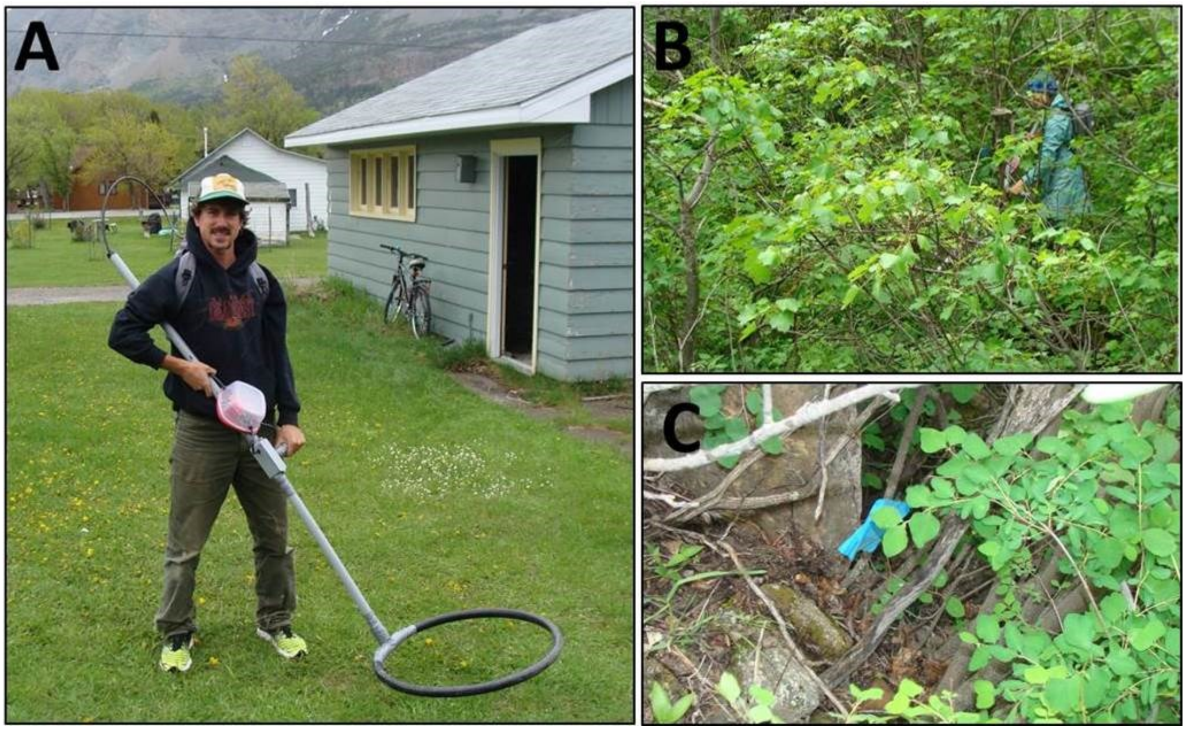

Figure 4.1 The senior author with the hand-made portable PIT tag scanner and backpack mounted reader (A) and habitat features at our study area in southern Alberta (B, C). Note the technician using the unit within dense stands of *Acer glabrum* and *Amelanchier alnifolia* at one of our study sites (B) and accumulations of rocks and woody debris on the forest floor (C). The blue flagging tape in C identifies the location of a long-toed salamander found in an accumulation of woody debris in fall 2013.

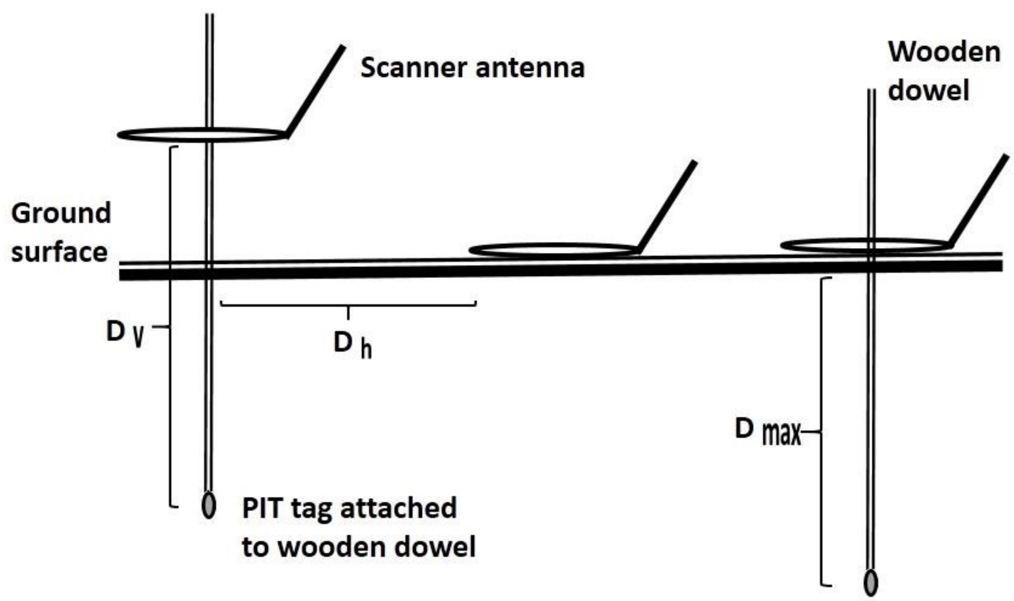

Figure 4.2 Vertical distances (D_v) and maximum detection depths (D_{max}) were measured from the center of the antenna to the PIT tag. Horizontal distances (D_h) were measured from the edge of the antenna to the point of PIT tag insertion.

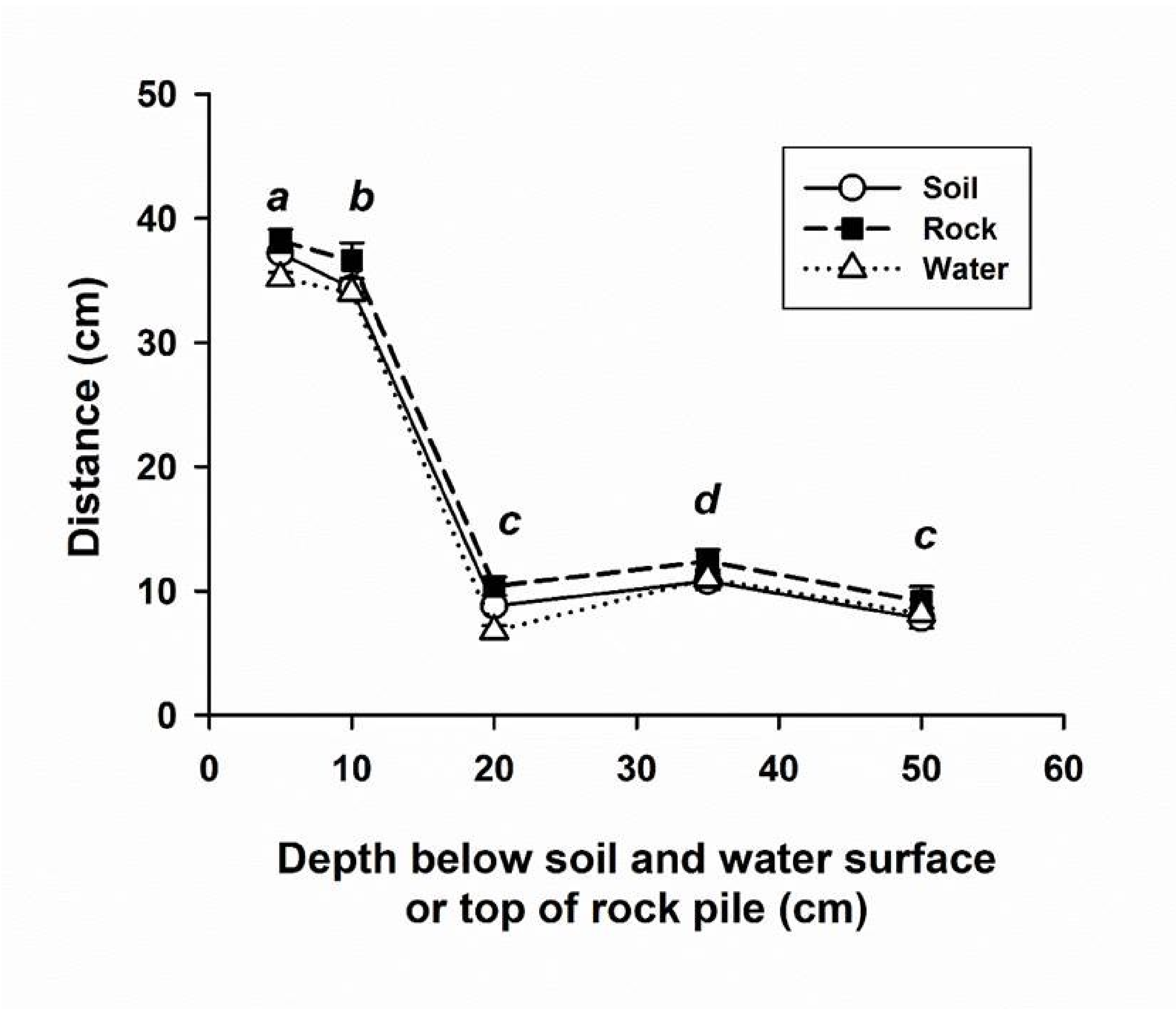

Figure 4.3 Maximum horizontal detection distance measured from the edge of the RFID antenna while resting on the substrate surface to the point of tag insertion.

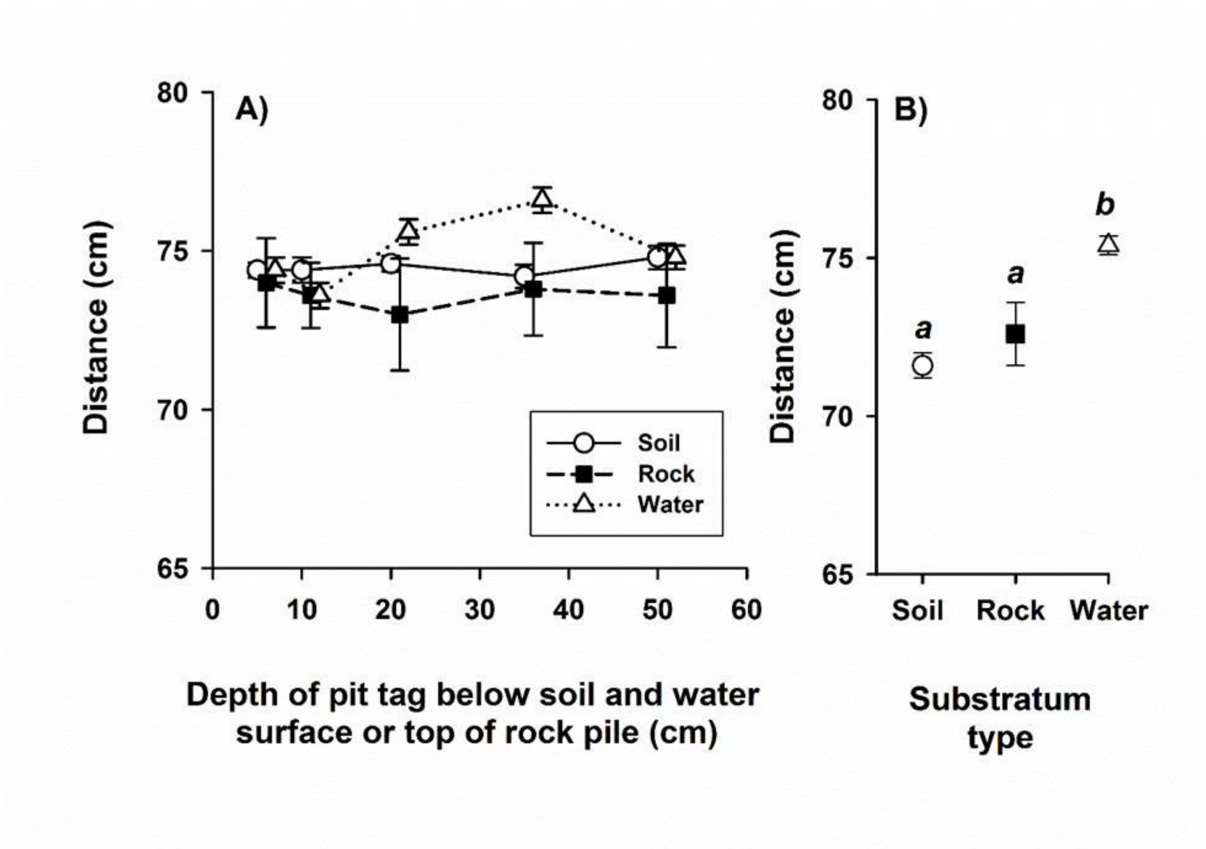

Figure 4.4 Maximum vertical detection distances measured from the center of the RFID antenna to the PIT tag inserted in each substrate at A) incremental depth and B) at the maximum depth of detection.

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Chapter 5: Summary of Findings and Management Recommendations

Summary of Main Findings

The population of long-toed salamanders (*Ambystoma macrodactylum*) at Linnet Lake has not recovered to previous (1994) levels since tunnels were installed in 2008 or fish were removed in 2008 – 2009. My estimates for the size of the breeding population in both 2013 (1380) and 2014 (706) were similar or smaller than those made by K. Pagnucco in 2008 (1492) and 2009 (1372). Comparing the size frequency distribution of adults at Linnet Lake with those at a nearby reference site (Stable Pond) gave no indication of successful recruitment in the previous 2 – 3 years, and the low number of juveniles I encountered at Linnet Lake provided further evidence for minimal recruitment. Adult long-toed salamanders at Linnet Lake were also larger on average than they were in 2008 – 2009, and were larger in 2014 than 2013, likely indicating an aging population. Aging of over 50 salamanders from Linnet Lake via skeltochronology supported this conclusion (J. Ma unpublished data). Road mortality rates have increased since 2009, the year immediately following tunnel installation, and relative rates (percentage of the breeding population) are similar to those observed by J. Fukumoto in 1994. However, migrating salamanders at Linnet Lake continue to use under-road tunnels in a pattern comparable to that observed in 2009. Cameras set to take pictures on a timed interval captured an average of 15% of PIT tagged salamanders using tunnels when compared to RFID antenna detections (33 of 215) in 2013 and 2014. This is far less than the 44% detection rate of cameras compared to pitfall traps reported by K. Pagnucco for 2009, and has implications for future monitoring of tunnel-use at this site.

Salamanders of all life stages oriented non-randomly during directed movements towards and away from breeding sites. At Stable Pond and Linnet Lake, orientation of immigrating adults near the shoreline was the same as orientation during emigration, and at Linnet Lake orientation patterns were the same in 2013 and 2014. At Stable Pond, emigrating adults oriented differently than dispersing young-of-year salamanders at fences 3-m and 50-m from the shoreline. The orientation of movements for both life-stages shifted similarly as salamanders moved further from the pond into forest, shrub, and grassland habitat, and adults were more concentrated in their movements than young-of-year.

PIT telemetry worked well as a method for detecting foraging salamanders during summer months and overwintering salamanders in below-ground refugia during the late fall with up 12 cm of snow cover. The deepest overwintering salamander detected was 38 cm below the ground surface, and all overwintering salamanders were in refugia associated with decomposing tree-root systems. Four of seven confirmed overwinter sites contained multiple individuals. Construction of a hand-made portable RFID antenna allowed me to overcome read-range limitations of commercially available portable antennas. I was able to detect 12-mm PIT tags 72 – 75 cm below the ground surface in soil, rock, and water substrates. Other than a 3-cm difference in maximum detection depth, there were no differences in the detectability of PIT tags among the three substrates tested (soil, rock, and water). Interestingly, horizontal read-range of the portable antenna declined non-linearly as tag depth increased, with a significant drop at depths > 10 cm.

Contributions to Knowledge of Long-toed Salamander Life-History

Generally, the life history of long-toed salamanders is similar to those of other ambystomid salamanders, and various aspects of their biology have been documented (Ferguson 1961, Anderson 1967, Sheppard 1977, Beneski at al. 1986, Giordano et al. 2007). However, because of their complex lifecycle and wide geographic range encompassing many diverse habitats, knowledge of the species is hardly complete. One of my research goals was to add to our understanding of long-toed salamander biology to aid in its conservation in the northeast portion of its range.

I found that comparing size structure between two sites offering different habitats and challenges was a useful diagnostic for detecting differences in recruitment (Chapter 2). I found that adult and young-of-year long-toed salamanders orient non-randomly and patterns differ between both age classes (Chapter 2, Figure 3.1). I also found grouped overwintering long-toed salamanders associated with decaying wood objects in all cases (n = 9), at moderate depths, up to 167 m from the breeding site, and usually not associated with a mammal burrows (Chapter 3, Table 3.2). It is possible that mammal burrows created the initial entry point to refugia in some cases, but I was not able to determine this.

My findings add to what is currently known about long-toed salamander ecology and highlight the variability of life-history strategies within a wide-ranging species. My results in concert with those of Sheppard (1977) can inform management decisions regarding conservation of long-toed salamanders in the Rocky Mountains by using measures of demographic composition and identifying important movement and overwintering areas on a site-by-site basis.

Ideally, these findings will encourage more work on this species throughout its range to better understand the variation in long-toed salamander life history strategies.

Contributions to RFID Techniques and Applications

For small vertebrates, specifically amphibians, tracking has historically been difficult due to size and battery life limitations of conventional radio transmitters. Small salamander species were tracked from the 1960s – 80s using radioactive tags (e.g. Madison and Shoop 1970, Shoop and Doty 1972, Sheppard 1977, Semlitsch 1981) but this technique has since been abandoned due to environmental concerns. In the intervening time, fine-scale habitat-use investigations for terrestrial salamanders were limited to studies of larger species that can physically handle surgically implanted radio transmitters (e.g. *A. trigrinum*, *A. jeffersonianum*, and *A. maculatum*). Recently, PIT telemetry has taken the functional place of radioactive tracking as a technique to relocate small salamander species in the terrestrial environment and allows investigations of fine-scale habitat-use (Cabarle et al. 2007). PIT telemetry was developed independently for both aquatic and terrestrial applications in the late 1990s (Kuhnz 2000, Roussel et al. 2000). Because the technique has not been widely applied, commercially available PIT telemetry scanners (portable RFID antennae) are not suitable for detecting animals more than ~ 30 cm below ground with small (12 mm) half-duplex (HDX) PIT tags (Hammed et al. 2008). The adaptable nature of RFID technology, however, has led several researchers to modify commercial scanners or construct their own to suit their needs in both aquatic and terrestrial systems (e.g. Cucherousset et al. 2005, Hill et al. 2006, Ryan et al. 2014). Still, open-air or below-ground detection of 12-mm HDX tags has not exceeded 36 cm with any system.

My handmade system detected 12-mm HDX PIT tags in optimal orientation at maximum depths of 72 –75 cm depending on the substrate. The deepest salamander I detected *in situ* was 38 cm below the ground surface, which exceeds the maximum read range of both commercial and modified portable RFID antennae reported in the literature. With this system I relocated 34 of 404 tagged individuals at Linnet Lake in 2013, and 87 of 629 individuals at Stable Pond in 2014 (two relocations were individuals tagged in 2013). I also located two dead salamanders, and one cluster of two “lost” PIT tags (within 8 cm of each other, 78 m and 110 m from their point of capture) that may have been passed through a salamander predator. Additionally, my study is the only one to my knowledge to monitor amphibian use of under-road crossing structures using RFID or that compares it with wildlife cameras. In 2013 and 2014, RFID in tunnels detected 152 of 643 tagged individuals at Linnet Lake; 15 detections in 2014 were individuals tagged in 2013. My study demonstrates that RFID technology can be adapted for multiple conservation applications and PIT telemetry can be used to detect moderately deep-dwelling animals in northern systems, allowing investigation of fine-scale habitat use of small terrestrial animals that inhabit moderately deep subsurface refugia.

Management Recommendations for Waterton Lakes National Park

Waterton Lakes National Park (WLNP) remains one of the only places in Canada with road-crossing structures designed specifically for amphibians. It is also one of the few cases where the system has been monitored post-installation in a rigorous fashion. The fence-tunnel system at Linnet Lake is valuable at multiple scales. First, it has the potential to protect a large portion of the breeding population of long-toed salamanders at Linnet Lake by reducing additive road mortality when fish predation limits salamander recruitment. Second, it is valuable for the conservation goals of WLNP by increasing ecological integrity via reduced road mortality for multiple taxa, and by educating visitors. And third, it provides an opportunity to employ post-mitigation monitoring of a wildlife population, which is seldom done, but critical for making informed decisions regarding how and where to install road-crossing structures under similar scenarios in the future.

Below, I provide a list of recommendations for maintaining and improving the fence-tunnel system at Linnet Lake, monitoring the Linnet Lake salamander population, and increasing public awareness (summarized in Table 5.1), which all have value as conservation strategies. These recommendations are largely based on my own observations and reflect my personal opinions, but also reflect recommendations currently proposed in recent publications on the subject of mitigating road mortality (Glista et al. 2009, Lesbarreres and Fahrig 2012, Beebee 2013, van der Grift et al. 2013) and previous studies of the Linnet Lake long-toed salamander population (Fukumoto 1995, Pagnucco 2010).

Fence-Tunnel Maintenance:

In both 2013 and 2014, I observed several places along the fence system where the fencing was no longer buried beneath the soil surface. Most often, this was due to erosion caused by runoff during spring snow melt and heavy seasonal rains. Occasionally, small mammal burrows also created gaps beneath fences. The most dramatic instance of a gap forming beneath fencing was in June 2013 when heavy rains caused the entire hillside beneath a section of fence to slump onto the road, leaving the fence passing > 1 meter above the ground surface. This gap was promptly fixed by grounds maintenance crews, but there were several other locations along the fencing which would have gone unrepaired by the Park had I not walked the length of the fence and patched gaps.

The fencing is just as important, if not more so, than the tunnels for preventing road mortalities of migrating salamanders. I recommend Parks fills in gaps along the fence at least twice per year, once after snow has sufficiently melted (late April/early May) and once after the first major rain in May. This could reduce the number of gaps along permanent fencing during the peak long-toed salamander movements and will reduce the number of salamanders that cross over the road surface.

Another area along the fence-tunnel system with problematic gaps was the junction between fences and tunnels (Figure 2.11). I observed the largest number of salamanders that crawled out of the fence-tunnel system onto the shoulder of the road at these junctions. The most effective junctions were those that used high planks of wood (2" x 8" or 2" x 12") tightly joined to the fencing with flat rubber material and back-filled with soil. The funneling nature of the directional fencing concentrates migrating salamanders at tunnel entrances. Often, salamanders will not immediately enter the tunnel, especially if there is no natural substrate along the floor of the tunnel. If an avenue exists by which they can avoid the tunnel, inevitably some salamanders will take it. This effectively creates a road mortality "hotspot" instead of a safe crossing. The section of road associated with high-use tunnels 3 and 4 incurred the highest road mortality rates second only to areas not protected by fencing. I recommend Parks reinforces the fence-tunnel junctions with tall, flat material to ensure salamanders funneled to entrances cannot bypass tunnels. Junctions should be inspected during regular fence-checks (recommended above) and fixed as needed.

The drainage system along the stretch of road containing salamander tunnels has not been maintained to function during heavy rain events. The drains are currently full of sand and silt from years of accumulation. Instead of runoff exiting the road through the intended drainage infrastructure, water running down the road enters the grill-like openings punctuating the length of salamander tunnels, effectively turning them into drains and rendering them impassable by salamanders moving against the flow of water. During the same storm that caused the hillside slump in June 2013, water flowing through tunnels washed the RFID monitoring antennas downhill into the woods and filled the camera cases mounted to entrance roofs with silt. Long-toed salamanders preferentially migrate during rain events. If tunnels are inaccessible to salamanders during heavy rains, they are prevented from moving when conditions are optimal. I recommend that Parks regularly clears the existing drainage system to reduce the amount runoff entering tunnels.

Monitoring the Linnet Lake Population:

Cameras have been employed to document salamander use of tunnels since they were installed in 2008. The cameras currently used are sufficient for this purpose if maintained, but to my knowledge, there is no plan for using the collected images to monitor the salamander population. I recommend that WLNP develop and implement a monitoring program using cameras.

K. Pagnucco demonstrated that cameras are useful, not only for determining the relative abundance of salamanders moving through tunnels, but also for collecting demographic information about the population. It has been difficult to determine the relationship between the number of salamander images collected in a season and the absolute abundance of salamanders that moved through tunnels (estimates range from 15 – 44% for the number of salamanders using tunnels that are photographed), but trends through time should reflect trends at the population level, giving an index of relative abundance. A monitoring program with goals stated *a priori* and plan designed to meet them will provide important information for determining the success of tunnel installation for protecting this population, and informing future tunnel installations not only in Canadian national parks, but elsewhere. Some aspects of the monitoring program protocol that should be held constant and determined *a priori* are length of time interval between photos, daily and seasonal monitoring period, inter-annual monitoring frequency, and how/which data will be recorded.

To create a sustainable and effective monitoring program, I recommend that cameras are maintained by performing checks of image quality before seasonal camera deployment. Four of eight cameras currently designated for tunnels were refocused to a 14” custom focal range by the manufacturer (Reconyx, Holmen, Wisconsin, USA) in 2014. The other four cameras are out of focus, which limits their use for monitoring tunnels. I also recommend that WLNP partners with local interest groups to process images. Image processing is time-consuming, but the Waterton nature festival organizers have expressed interest in assisting with fence-tunnel system maintenance and may be interested in joining in monitoring efforts. Local high schools and youth clubs, such as 4-H or Scouts Canada, are another potential source of volunteers for implementing a sustainable and rigorous monitoring program.

I demonstrated that PIT tags and RFID can also be used to monitor tunnel-use by long-toed salamanders successfully. This method is much more costly, labor-intensive, and requires more technical skill and training than using cameras. I do not think it is suitable as a yearly, long-term monitoring strategy unless the conservation of salamanders at Linnet Lake becomes a higher priority for the Park than it currently is and the appropriate resources can consistently be invested.

Road mortality surveys at Linnet Lake during breeding migrations would be useful for assessing maintenance efforts for the tunnel-fence system (see above). Conducting surveys at Linnet Lake and Stable Pond would also be informative for monitoring changes in road mortality "hotspots" over time. I recommend road mortality surveys be done for more than one year prior to any future tunnel or fence installation intended to reduce road mortality using a consistent protocol.

Increasing Public Awareness

There are many misconceptions among local residents of Waterton Lakes and the surrounding area as to the purpose and functionality of the tunnel-fence system, and even as to the existence of long-toed salamanders at Linnet Lake. Here, I offer recommendations that will boost awareness and ultimately protect more salamanders and other amphibians from road mortality.

The salamander-crossing sign at Linnet Lake was stolen in the summer 2013. As of July 2015, it has not been replaced. Signage alone has been shown to be fairly ineffective (Glista et al. 2009) for changing driver behavior and I observed many nighttime drivers exceeding the 30 km/h speed limit through the fence-tunnel area throughout the course of my study. Signs do, however, increase public awareness. Unfortunately for salamanders, they are seldom seen crossing the road due to their small size and poor visibility during times with large movements. Drivers may initially drive with caution, but quickly become habituated to signage because they never see salamanders on roads. This problem is exacerbated by the fact that Park visitors are not aware that peak migratory movements occur primarily on rainy nights for only ~ 1 mo of the year (May), and thus do not realize that cautious driving is most important during this time.

I recommend that the original signs be maintained and additional signs be placed near the visitor center for traffic passing in the opposite direction, and at Stable Pond where road mortality also occurs (Figure 5.1). Additionally, I recommend that signs be enhanced with battery or solar-powered lighting systems during the peak migration season (Chapter 2) or removed for the non-migratory season. A lighting system activated from mid-April – end of May only when rain is forecasted would likely be the most effective strategy. This will draw attention to signage when it is most useful and teach residents and returning visitors under what conditions they should be most aware of salamander road-crossing. An alternative to lighting systems is reducing the speed limit along these sections of road with temporary signs (and enforcing it) during the migration season or when rain is forecasted during that time period.

The interpretive poster in the visitor center intended to educate the public about long-toed salamanders in the Park and their conservation was inaccurate and was removed completely 2014. I recommend replacing this poster or creating some other better researched display to educate visitors about the salamander population at Linnet Lake. Additionally I recommend that the Park continues to include information about long-toed salamanders in its interpretive programs. I do not know if this is currently done on a yearly basis, but in 2013 I attended a public lecture by S. Gallagher (Park interpreter) about wetlands which included information about the Linnet Lake system.

Inevitably, some salamanders will bypass the tunnel fence-system and cross the road surface, especially along the section of road in front of the visitor center that is not protected and consequently incurs the highest salamander road mortality rates. I recommend that Parks partners with the residents of the town site, local volunteer groups, and local conservation organizations to re-implement one or more “bucket-brigade” events during which volunteers go out on one or more rainy nights during the migration season (May) and walk the length of the road bordering Linnet Lake to assist salamanders found on the road surface in crossing. This will reduce road mortalities, give the local community a sense of involvement and ownership, and will help educate locals and Parks staff.

Habitat Enhancement and Supplementation

Below I provide some brief recommendations as to how managers can improve habitat in the Linnet Lake system for long-toed salamanders and possible strategies for enhancing the population through translocation.

Fish removals in 2010 and 2011 resulted in the relocation of > 35000 fish in only 10 d of trapping, and I captured >14000 fish over 5 d of trapping in 2013. It is unknown what percentage of the fish populations in Linnet Lake these numbers represent, but indicate that a large number of fish can be removed from Linnet Lake in a short amount of time. It is unlikely that the amount of fish removed in 2010 and 2011 had the desired effect of significantly increasing salamander recruitment, but fish reduction by natural or other means has increased amphibian recruitment in other systems (e.g. Eaton et al. 2005, Knapp et al. 2007). I recommend that if WLNP wants manage fish in Linnet Lake in the future, removals should be conducted multiple times in the same year and continue until the number of fish being captured is below a pre-defined threshold.

Efforts should focus on large fish that are more likely to reproduce and predate larval salamanders and should take advantage of seasonal schooling activity if it occurs in target fish species to facilitate efficient removal. If mechanical removal does not achieve the desired effects, chemical removal of fish with a piscicide, which has been done at Linnet Lake in the past to remove sucker (*Catostomus* spp.) (Fukumoto 1995), is another option, but one with much greater negative impact on the Linnet Lake ecosystem.

Breeding habitat of salamanders in Linnet Lake could be enhanced several ways. The addition of structure (e.g. large tree branches) to the shallow water along the lake's shoreline would provide both egg-laying substrate and refuge for salamander larvae from predators. Currently, most woody debris in the lake is limited to the southwest corner. Additionally, and with significantly more effort, temporary or semi-permanent fish exclosures composed of fine mesh or other material that allows passage of small aquatic invertebrates could be used along the shoreline to protect areas with salamander eggs and larvae from fish. Fish could be removed from exclosures mechanically with minnow traps. Once larvae reach a large size (> 40 mm SVL, Pagnucco et al. 2011), exclosures could be removed (or opened if semi-permanent) to allow larvae to exit the lake upon metamorphosis. I would recommend egg-laying structure supplementation and/or using fish exclosures as two possible alternatives to whole-lake fish removal if fish removal is no longer supported by WLNP.

Finally, supplementing the Linnet Lake long-toed salamander population by translocating larvae or egg masses from nearby sites is an option for combatting the population's decline. The population at Linnet Lake likely exists as a member of a larger metapopulation that includes nearby breeding sites. The recent decline is likely caused by natural fish invasion and enhanced by road mortality, and leads me to believe that salamanders may be extirpated occasionally from Linnet Lake. Naturally, Linnet Lake may be recolonized by individuals from nearby Stable Pond (1.2 km), Lonesome Lake (1.3 km), or the WLNP wastewater ponds (0.9 – 1.1 km, unknown long-toed salamander occupancy, but the western tiger salamander (*A. mavortium*) are present). Colonization rates are not well-known for long-toed salamanders, but appear to be faster at low-elevation sites (Funk and Dunlap 1999). I would recommend translocating egg masses (or larvae) from Stable Pond or Lonesome Lake to prevent extirpation by enhancing recruitment, or speed recolonization of this site if the current decline continues.

Conclusion

These proposed conservation actions vary in the amount of effort and financial investment required, and in the strength and broadness of their effects. Maintaining the fence-tunnel system is relatively low-cost and potentially protects many other amphibian, reptile, and mammal species documented to use tunnels and/or suffer from road mortality in this location (Table 2.1, Pagnucco 2010). Monitoring the Linnet Lake long-toed salamander population using tunnel cameras requires more effort thorough the formation of a sustainable monitoring protocol and its implementation, but is valuable for determining the success of the tunnel installation, informing future efforts, and collecting rare long-term data on a fluctuating amphibian population. Managing the fish in Linnet Lake to promote salamander recruitment may be the most labor intensive, but also the most likely to have a positive impact on the salamander population at this site. Ultimately, balancing conservation goals with logistic feasibility is necessary and all efforts, regardless of the scale, can be utilized for education, whether it is for one Park visitor or the greater scientific community.

Tables and Figures

Table 5.1 Summary of management recommendations for conserving the Linnet Lake long-toed salamander population and reducing long-toed salamander road mortality at Stable Pond.

Conservation Problem	Cause	Management Action	Method	Monitoring Strategy
Road mortality*	High traffic volume and speed	Reduce speed limit	Signage	Seasonal road mortality surveys
		Increase speed limit enforcement	Night patrols	
	Public is unaware	Educate public	Interpretive programs,	
			Enhanced signage	
	Gaps in fence-tunnel system	Seasonal maintenance	Filling gaps and patching fence	Seasonal fence checks
		Enhance existing infrastructure	Reinforce fence-tunnel junctions	
		Assisted crossing	"Bucket brigade" by volunteers	Annual records
	Shifted movement patterns	Additional fencing or tunnels	-	
		Assisted crossing	"Bucket brigade" by volunteers	Annual records
Low Recruitment*	Fish predation	Monitor and remove fish	Mechanical removal	Fish trapping
			Piscicide	
			Fish exclosures	Regular checks for fish
		Enhance breeding habitat	Add structure	Regular checks for eggs/larvae
			Fish exclosures	Regular checks for fish
		Supplement salamander population	Translocate eggs or larvae from nearby site (into fish exclosure if present)	Regualar checks for eggs/larvae and fish
	Lack of breeding adults	Reduce road mortality (see above)		

* These recommendations are designed to supplement a scientific monitoring program for the long-toed salamander population at Linnet Lake using cameras in tunnels.

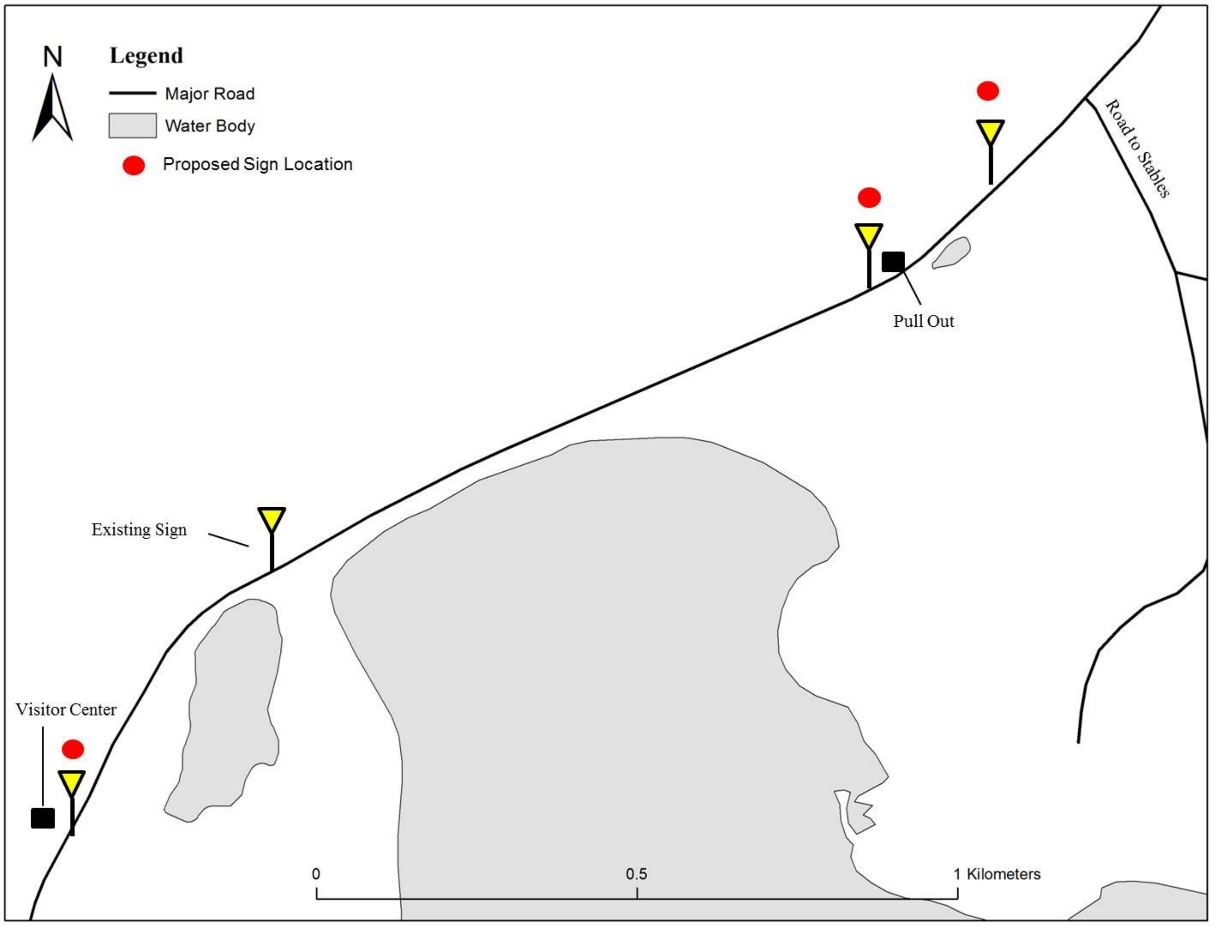

Figure 5.1 Proposed locations for three additional salamander-crossing signs to reduce road mortality and increase public awareness of road-crossing “hot spots”. Proposed locations are: in front of the visitor center, at the Stable Pond pullout, and between Stable Pond and the turnoff to the stables.

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Appendix A

Fish Removals and Sampling

In 2010 and 2011 fish (lake chub: *Couesius plumbeus* and sucker: *Catostomus commersonii* and *Ca. catostomus*) were removed from Linnet Lake using a combination of Gee minnow-traps and either a box trap or fyke nets and relocated to Middle Waterton Lake (Table A.1). In 2013 I sampled fish in Linnet Lake using the same fyke nets and different minnow traps (Chapter 2) to estimate relative abundance using mark-recapture and did not relocate fish. For all sampling sessions, data collection began the day after traps were initially set and each trap-set approximated a 24 h interval. The box trap and minnow trap dimensions used in 2010 and 2011 are unknown to me, but the fyke nets (Filmar VX-4, Montreal, Quebec, Canada) were made of 0.32-mm mesh and composed of four 76.2-cm circular hoops and one square 76.2-cm hoop for the opening spaced 76.2 cm apart . Each net had one leader 50.8 cm tall, weighted on the bottom and floated on the top, that was either 6.1 m (four nets) or 8.5 m (three nets) long. The Gee minnow-traps I used were 42 x 19 cm metal traps with 3.2-mm mesh and 2.5 cm openings.

2010

Fish were removed daily from Linnet September 28 – October 3 2010 using 58 – 62 minnow traps and one box trap. The number of minnow traps used was not recorded for day 4 and 6, and the box trap was not set on day 1 or checked on day 5 (Table A.1). Over the 6 days 6500 lake chub (1381 minnow-trapped, 5119 box-trapped) and 19403 sucker (15478 minnow trapped, 3925 box-trapped) were relocated to Middle Waterton Lake. Also, the total length of

152 lake chub (70 from minnow traps and 82 from box traps) and 162 sucker (93 from minnow traps and 69 from box traps) were measured (Figure A.1).

2011

Fish were removed daily from Linnet Lake May 9 – 13 2011 using minnow traps and fyke nets. Minnow traps (nine) were set only for the first day. Over 5 days, 9811 fish were removed (662 from minnow traps, 9149 from fyke nets) (Table A.1) and 210 were measured (100 chub and 110 sucker) (Figure A.1). During 2011 fish removals, 11 adult long-toed salamanders were also captured in fyke nets.

2013

I captured fish August 6 –10 2013 using 68 minnow traps and three to four fyke nets (Chapter 2). I marked fish with a caudal fin clip and then released them. I captured a total of 14074 fish (6550 chub and 7524 sucker, Table A.1) and measured 460 of them (210 chub and 250 sucker, Figure A.1).

Table A.1 Summary of fish capture methods and number of fish captured each year. Capture began on September 28, 2010; May 9, 2011; and August 6, 2013. 25903 fish were removed in 2010, 9811 in 2011, and 14074 unique individuals were captured in 2013.

		Fall 2010						Spring 2011					Fall 2013				
Trap Type	Sample Day →	1	2	3	4	5	6	1	2	3	4	5	1	2	3	4	5
Minnow	*# traps*	*62*	*61*	*60*	*?*	*?*	*58*	*9*					*68*	*64*	*66*	*68*	*68*
	Sucker	2116	2934	2537	2567	3090	2234	214	—	—	—	—	885	550	660	508	741
	Chub	245	348	310	198	52	228	448	—	—	—	—	1294	1129	1387	1461	1206
Fyke	*# traps*							*6*	*7*	*7*	*7*	*7*	*4*	*3*	*3*	*4*	*4*
	Sucker	—	—	—	—	—	—	380	704	760	2370	1185	2970	654	371	469	1913
	Chub	—	—	—	—	—	—	1440	305	240	1200	565	1412	228	195	412	1923
Box Trap	Sucker	—	2043	1078	546	—	258	—	—	—	—	—	—	—	—	—	—
	Chub	—	3074	565	407	—	1073	—	—	—	—	—	—	—	—	—	—

?missing data

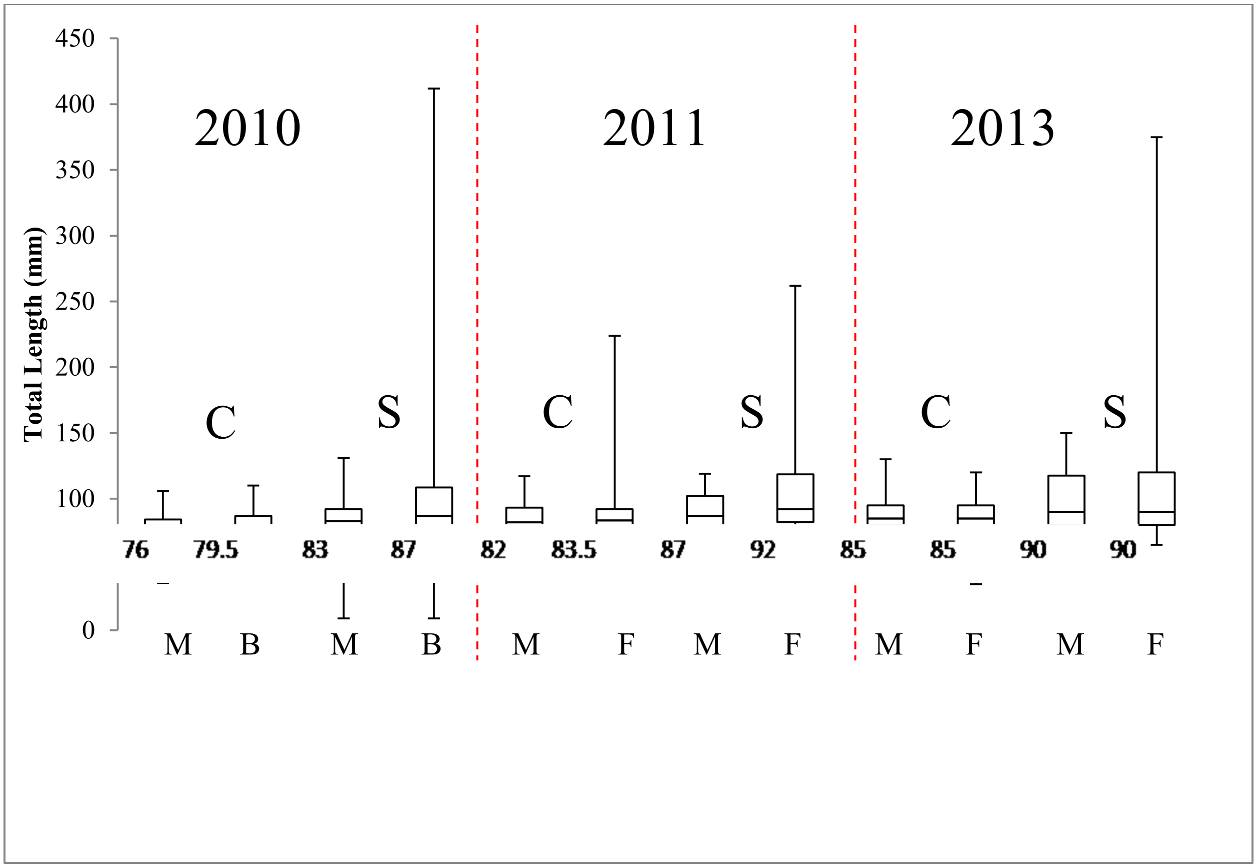

Figure A.1 Size comparison of fish captured in all years (2010, 2011, 2013), grouped by species (C) chub, (S) sucker, and by trap type (M) minnow trap, (B) box trap, (F) fyke net. Median lengths are given below and to the left of each box plot. Boxes show the median, 25th, and 75th percentile, and whiskers indicate the range. From left to right, sample sizes are (2010: 69, 81, 92, 68), (2011: 49, 49, 49, 59), and (2013: 141, 67, 116, 132).