University of Alberta

Using under-road tunnels to protect a declining population of long-toed salamanders (*Ambystoma macrodactylum*) in Waterton Lakes National Park.

by

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ABSTRACT

I investigated the value of under-road tunnels as a conservation strategy to protect a long-toed salamander population, in south-west Alberta, whose overwintering sites and breeding habitat (Linnet Lake) are separated by a road. I conducted a mark-recapture study from 2008-2009, capturing salamanders using roadside fences and pitfall traps. Four tunnels were monitored in 2009 using traps and cameras. A 2008 estimate indicated that the population declined by 60% since 1994, however, road mortality was dramatically reduced following installation of fences and tunnels. Camera and trap data documented 130 salamanders navigating tunnels in 2009. I found little evidence of juvenile recruitment from Linnet Lake, likely because of predation by lake chub. Experiments showed that lake chub consumed salamander larvae, and fish presence altered larval behaviour. Continued monitoring is needed to determine if reduced road mortality translates into population gains, and whether fish predation threatens the persistence of the long-toed salamander population.

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1.1 Introduction

1.1.1 Effects of roads on amphibian movement patterns

The negative effects of roads on wildlife are well-documented. Construction of roads reduces the amount and quality of habitat (Jaeger and Fahrig 2004). In addition, roads and associated traffic can act as barriers to animal movement, leading to reduced population connectivity, which in turn can lead to diminished gene flow and ultimately a loss of genetic diversity (Ferreras 2001).

Roads also result in animal–vehicle collisions (e.g. Ferreras et al. 1992; Taylor and Goldingay 2004; Glista et al. 2009), which represents one of humankind's most visible impacts upon wildlife. Studies have shown that a wide range of species may be affected by vehicle-caused mortalities, occasionally leading to local population extinction (Fahrig et al. 1995). Rates of road-kill can exceed natural mortality (Ferreras et al. 1992), affecting the sex structure (Gibbs and Steen 2005) and size (Fahrig et al. 1995; Huijser and Bergers 2000) of animal populations. A variety of factors can influence the species and number of individuals killed on roads, including vehicle speed, traffic volume, local topography, and accessibility of cover (Clevenger et al. 2003). A species' behavioural traits may also affect probability of mortality on roads, including foraging patterns (Bonnet et al. 1999), vagility (Carr and Fahrig 2001) and inclination to cross open habitats (deMaynadier and Hunter 2000).

Amphibians require a complex set of habitats connected by suitable corridors to satisfy their life histories. The susceptibility of amphibians to injury or death while crossing roads has been well-documented (Fahrig et al. 1995;

Trombulak and Frissel 2000; Carr and Fahrig 2001). The highest rates of mortality for amphibians occur when roads disrupt the spatial connectivity of essential resources and habitats (e.g. Ashley and Robinson 1996; Smith and Dodd 2003). Adult ambystomid salamanders, which often migrate on rainy nights in large numbers during their breeding season, are at risk if they encounter busy roads.

1.1.2 Wildlife crossing structures

One strategy used by conservationists to reduce the negative effects of roads is the installation of wildlife crossing structures, such as overpasses and tunnels, which are meant to improve road permeability to animal movements. Specific wildlife crossing structures have been designed for a variety of target species and have been incorporated into road planning and mitigation programs throughout the world (Clevenger and Waltho 2000; Goosem et al. 2001; Cain et al. 2003).

The effectiveness of wildlife crossing structures is usually evaluated by documenting their rate of use by various species, primarily through photographic records and/or detection of footprints using a suitable medium on track-plates. Relatively few studies have documented structure use by specific classes of individuals (e.g. age and sex) and the type of use (e.g. dispersal, migration), which would provide a more comprehensive assessment of effectiveness of mitigation structures and gauging their success (van der Ree et al. 2009).

The first purpose-built amphibian tunnels in North America were constructed at a site in Amherst, Massachusetts in 1987 to aid movement of the spotted salamander (*Ambystoma maculatum*; Jackson and Tyning 1989). Although amphibian tunnels have been installed under roads throughout Europe and the United States, success in terms of use by the target species has been variable (Brehm 1989; Jackson and Tyning 1989; Allaback and Laabs 2003).

1.1.3 Effects of native fish on amphibians

Along with road construction and other forms of habitat fragmentation, another factor responsible for global amphibian declines is thought to be the introduction of exotic predators, such as fish (Wake 1991). Evidence that introduced fish can lead to severe declines in amphibian populations is rapidly accumulating (Stuart et al. 2004). Fish are often the principal predators on amphibians in permanent water bodies (Petranka et al. 1987; Kats et al. 1988), and are capable of eliminating amphibian populations (Heyer et al. 1975). Fish can affect amphibians not only through predation (Leu et al. 2009), but also competition (Finlay and Vredenburg 2007) and disease transmission (Kiesecker et al. 2001). In addition to mortality from predation, fish can induce antipredator behaviour in amphibian larvae including reduced activity, shifted diel patterns, or changes in habitat use including increased refuge use (Kats et al. 1988; Lawler 1989; Resetarits 1991). These defences incur costs such as decreased growth, extended larval periods, and reduced size at metamorphosis (Resetarits 1991), which may affect fitness (Binckley and Resetarits 2002).

Although Scott and Crossman (1973) estimated that 93% of Canadian freshwater fish species are capable of consuming amphibian larvae, the vast majority of research has focused on large, piscivorous fish, such as trout, which are well-established predators of amphibians (Funk and Dunlap 1999; Pilliod and Peterson 2001; Pearson and Goater 2009). It is often assumed that small-bodied, gape-limited fish are not significant amphibian predators. However, in recent years, a few studies have shown that small-bodied, nonpiscivorous fish can also have negative effects on amphibian populations by consuming their eggs and larvae (Monello and Wright 2001; Baber and Babbitt 2003; Laurila et al. 2006; Leu et al. 2009), occasionally leading to population reductions (Kats and Ferrer 2003).

1.1.4 Study species

The long-toed salamander (*Ambystoma macrodactylum*) is found throughout North America, in a wide variety of habitats and elevations, from the Alaskan panhandle throughout British Columbia, Canada, and down the eastern slopes of the Rocky Mountains in Alberta. Within Alberta, the long-toed salamander is currently listed as a “species of special concern” (Government of Alberta 2009). In the United States, its range extends south through Washington and Oregon to northern California, and as far east as central Idaho and the eastern slopes of the Rockies in Montana.

Long-toed salamanders require both aquatic breeding habitat and terrestrial foraging and overwintering habitat to complete their life histories (Graham and Powell 1999). Adult and juvenile long-toed salamanders spend the vast majority of their time in terrestrial habitat. During the winter, long-toed salamanders overwinter in abandoned mammal burrows (Anderson 1967; Sheppard 1977). In Alberta, adults emerge from their overwintering sites and migrate to their breeding sites shortly after spring melt occurs (Ferguson 1961; Anderson 1967; Beneski et al. 1986). As with many other pond-breeding amphibians, long-toed salamanders show a high-degree of breeding site fidelity (Anderson 1967). After laying eggs on emergent vegetation, adult long-toed salamanders disperse throughout the terrestrial landscape to forage during the summer, eventually migrating back to overwintering sites in summer or fall

(Anderson 1967). Eggs hatch 5-7 weeks after being laid (Fukumoto 1995), and larvae metamorphose into young-of-the-year during late summer (Graham and Powell 1999). Young-of-the-year disperse throughout the terrestrial landscape in search of overwintering sites in the fall (Beneski et al. 1986). Adult migration and juvenile dispersal usually takes place nocturnally, and primarily when precipitation or surface moisture occurs (Fukumoto 1995).

Typical long-toed salamander breeding sites include ponds and small lakes that lack fish (Semlitsch 1987). Of the 31 wetlands where long-toed salamanders were observed in the Castle and Waterton River drainages, Alberta, in 2001-2002, 100% lacked trout and 94% lacked minnows (Pearson 2003). Under experimental settings, fathead minnows (*Pimephales promelas*) reduced larval long-toed salamander survival to the same extent as rainbow trout (*Oncorhynchus mykiss*; Pearson and Goater 2009). Although they concluded that trout reduced larval survival through consumption, Pearson and Goater (2009) assumed that some other mechanism must have caused the observed reduction in survival in mesocosms containing fathead minnows, such as interspecific competition. Although the small gape of most minnows is thought to limit them to feeding mainly on algae, zooplankton, and small insects (Scott and Crossman 1973), the ability of minnows to consume larval amphibians has not been thoroughly examined.

1.1.5 Study system

Linnet Lake (49°04’N, 113°54’W) is a small (3.5 ha), shallow (max depth = 5 m) water body situated between Middle and Upper Waterton Lakes in Waterton Lakes National Park (WLNP), Alberta, Canada. Linnet Lake was once home to a large population of long-toed salamanders that recently underwent significant declines. In 1994, this population was estimated to be 3856 adults (95%CI: 3274 – 4690; Fukumoto 1995), and occasional surveys suggested a continued decline in subsequent years (Pearson 2002). This decline was linked to mortalities of adults on the Park's Entrance Road, which separates salamanders breeding in Linnet Lake from known overwintering habitat (Figure 1.1). In May 2008, four amphibian tunnels were installed by Parks Canada under the Entrance Road in an effort to reduce road mortality of long-toed salamanders and increase connectivity between terrestrial and breeding habitats. These are the first amphibian tunnels installed in a Canadian National Park, and to my knowledge, only the second set of tunnels constructed in Canada (Gartshore et al. 2006).

Although the installation of tunnels may reduce vehicle-caused mortality, this is not the only additive source of mortality that the Linnet Lake population of long-toed salamanders currently faces. Preliminary minnow-trapping in 2008 revealed that a large number of lake chub (*Couesius plumbeus*) are also resident in Linnet Lake. This site therefore represents the rare situation where populations of long-toed salamanders and fish seemingly coexist. However, given the virtually complete allopatry between fish species and long-toed salamanders in WLNP, and the well-documented ability of many fish species to consume long-toed salamander larvae, it is possible that lake chub in Linnet Lake are also contributing to the observed long-toed salamander population decline.

1.2 Thesis goals and outline

The central goal of my study is to test the effectiveness of under-road tunnels as a conservation strategy to protect amphibian populations that have essential seasonal habitats separated by roads. My thesis uses the population of long-toed salamanders breeding at Linnet Lake in Waterton Lakes National Park as a model system to address this problem. In Chapter 2, I examine the ability of under-road tunnels to reduce road mortalities and maintain habitat connectivity for this apparently declining population of salamanders. To do this, I quantified the extent that road tunnels, and associated drift fences that direct individuals to the tunnels, reduced vehicle-caused mortality of long-toed salamanders. In addition, I derived empirically-based models predicting the probability of tunnel use by individual long-toed salamanders. To better understand temporal and spatial variation in long-toed salamander migrations, I also determined the extent that immigration and emigration were influenced by the occurrence of precipitation events, and derived empirically-based models explaining variance in the presence of long-toed salamanders based on habitat characteristics adjacent to the tunnels and Linnet Lake. In Chapter 3, I determine if cameras can be used to monitor tunnel use by long-toed salamanders and other amphibians as an alternative to direct observation or trapping. In Chapter 4, I assess the role of lake chub in the decline of the Linnet Lake long-toed salamander population by combining field observations and laboratory experiments. In the field, I used mark-recapture techniques to establish population estimates for long-toed salamander and lake chub populations at Linnet Lake. I conducted laboratory experiments to determine if lake chub had direct (consumptive) and/or indirect (non-consumptive, behavioural) effects on long-toed salamander larvae. Chapter 5 discusses the main conclusions of my study, as well as offering recommendations for the conservation of long-toed salamanders and other pond-breeding amphibians.

Even though wildlife crossing structures have been installed in North America since the 1970s, the installation of amphibian tunnels is an uncommon and expensive proposition. Thus, the opportunity to assess this technology at Waterton Lakes National Park is of broad interest. By studying patterns of tunnel use by the long-toed salamander population at Linnet Lake, I will determine factors that promote tunnel use by other amphibian species and other small vertebrates for which roads pose a threat. As the demands of forestry, energy, urbanization, and agriculture result in continued road-building throughout Canada, the need for measures to protect amphibians from road mortalities will continue to grow. Although tunnels may reduce adult long-toed salamander mortalities, if fish predation in Linnet Lake reduces survival of salamander larvae to metamorphosis, the population may still be at risk of extirpation.

1.3 Literature cited

Allaback ML, Laabs DM (2003) Effectiveness of road tunnels for the Santa Cruz long-toed salamander. 2002-2003 Transactions of the Western Section of the Wildlife Society 38/39:5-8.

Anderson JD (1967) A comparison of the life histories of coastal and montane populations of *Ambystoma macrodactylum* in California. American Midland Naturalist 77:323-355.

Ashley EP, Robinson JT (1996) Road mortality of amphibians, reptiles and other wildlife on the Long Point Causeway, Lake Erie, Ontario. Canadian Field Naturalist 110:403-412.

Baber MJ, Babbitt KJ (2003) The relative impacts of native and introduced predatory fish on a temporary wetland tadpole assemblage. Oecologia 136:289-295.

Beneski JT, Zalisko EJ, Larsen JH Jr (1986) Demography and migratory patterns of the eastern long-toed salamander, *Ambystoma macrodactylum columbianum*. Copeia 1986:398-408.

Binckley CA, Resetarits WJ Jr (2002) Reproductive decisions under threat of predation: squirrel treefrog (*Hyla squirella*) responses to banded sunfish (*Enneacanthus obesus*). Oecologia 130:157-161.

Bonnet X, Naulleau G, Shine R (1999) The dangers of leaving home: dispersal and mortality in snakes. Biological Conservation 89:39-50.

Brehm K (1989) The acceptance of 0.2 m tunnels by amphibians during their migration to the breeding site. In: Langton TES (ed) Amphibians and Roads: Proceedings of the toad tunnel conference. ACO Polymer Products, Shefford, UK, pp 29-42.

Cain AT, Tuovila VR, Hewitt DG, Tewes ME (2003) Effects of a highway and mitigation projects on bobcats in Southern Texas. Biological Conservation 114:189-197.

Carr LW, Fahrig L (2001) Effect of road traffic on two amphibian species of differing vagility. Conservation Biology 15:1071-1078.

Clevenger AP, Chruszcz B, Gunson KE (2003) Spatial patterns and factors influencing small vertebrate fauna road-kill aggregations. Biological Conservation 109:15-26.

Clevenger AP, Waltho N (2000) Factors influencing the effectiveness of wildlife underpasses in Banff National Park, Alberta, Canada. Conservation Biology 14:47-56.

deMaynadier PG, Hunter ML Jr (2000) Road effects on amphibian movements in a forested landscape. Natural Areas Journal 20:56-65.

Fahrig L, Pedlar JH, Pope SE, Taylor PD, Wegner JF (1995) Effect of road traffic on amphibian density. Biological Conservation 73:177-182.

Ferguson DE (1961) The geographic variation of *Ambysoma macrodactylum* Baird, with the description of two new subspecies. American Midland Naturalist 65:311-338.

Ferreras P (2001) Landscape structure and asymmetrical interpatch connectivity in a metapopulation of the endangered Iberian lynx. Biological Conservation 100:125-136.

Ferreras P, Aldama JJ, Beltrán JF, Delibes M (1992) Rates and causes of mortality in a fragmented population of Iberian lynx *Felis pardina* Temminck, 1824. Biological Conservation 61:197-202.

Finlay JC, Vredenburg VT (2007) Introduced trout sever trophic connections in watersheds: consequences for a declining amphibian. Ecology 88:2187-2198.

Fukumoto J (1995) Long-toed salamander (*Ambystoma macrodactylum*) ecology and management in Waterton Lakes National Park. MSc. thesis, University of Calgary, Calgary, AB.

Funk WC, Dunlap WW (1999) Colonization of high-elevation lakes by long-toed salamanders (*Ambystoma macrodactylum*) after the extinction of introduced trout populations. Canadian Journal of Zoology 77:1759-1767.

Gartshore RG, Purchase M, Rook RI, Scott L (2006) Bayview Avenue extension, Richmond Hill, Ontario, Canada habitat creation and wildlife crossings in a contentious climatic setting: a case study. In: Irwin CL, Garrett P, McDermott KP (eds) Proceedings of the 2005 International Conference on Ecology and Transportation. Center for Transportation and the Environment, North Carolina State University, Raleigh, NC, pp 55-76.

Gibbs JP, Steen DA (2005) Trends in sex ratios of turtles in the United States: implications of road mortality. Conservation Biology 19:552-556.

Glista DJ, DeVault TL, DeWoody JA (2009) A review of mitigation measures for reducing wildlife mortality on roadways. Landscape and Urban Planning 91:1-7.

Goosem M (2001) Effects of tropical rainforest roads on small mammals: inhibition of crossing movements. Wildlife Research 28:351-364.

Government of Alberta (2009) Species assessed by Alberta's Endangered Species Conservation Committee: short list. Available online: http://www.srd.alberta.ca/BioDiversityStewardship/SpeciesAtRisk/SpeciesSummaries/documents/SpeciesAssessedbyESCC-Short%20List-Dec03-2009.pdf.

Graham KL, Powell GL (1999) Status of the long-toed salamander (Ambystoma macrodactylum) in Alberta. Alberta Wildlife Status Report No. 22. Alberta Environmental Protection, Fisheries and Wildlife Management Division, and Alberta Conservation Association, Edmonton, Alberta, Canada.

Heyer WR, McDiarmid RW, Weigmann DL (1975) Tadpoles, predation and pond habitats in the tropics. Biotropica 7:100-111.

Huijser MP, Bergers PJM (2000) The effect of roads and traffic on hedgehog (*Erinaceus europaeus*) populations. Biological Conservation 95:111-116.

Jackson SD, Tyning TF (1989) Effectiveness of drift fences and tunnels for moving spotted salamanders *Ambystoma maculatum* under roads. In: Langton TES (ed) Amphibians and Roads: Proceedings of the toad tunnel conference. ACO Polymer Products, Shefford, UK, pp 93-99.

Jaeger JA, Fahrig L (2004) Effects of road fencing on population persistence. Conservation Biology 18:1651-1657.

Kats LB, Ferrer RP (2003) Alien predators and amphibian declines: review of two decades of science and the transition to conservation. Diversity and Distributions 9:99-110.

Kats LB, Petranka JW, Sih A (1988) Antipredator defences and the persistence of amphibian larvae with fishes. Ecology 69:1865-1870.

Kiesecker JM, Blaustein AR, Miller CL (2001) The transfer of a pathogen from fish to amphibians. Conservation Biology 15:1064-1070.

Laurila A, Pakkasmaa S, Merilä J (2006) Population divergence in growth rate and antipredator defences in *Rana arvalis*. Oecologia 147:585-595.

Lawler SP (1989) Behavioural responses to predators and predation risk in four species of larval anurans. Animal Behaviour 38:1039-1047.

Leu T, Lüscher B, Zumbach S, Schmidt BR (2009) Small fish (*Leucaspius delineates*) that are often released into garden ponds and amphibian breeding sites prey on eggs and tadpoles of the common frog (*Rana temporaria*). Amphibia-Reptilia 30:290-293.

Monello RJ, Wright RG (2001) Predation by goldfish (*Carassius auratus*) on eggs and larvae of the eastern long-toed salamander (*Ambystoma macrodactylum columbianum*). Journal of Herpetology 35:350-353.

Pearson KJ (2002) Linnet Lake long-toed salamander (*Ambystoma macrodactylum*) roadkill prevention and population estimation project summary report, Waterton Lakes National Park, 2001. Unpublished technical report. Parks Canada Agency, Waterton Park, AB.

Pearson KJ (2003) Distribution and habitat associations of the long-toed salamanders (*Ambystoma macrodactylum*) in the Oldman River Drainage.

Alberta Sustainable Resource Development, Fish and Wildlife Division, Alberta Species at Risk Report No. 75, Edmonton, AB, 25 pp.

Pearson KJ, Goater CP (2009) Effects of predaceous and nonpredaceous introduced fish on the survival, growth, and antipredation behaviours of long-toed salamanders. Canadian Journal of Zoology 87:948-955.

Petranka JW, Kats LB, Sih A (1987) Predator-prey interactions among fish and larval amphibians: use of chemical cues to detect predatory fish. Animal Behaviour 35:420-425.

Pilliod DS, Peterson CR (2001) Local and landscape effects of introduced trout on amphibians in historically fishless watersheds. Ecosystems 4:322-333.

van der Ree R, Heinze D, McCarthy M, Mansergh I (2009) Wildlife tunnel enhances population viability. Ecology and Society 14:7-16.

Resetarits WJ Jr (1991) Ecological interactions among predators in experimental stream communities. Ecology 71:1782-1793.

Scott WB, Crossman EJ (1973) Freshwater fishes of Canada. Bulletin of the Fisheries Research Board of Canada No. 184.

Semlitsch RD (1987) Interactions between fish and salamander larvae. Costs of predator avoidance or competition? Oecologia 72:481-486.

Sheppard RF (1977) The ecology and home range movements of *Ambystoma macrodactylum krausei* (Amphibia: Urodela). MSc. thesis, University of Calgary, Calgary, AB.

Smith LL, Dodd CK Jr (2003) Wildlife mortality on U.S. highway 441 across Paynes Prairie, Alachua County, Florida. Florida Scientist 66:128-140.

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW (2004) Status and trends of amphibian declines and extinctions worldwide. Science 306:1783-1786.

Taylor BD, Goldingay RL (2004) Wildlife road-kills on three major roads in north-eastern New South Wales. Wildlife Research 31:83-91.

Trombulak SC, Frissell CA (2000) Review of ecological effects of roads on terrestrial and aquatic communities. Conservation Biology 14:18-30.

Wake DB (1991) Declining amphibian populations. Science 253:860.

Figure 1.1. Aerial photo of Linnet Lake and surrounding habitat in Waterton Lakes National Park, Alberta, Canada. The Park Entrance Road and known long-toed salamander overwintering habitat are indicated.

CHAPTER 2: CHARACTERIZING THE SPATIO-TEMPORAL USE OF TUNNELS AND MOVEMENT PATTERNS OF LONG-TOED SALAMANDERS IN WATERTON LAKES NATIONAL PARK

2.1 Introduction

Construction of roads can drastically change natural landscapes by creating barriers to animal movement and consequently reducing population connectivity (Ascensão and Mira 2007). Roads also promote animal–vehicle collisions, which is the leading human-induced source of direct mortality in terrestrial vertebrates (Forman and Alexander 1998) and the greatest non-natural source of vertebrate death in protected areas, such as parks and reserves (Bernardino and Dalrymple 1992; Kline and Svann 1998).

Temperate pond-breeding amphibians may be especially vulnerable to road mortality because their life histories often involve mass movements between aquatic breeding habitat and terrestrial habitats. They are inconspicuous and slow-moving, which increases their susceptibility and exposure to vehicles. For example, Clevenger et al. (2001) documented the movement of tiger salamanders (*Ambystoma mavortium*) across the Trans-Canada highway in Kananaskis, Alberta, and found 180 road-killed individuals along a 1 km stretch of road over a 5 day migration period.

One conservation strategy used to minimize the negative effects of roads on wildlife is the installation of wildlife crossing structures, such as overpasses and tunnels, which are designed to improve road permeability to animal movements. The inclusion of wildlife crossing structures in the design and construction of roads is becoming increasingly common around. However, relatively few studies have examined the efficacy of these structures, and for those that have, success has proven to be variable (Brehm 1989; Jackson and Tyning 1989; Allaback and Laabs 2003).

Linnet Lake, located in Waterton Lakes National Park (WLNP), is the breeding site of a population of long-toed salamanders (*Ambystoma macrodactylum*) that has decreased over the past decade. I documented a 60% decline in the estimated number of breeding adults in 2008 (mean =1492, 95%CI=1243–1865; see Chapter 4) compared to a population estimate in 1994 (Fukumoto 1995). Parks biologists attributed this decline to road mortalities occurring on the Entrance Road, which separates long-toed salamanders breeding in Linnet Lake from terrestrial overwintering habitat to the west of the road (Pearson 2002). In May 2008, Parks Canada installed 4 concrete amphibian tunnels under the Entrance Road adjacent to Linnet Lake to reduce vehicle-caused mortalities of migrating long-toed salamanders and improve habitat connectivity. These are the first amphibian tunnels installed in a Canadian National Park, and to my knowledge, only the second set of tunnels constructed in Canada (Gartshore et al. 2006).

My primary objectives were to: i) quantify the extent that road tunnels, and associated drift fences, reduce vehicle-caused mortality of long-toed salamanders, ii) derive empirically-based models predicting the probability of use of tunnels by individual long-toed salamanders, and iii) determine the extent to which immigration and emigration are influenced by the occurrence of precipitation events. Preliminary analyses showed appreciable variance in the use of the four tunnels by long-toed salamanders that likely reflected variance in habitat characteristics of areas adjacent to each tunnel. Thus, a secondary objective was to derive empirically-based models explaining variance in the presence of long-toed salamanders along migration corridors based on habitat characteristics. This included defining linkages between habitat use during the main periods of immigration from overwintering areas to Linnet Lake in the early spring, and from Linnet Lake to overwintering areas during the late spring and summer.

I predicted that the installation of road tunnels would result in reduced levels of vehicle-caused mortality in 2008 and 2009 compared to that observed in 1994 prior to the establishment of tunnels and arrays of directional fencing. I predicted that larger salamanders would be more likely to use tunnels than smaller salamanders. Since terrestrial salamanders are vulnerable to desiccation (Feder 1983) and resistance to desiccation is positively-correlated with size (Ray 1958), larger salamanders should be more tolerant of moving along a cement tunnel, which represents a 12 m stretch of dry, unvegetated habitat. I predicted that capture rates of salamanders would be strongly and positively related to precipitation events, which is true for other ambystomids, such as small-mouthed (*Ambystoma texanum*) and eastern tiger salamanders (*A. tigrinum tigrinum*; Williams et al. 2009). Given that previous studies on habitat selection of other ambystomid salamanders have identified soil moisture and canopy cover as important habitat variables for salamander use (deMaynadier and Hunter 1998; Jenkins 2006), I predicted that salamander capture rates would be relatively higher in habitats with high soil moisture and extensive tree canopy. Determining how habitat variables and precipitation contribute to the spatial and temporal variation in salamander movement patterns will inform Parks staff when and where most long-toed salamanders use tunnels, which will allow optimization of monitoring methods.

2.2 Methods

2.2.1 Study site

Field work was conducted at Linnet Lake (49°04’N, 113°54’W) in Waterton Lakes National Park. Linnet Lake is a small (3.5 ha), shallow (max depth = 5 m) water body situated between Middle and Upper Waterton Lakes. Aspen (*Populus tremuloides*) and various conifer species, including lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*), dominate the forest that immediately surrounds the lake. However, portions of the slope to the west of Linnet Lake are grass-covered and exposed. Coarse woody debris (CWD; e.g. fallen branches and logs), which can be used by salamanders for cover, was commonly found throughout areas around the majority of the shoreline and to the west of Linnet Lake.

Four amphibian tunnels were installed under the Entrance Road that runs parallel to Linnet Lake from 26 May – 4 June 2008 (Smith et al. 2010). Each tunnel is a “box culvert” 60 cm wide by 52 cm high (AT500 Amphibian Tunnels, ACO Technologies, Shefford, UK). Each concrete section has slots along the top that allow air, moisture, and light into the tunnel. Tunnel segments were placed together to span the width of the road and the sidewalk which runs along the side of the road that abuts Linnet Lake. Tunnels were approximately 12 m in length and were placed 80-110 m apart (Figure 2.1). The minimum distance that salamanders travelled between the upland habitat on the west side of the road and Linnet Lake was 30 m.

2.2.2 Monitoring road mortality

I conducted visual surveys from 14 April – 14 October 2008 and from 2 May – 20 June 2009 daily before dawn to document road mortality of amphibians. During surveys, I walked 500 m along the Entrance Road, the length that parallels Linnet Lake, and identified each amphibian encountered, alive or dead. Annual road mortality rates were calculated as the number of long-toed salamanders found killed along the Entrance Road divided by the total number of salamanders captured either on the road or in roadside traps.

2.2.3 Monitoring long-toed salamander movement and tunnel use

To collect data on long-toed salamander movements, I designed a drift fence and pitfall trap array system to intercept animals immigrating to Linnet Lake from terrestrial habitats and emigrating from Linnet Lake to terrestrial habitats. In April 2008, I installed drift fencing on both sides of a 500 m section of the Entrance Road that parallels Linnet Lake.   Since the tunnels were not installed until after the spring migration period, I could not install directional fencing in 2008, and consequently fences were oriented parallel to the road. Fences consisted of 1 m high silt fencing buried 15 cm to prevent salamanders from digging beneath. I installed 44 pitfall traps along the fences, 24 along the west and 20 along the east side of the road. Pitfall traps were 8 L plastic buckets (25 cm in diameter, 25 cm in height) buried flush with the soil surface, and were checked daily between 0600-1000 h from 14 April - 14 October. Trapping during September and October was carried out in an effort to capture young-of-the-year long-toed salamanders dispersing from Linnet Lake into upland habitat. I did not capture any young-of-the-year, only 1 juvenile, and 31 adult long-toed salamanders from 1 September – 14 October 2008. Because of the low number of salamanders captured during this time period, I decided not to monitor the fall migration in 2009.

In an effort to maximize tunnel use by long-toed salamanders in 2009, traps were removed and I installed directional fencing angled towards tunnel entrances (Figure 2.1). The total lengths of drift fences leading to each tunnel were 132.7 m (Tunnel 1), 159.2 m (Tunnel 2), 222.4 m (Tunnel 3), and 274.0 m (Tunnel 4). I collected salamanders during nightly searches from 3 May – 16 June by patrolling the fences, beginning at 2200 h each night and lasting anywhere from 30 min to 4 h. During night searches, I would walk along each fence once, measuring and marking every salamander I found as detailed below, as well as marking the location with the individual's identification number so I could determine initial distance from the nearest tunnel entrance.

In order to monitor tunnel use by long-toed salamanders in 2009, I installed a rectangular pitfall trap (76 cm in length, 20 cm in width, 18 cm in depth) at the "exit" of each tunnel based on the dominant direction of salamander movement. From 22 April – 19 May, exit traps were located on the east side of the road to catch individuals immigrating to Linnet Lake to breed. Traps were moved to the west side of the road to catch salamanders emigrating from 20 May – 19 August. Traps were checked daily between 0600-1000 h, and captured long-toed salamanders were measured and marked as detailed below, then released 2 m upland under cover. Captures from exit traps were used to calculate "fence/tunnel efficiency", defined as the percentage of animals encountering the fence that eventually passed through the tunnels (Jackson and Tyning 1989) and were captured in exit pitfall traps.

In both years, I determined the age-class, sex, snout-vent-length (SVL; mm), total length (TL; mm) and mass (g ± 0.1 g) of each captured salamander. I used Visible Implant Elastomer (VIE; Northwest Marine, Shaw, WA) to mark every captured salamander. In 2009, each long-toed salamander was given a unique individual mark, whereas in 2008, due to the large number of salamanders being caught simultaneously, all salamanders caught after 8 May were batch-marked with a unique mark for each capture date. I also recorded the trap number and Julian date of capture for each salamander prior to releasing it on the opposite side of the road, in the direction that it was headed.

In 2009, I installed a Reconyx™ PC85 wildlife camera at each tunnel entrance on both sides of the road (8 cameras) as a secondary method of monitoring tunnel use. Cameras captured images from 22 April – 24 August 2009. Cameras were set to take 3 images at 1 second intervals every time movement was detected, regardless of the time-of-day (motion-triggered images). Cameras were also programmed to take 1 image every minute from 2100 to 0600 h every day (timed-interval images).

Precipitation data used in this study were recorded by the Waterton Park Gate weather station (situated 7 km from Linnet Lake), which recorded daily precipitation totals.

2.2.4 Habitat characteristics

I measured five categories of habitat variables at all pitfall trap locations in 2008: slope (°), canopy cover (%), soil moisture content, leaf litter depth (mm) and ground cover characteristics. Slope was measured using a clinometer. At each trap, the observer took 4 estimates of canopy cover using a densiometer by facing each of the 4 cardinal directions, and averaged them. Due to disturbance caused by installing pitfall traps and fences, measurements of soil moisture, leaf litter depth and ground cover were taken 2 m from the centre of each pitfall trap in the direction perpendicular from the fence. I measured soil moisture content using a Kelway soil pH and moisture meter. I estimated ground cover by placing one 1 m^2 plot at each trap location and visually estimated the percent cover of shrubs, grasses and forbs, leaf litter, coarse woody debris (CWD) and bare soil in each plot (Jenkins 2006). Shrubs were distinguished from trees by having multiple stems arising from the base and by being shorter, usually less than 8 m. Coarse woody debris is dead woody material in various stages of decomposition, including fresh and rotting logs, snags, stumps and branches (Harmon and Sexton 1996). In 2008, I measured habitat characteristics for roadside pitfall trap locations on 15 May. Because pitfall traps were absent along road fences in 2009, I measured the same habitat variables at locations every 15 m along each road fence between 22 – 30 May. I used habitat measurements from 2008 to model attributes of immigration/emigration movement corridors, whereas I used 2009 measurements to analyze habitat attributes associated with tunnels.

In order to reduce the number of variables used in analyses, I ran Pearson correlation tests to determine if any of the habitat variables were correlated with one another. When 2 variables had a correlation of ≥0.7, the variable with the largest *F*-statistic in a univariate regression was retained (Jenkins et al. 2006). There was a strong negative correlation between shrub cover and 3 other ground cover measurements (grasses and forbs, leaf litter, and bare soil). Shrub cover had the highest *F*-value and was therefore used in analyses.

2.2.5 Statistical analyses

I used a chi-square test to determine if the proportion of long-toed salamanders captured in exit traps differed between the four tunnels. I used Akaike’s Information Criteria (AIC) and the information-theoretic approach (Burnham and Anderson 1998) to identify hypotheses important in determining tunnel use by individual long-toed salamanders. Models included various combinations of the following parameters: SVL, sex, distance (from nearest tunnel entrance), direction (immigrating or emigrating), as well as the interaction between distance and direction. Based on *a priori* predictions, I evaluated 10 potential models using logistic regressions. Models with $\Delta\ i < 2$ were considered supported, and Akaike weights (*w*) were used to rank variable importance and adjust coefficients and odds ratios (Burnham and Anderson 1998).

I identified peak adult immigration and emigration periods in 2008 and 2009 based on the number of days between the 5th and 95th percentile of total captures (Paton and Crouch 2002). Julian date of capture was converted to day of capture, with the first day of capture being coded as 1 within each breeding year (4 May 2008, 3 May 2009). I tested for differences in long-toed salamander capture rates (captures/d) between migration directions (immigration and emigration) and among years (1994, 2008 and 2009) on days with or without precipitation, as well as the interactions between these factors, with the G-test of independence (Sokal and Rohlf 1981).

I again used an AIC approach to evaluate the multiple regression models and select the most parsimonious model of habitat use during immigration and emigration. Models included combinations of the following parameters: slope (°), leaf litter depth (mm), soil moisture content, canopy cover (%), and ground cover by shrub (%), and CWD (%). Data on long-toed salamander captures, canopy cover, leaf litter depth, and soil moisture were natural log transformed to meet with the assumptions of normality. Based on *a priori* predictions, I evaluated 11 potential models for each movement direction. Again, models with $\Delta\ i < 2$ were considered supported, and Akaike weights (*w*) were used to rank variable importance and adjust coefficients (Burnham and Anderson 1998).

I used analyses of variance (ANOVAs) to determine how habitat characteristics (leaf litter depth, canopy cover, and soil moisture) differed between the 4 tunnels, using fence section habitat data that was collected in 2009. Multiple linear regressions were conducted to examine the relationship between habitat measurements (independent variables) and the spatial variation in the number of salamanders captured (dependent variable) either immigrating or emigrating across the road. Tests were deemed to be statistically significant at $P < 0.05$.

All statistical tests were executed with SPSS v.16 (SPSS Inc., Chicago, USA). Akaike's Information Criterion values were obtained using formulas from Burnham and Anderson (1998) in Excel 2007 (Microsoft, Redmund, USA).

2.3 Results

2.3.1 Road mortalities

In 2008, I found 6 live amphibians [5 long-toed salamanders, 1 western toad (*Anaxyrus boreas*)], 16 road-killed amphibians (10 long-toed salamanders, 6 western toads) and 2 road-killed wandering garter snakes (*Thamnophis elegans vagrans*) along the Entrance Road. Seven of the road-killed long-toed salamanders were found during the spring migration period, while the other 3 were found during the fall migration period. In 2009, I found 2 road-killed long-toed salamanders, both in early May. The percentage of road-killed adult long-toed salamanders (calculated as the number of road-killed individuals divided by the total number of salamanders migrating across the road) was 1.6% in 2008 and 0.6% in 2009. By contrast, road-killed long-toed salamanders represented 10% of long-toed salamanders (67 of 690) observed at Linnet Lake in 1994 (Fukumoto 1995), when drift fences and tunnels were absent. In addition, 1 road-killed western toad and 3 road-killed garter snakes were found along the Entrance Road in 1994.

2.3.2 Tunnel use by long-toed salamanders

In 2009, a total of 104 adult long-toed salamanders were captured in exit traps, presumably after successfully moving through the tunnels, 74% of which were captured immigrating to Linnet Lake. Of the 91 long-toed salamanders captured along the fence during night surveys during the peak immigration period, 21 were later captured in “exit” traps after using the tunnels, resulting in a fence/tunnel efficiency rating of 23%. During the peak emigration period, 2 of 179 salamanders caught along fences were caught in tunnel “exit” traps, for a fence/tunnel efficiency rating of 1%. In addition, 4 grey tiger salamanders (*Ambystoma mavortium*) and 7 western toads were also captured in exit traps.

The tunnels were not equally-used by long-toed salamanders (χ^2= 38.0, df=3, *P*<0.001). Of the 104 long-toed salamanders captured exiting tunnels, almost half were detected at Tunnel 3 (49%), compared to Tunnel 1 (7%), 2 (26%), and 4 (18%). This pattern of differential use is retained even when total exit trap captures are corrected for total length of drift fences leading to each tunnel: long-toed salamanders captured per metre fencing = 0.05 (Tunnel 1), 0.17 (Tunnel 2), 0.23 (Tunnel 3), and 0.07 (Tunnel 4). On average, long-toed salamanders spent 3.5 ± 0.1 nights moving along fences before using tunnels (n = 23, range: 1 – 15 d).

Only 1 of 10 candidate models for predicting tunnel use was supported (Table 2.1a). This model (w_i = 0.95) included the parameters distance and direction. Model-averaged parameter estimates for covariates of tunnel use, odds ratios and confidence intervals are presented in Table 2.1b. Akaikeweighted odds ratios for these variables indicated that salamanders immigrating to Linnet Lake were 19.5 times more likely to use tunnels than emigrating salamanders, and females were 1.2 times more likely to use tunnels than males (Table 2.1b). Immigrating salamanders were more likely to move longer distances along fences to reach the tunnel than were emigrating salamanders (Table 2.1b). The odds of successful tunnel use seemed to decrease slightly with body size and distance from the nearest tunnel entrance (Table 2.1b), however, because the 95%CI of the odds ratios for these parameters bound zero, it is impossible to conclude whether there is any real effect of these variables on probability of tunnel use. Long-toed salamanders were capable of travelling up to 78 m along fences before successfully using tunnels (mean ± SE, 26.9 ± 4.45 m; n=23).

From 22 April – 24 Aug 2009, tunnel cameras took over 500,000 digital images, at timed intervals and when triggered by motion. Although most of these images displayed an unoccupied tunnel floor, or a non-target vertebrate using the tunnel, 55 long-toed salamanders were photographed using tunnels. Twenty-six (47%) of the salamanders that were photographed using tunnels were not captured in exit traps. Cameras also documented adult western toads (4 events), grey tiger salamanders (2 events), and wandering garter snakes (18 events) using the tunnels.

2.3.3 Timing of long-toed salamander migrations

The peak spring immigration period in 1994 started on 12 April, 22 d earlier than in 2008 and 21 d earlier than in 2009, when the peak immigrations began in early May. In all 3 years, the peak immigration period ended between 12 –15 May. The peak spring emigration period in 1994 began 7 days and 19 days earlier than in 2008 and 2009, respectively. Across the 3 years, the peak emigration period lasted 21 – 29 d (Figure 2.2). The peak fall migration period in 2008 lasted 41 d, and occurred from 3 Sept – 14 Oct. Table 2.2 presents mean (± SE) snout-vent-length (SVL; mm), total length (mm), and mass (g) of female and male long-toed salamanders captured in pitfall traps during immigration to and emigration from Linnet Lake in spring (2008 and 2009) and fall (2008).

I captured 16 long-toed salamanders in both 2008 and 2009. Of these, 11 were captured emigrating from the lake in spring 2009 and 6 were individually-marked. All 6 of these individually-marked, emigrating salamanders were initially captured immigrating to Linnet Lake in 2008 (3 during the fall), and never caught again till 2009, suggesting that they had overwintered on the east side of the road, close to the breeding site. In addition, I captured 80 long-toed salamanders at pitfall traps located along fence transects immediately surrounding Linnet Lake in 2008 and 2009, the majority of which were captured far from the Entrance Road along the south and east sides of the lake (K. Pagnucco, unpublished data). These individuals were all unmarked and were never captured attempting to cross the road, indicating that they overwintered close to Linnet Lake.

2.3.4 Relationship between precipitation and daily variation in long-toed salamander captures

The effect of precipitation on immigration and emigration varied between years (G-test of independence, χ^2=61.8, df = 7, *P*<0.001; Figure 2.3). Salamander capture rates were typically higher on days with precipitation, and this relationship was stronger during emigration than immigration (G-test of interaction, χ^2=25.1, df = 2, *P*<0.001; Figure 2.3). An exception was observed in

2009, when the capture rate during immigration was higher on days without precipitation than on days when there was precipitation (Figure 2.3). In 1994, 2008 and 2009, 75% 63% and 43% of all immigrating salamanders were captured on days with precipitation, respectively, whereas 99%, 95% and 90% of all emigrating salamanders were captured on days with precipitation during the same years.

2.3.5 Effects of habitat characteristics on spatial variation in long-toed salamander captures

Table 2.3 contains values for habitat variables measured at roadside pitfall trap locations in 2008. The most parsimonious regression model for habitat use during immigration was supported by 48% of the total weight, and consisted of the parameters “shrub cover” and “leaf litter depth” (Table 2.4a). Salamander captures were positively-related to shrub cover (%), and negatively-related to leaf litter depth (mm; Table 2.5a).

The most parsimonious regression model of habitat use during emigration was supported with 35% of the total weight, and included soil moisture content and CWD cover (%; Table 2.4b). Although shrub cover was the most important habitat variable predicting long-toed salamander use during immigration (Table 2.5a), it was the least important variable during emigration (Table 2.5b). Soil moisture content was the most important predictor of habitat use during emigration, followed by CWD; both were positively-correlated with salamander capture rates (Table 2.5b).

Habitat surrounding the four tunnels differed in soil moisture content ($F_{3,54}$= 3.37; P=0.02; Figure 2.4), but not in leaf litter depth ($F_{3,54}$= 2.17; P=0.10), or canopy cover ($F_{3,54}$= 1.91; P=0.14; Figure 2.4). Soil moisture content was highest at Tunnel 3, but was only significantly higher than moisture at Tunnel 2 (Figure 2.4).

2.4 Discussion

In this study, I evaluated the effectiveness of newly-installed under-road amphibian tunnels in WLNP at mitigating road mortalities of long-toed salamanders. To determine how fences and tunnels interacted to direct salamanders across the road, I calculated “fence/tunnel efficiency.” I modelled probability of tunnel use based on salamander size, sex, direction of movement, and initial distance from tunnel entrance in order to determine if only certain individuals would use tunnels. To determine the importance of precipitation during peak long-toed salamander migration periods, I tested for differences in long-toed salamander capture rates on days with or without precipitation based on migration direction and study year. To predict potential movement corridors of long-toed salamanders, I modelled spatial variation in salamander captures based on habitat characteristics.

2.4.1 Drift fence/tunnel system reduced road mortality of long-toed salamanders

The extensive drift fence/tunnel system built along the Entrance Road in WLNP was successful at reducing access by long-toed salamanders and other amphibians to the road surface, consequently reducing road mortalities to less than 2% of long-toed salamanders that attempted to cross the road in both 2008 and 2009. In contrast, a study at the same site that was conducted using night surveys and no drift fencing in 1994 found that 10% of long-toed salamanders that attempted to cross the Entrance Road were killed by vehicles (Fukumoto 1995). Similarly, the installation of barrier fences to guide animals to a single culvert near Lake Jackson in Florida led to a decrease in turtle road mortalities from 11.9 to 0.01 individuals/km/day (Aresco 2005).

2.4.2 Factors affecting tunnel use by long-toed salamanders

Although the deployment of amphibian tunnels is new in Canada, they have been installed throughout the United States and to a greater extent throughout Europe with varying degrees of success. In California, only 9% of the endangered Santa Cruz long-toed salamanders (*Ambystoma macrodactylum croceum*) that encountered drift fences passed through tunnels during immigration (Allaback and Laabs 2003), which was similar to the 12% effectiveness reported for smooth newts (*Triturus vulgaris*) in Germany (Brehm 1989). However, tunnel effectiveness was considerably higher for a population of spotted salamanders (*A. maculatum*) crossing a street in Massachusetts: 68% of spotted salamanders captured along fences used tunnels (Jackson and Tyning 1989). In my study, 23% of immigrating long-toed salamanders and 1% of emigrating salamanders that encountered drift fences were subsequently captured in exit traps after presumably moving through the tunnels. Discrepancies in reported tunnel effectiveness can at least partially be attributed to fence orientation; in both my study and that of Jackson and Tyning (1989), fences were angled towards tunnel entrances, whereas the fences in Allaback and Laabs (2003) were parallel to the road. Therefore, angling fences towards entrances appears to be important in encouraging tunnel use by amphibians (Jackson 2003).

An alternative explanation for differences in tunnel effectiveness may be the different monitoring techniques used. Both Allaback and Laabs (2003) and I used pitfall traps to monitor tunnel use, whereas Jackson and Tyning (1989) visually-monitored tunnel exits for salamanders. This latter method ensures that the vast majority of salamanders using tunnels are documented. In contrast, my tunnel cameras documented 26 salamanders using tunnels that were not subsequently captured in exit traps. This represented 47% of all salamanders photographed in the tunnels and 25% of the animals captured in exit traps, suggesting low detectability using this monitoring technique (MacKenzie et al. 2005). Combining camera and tunnel exit trap data, at least 130 long-toed salamanders used the tunnels when migrating between overwintering and breeding habitat in 2009, which represents 10% of the total breeding population (see Chapter 4). Given the low number of road-kills, and the number of long-toed salamanders encountered along the fences, use of the tunnels was likely much higher, given that some salamanders likely completely escaped detection.

The length of drift fences leading to tunnel entrances has been thought to be an important factor in determining amphibian use. Allaback and Laabs (2003) did not observe any Santa Cruz long-toed salamanders passing through tunnels after following drift fences for more than 16 m. Although my results showed that long-toed salamanders travelled an average 27 m, and up to 78 m, along fences before successfully using tunnels, my models suggest that individuals found closer to tunnel entrances were more likely to use tunnels. This suggests that roadside drift fences may have been acting as a barrier to long-toed salamander movement. If the majority of salamanders are failing to reach Linnet Lake because they are being deterred by fencing, this can lead to decreased reproductive output.

Tunnel use by long-toed salamanders may increase over time as salamanders adapt to the presence of these structures within their terrestrial habitat. One of the major failures of short-term studies of crossing structure use is that they fail to address the need for wildlife habituation to such large-scale landscape changes (Opdam 1997). Individuals require time to accept crossing structures (Land and Lotz 1996; Clevenger and Waltho 2000; Donaldson 2005), and habituation periods may take several years as individuals experience, learn, and adjust behaviour (Clevenger et al. 2002). Although wildlife passage use has been shown to increase as mammals learn a structure's location and become accustomed to it over time (Land and Lotz 1996), such adaptive behaviour has yet to be documented for amphibians.

2.4.3 Effects of precipitation on timing of long-toed salamander migrations

Movement of long-toed salamanders to Linnet Lake in the spring was not dependent on precipitation, which is contrary to the majority of cases reported in the literature on amphibian migrations (Semlitsch 1985; Beneski et al. 1986; Sexton et al. 1990; Greenberg and Tanner 2004; Todd and Winne 2006). Although salamanders likely require moist substrates to migrate, in WLNP moisture during immigration often results from snowmelt as opposed to rainfall. Seepage from snowmelt was proposed as the catalyst for emergence from winter hibernacula for a long-toed salamander population in Canmore, Alberta (Sheppard 1977). In contrast, I found that virtually all emigration later in the active season occurred on days with rain. In both 2008 and 2009, emigration at Linnet Lake began in mid-May after long dry periods (K. Pagnucco, personal observation), at which point, any amount of precipitation appeared to trigger movement. Also, salamanders must arrive at the breeding site early enough to assure that larvae have time to metamorphose (Werner 1986) but no such time constraint exists for emigration during the late spring, thus precipitation should be more strongly correlated to emigration than to immigration.

2.4.4 Access of long-toed salamanders to overwintering habitats

Almost 75% of all salamanders captured in tunnel exit traps were individuals immigrating to the breeding site. Of the 16 long-toed salamanders I captured in both years, 38% were individuals that I captured immigrating to Linnet Lake in 2008 and recaptured emigrating in 2009. I also captured 80 individuals around Linnet Lake, none of which were subsequently captured crossing the Entrance Road. These data suggest that some adult salamanders successfully overwinter in areas close to the breeding site and do not annually migrate across the road. Whether a salamander migrates or not may depend on the availability of suitable overwintering sites and the size of the population. As the population grows and the more-desirable overwintering sites close to the lake become occupied, more salamanders may be forced to migrate further to access overwintering sites on the west side of the road. If overwintering conditions are more suitable on the west side of the road and/or overwintering sites close to the lake are limited, and emigrating salamanders do not use the tunnels readily and are confined to Linnet Lake, the population may decline through time.

2.4.5 Effects of habitat characteristics on spatial variation in long-toed salamander captures

Habitat analyses suggested that long-toed salamanders move through habitats with moist substrate and understory cover, although patterns differed depending whether individuals were moving to or from Linnet Lake. Shrub cover was the most important variable predicting use of an area by immigrating long-toed salamanders and tree canopy cover was much less important. At the spatial scale that salamanders operate, understory vegetation rather than canopy may be more closely related to substrate moisture, which is critical for amphibians that depend on moist skin for respiration (Duellman and Trueb 1994). Graham (1997) reported that long-toed salamanders near Hinton, Alberta, were most often caught in shrubby clearcuts, without tree canopy cover. Density of understory vegetation was positively-correlated with above-ground activity of red-spotted newts (*Notopthalmus viridescens*) and red-backed salamanders (*Plethodon cinereus*; Pough 1987) in central New York State. The higher temperatures and lower soil moisture in open habitats may limit use by amphibians because of the threat of desiccation (Spight 1968; Spotila 1972). Use of forested habitat may have also been a strategy to avoid garter snakes, which are known to prey on amphibians (Johnson 2000) and are more common in open, grassy habitats (Rothermel and Semlitsch 2002).

My results also revealed the lack of strong correlations between habitat characteristics and patterns of habitat use along emigration routes as compared to immigration routes. Given that immigration often occurred in the absence of precipitation, salamanders may be more selective of habitat containing substrates capable of holding moisture during these movements as opposed to during emigration, where the vast majority of salamanders move during rainfall and all habitats are likely to be moist. No correlation between habitat attributes and use by long-toed salamanders was also documented in northwest Idaho by Beneski et al. (1986), who compared total expected and observed long-toed salamander captures and found no significant differences for habitat type (dam, spillway, wet wooded, wet field, dry wooded), soil (wet, dry), or vegetation type (closed wooded, open fields). Beneski et al. (1986) concluded that long-toed salamanders do not use preferred migration routes, but instead move indiscriminately through all available habitats.

The four tunnels were not used equally: almost 50% of all salamanders caught in tunnel exit traps used Tunnel 3 (Figure 2.4). Immigration and emigration corridors used by salamanders when travelling to or from Linnet Lake were similar in 2008 and 2009 (Figure 2.5). I found that the soil moisture content was highest in the habitats surrounding the entrances of Tunnel 3, which may explain higher use of this tunnel. Natural vegetation can enhance the “attractiveness” of crossing structures to animals and allow habitat continuity (Glista et al. 2009). This study revealed that shrub cover appeared to be an important habitat feature during salamander immigration movements, and consequently, planting shrubs adjacent to tunnel entrances may increase use. A variety of studies have shown that cover in the vicinity of passage entrances increases their use by target vertebrate species (e.g. Bennett 1991; Rodriguez et al. 1996; Clevenger and Waltho 2005), primarily because of the protection from predators that cover provides.

2.4.6 Conclusions

My results showed that: i) deployment of road tunnels and accompanying directional drift fencing was accompanied by appreciable reductions in vehicle-caused mortality of long-toed salamanders and, ii) that salamanders and other amphibians used the tunnels to a modest extent even during the first full year following installation. Continued monitoring will be needed to determine if tunnel use by long-toed salamanders increases through time, as individuals become increasingly familiar with these new structures and tunnels accumulate organic matter, providing conditions more closely resembling natural substrates. Long-term monitoring is also required to determine the rate at which reductions in vehicle-caused mortality of salamanders are translated into population gains.

My results also showed that while spatial distribution of long-toed salamanders during early spring immigration to Linnet Lake was strongly and positively related to percent shrub cover, salamander presence during late spring emigration from Linnet Lake was not strongly related to any habitat characteristics. Some areas adjacent to the entrance road and Linnet Lake are actively managed to control invasive plant species. My study suggests that spraying of herbicides and mechanical removal of plants could influence migration routes by long-toed salamanders.

2.5 Literature cited

Allaback ML, Laabs DM (2003) Effectiveness of road tunnels for the Santa Cruz long-toed salamander. 2002-2003 Transactions of the Western Section of the Wildlife Society 38/39:5-8.

Aresco MJ (2005) Mitigation measures to reduce highway mortality of turtles and other herpetofauna at a North Florida lake. Journal of Wildlife Management 69:549-560.

Ascensão F, Mira A (2007) Factors affecting culvert use by vertebrates along two stretches of road in southern Portugal. Ecological Research 22:57-66.

Beneski JT, Zalisko EJ, Larsen JH Jr (1986) Demography and migratory patterns of the eastern long-toed salamander, *Ambystoma macrodactylum columbianum*. Copeia 1986:398-408.

Bennett AF (1991) Roads, roadsides and wildlife conservation: a review. In: Saunders DA, Hobbs RJ (eds) Nature Conservation 2: the role of corridors. Surrey Beatty & Sons, Chipping Norton, pp 99-118.

Bernardino FS Jr, Dalrymple GH (1992) Seasonal activity and road mortality of the snakes of the Pa-hay-okee wetlands of everglades National Park, USA. Biological Conservation 61:71-75.

Brehm K (1989) The acceptance of 0.2 m tunnels by amphibians during their migration to the breeding site. In: Langton TES (ed) Amphibians and Roads: Proceedings of the toad tunnel conference. ACO Polymer Products, Shefford, UK, pp 29-42.

Burnham KP, Anderson DR (1998) Model selection and inference: a practical information-theoretic approach. Springer-Verlag, New York, NY.

Clevenger AP, Waltho N (2005) Performance indices to identify attributes of highway crossing structures facilitating movement of large mammals. Biological Conservation 121:453-464.

Clevenger AP, Chruszcz B, Gunson K, Wierzchowski J (2002) Roads and wildlife in the Canadian Rocky Mountain Parks – movements, mortality and mitigation. Final report. Prepared for Parks Canada, Banff, Alberta, Canada.

Clevenger AP, McIvor M, McIvor D, Chruszcz B, Gunson K (2001) Tiger salamander, *Ambystoma tigrinum*, movements and mortality on the Trans-Canada Highway in southwestern Alberta. Canadian Field-Naturalist 115:199-204.

Clevenger AP, Waltho N (2000) Factors influencing the effectiveness of wildlife underpasses in Banff National Park, Alberta, Canada. Conservation Biology 14:47-56.

deMaynadier PG, Hunter ML Jr (1998) Effects of silvicultural edges on the distribution and abundance of amphibians in Maine. Conservation Biology 12:340-352.

Donaldson BM (2005) Use of highway underpasses by large mammals and other wildlife in Virginia and factors influencing their effectiveness. In: Proceedings of the International Conference of Ecology and Transportation, San Diego, CA, pp 433-441.

Duellman WE, Trueb L (1994) Biology of Amphibians. Johns Hopkins University Press, Baltimore, MD.

Feder ME (1983) Integrating the ecology and physiology of plethodontid salamanders. Herpetologica 39:291-310.

Forman RTT, Alexander LE (1998) Roads and their major ecological effects. Annual Review of Ecology and Systematics 29:207-231.

Fukumoto J (1995) Long-toed salamander (*Ambystoma macrodactylum*) ecology and management in Waterton Lakes National Park. MSc. thesis, University of Calgary, Calgary, AB.

Gartshore RG, Purchase M, Rook RI, Scott L (2006) Bayview Avenue extension, Richmond Hill, Ontario, Canada habitat creation and wildlife crossings in a contentious climatic setting: a case study. In: Irwin CL, Garrett P, McDermott KP (eds) Proceedings of the 2005 International Conference on Ecology and Transportation. Center for Transportation and the Environment, North Carolina State University, Raleigh, NC, pp 55-76.

Glista DJ, DeVault TL, DeWoody JA (2009) A review of mitigation measures for reducing wildlife mortality on roadways. Landscape and Urban Planning 91:1-7.

Graham KL (1997) Habitat use by long-toed salamanders (*Ambystoma macrodactylum*) at three different scales. MSc. thesis, University of Guelph, Guelph, ON.

Greenberg CH, Tanner GW (2004) Breeding pond selection and movement patterns by eastern spadefoot toads (*Scaphiopus holbrookii*) in relation to weather and edaphic conditions. Journal of Herpetology 38:569-577.

Harmon ME, Sexton J (1996) Guidelines for measurements of woody detritus in forest ecosystems. US LTER publication No. 20, US LTER network office, University of Washington, Seattle, WA, pp 1-34.

Jackson SD (2003) Proposed design and considerations for use of amphibian and reptile tunnels in New England. Department of Natural Resources Conservation, University of Massachusetts, Amherst, MA, 4 pp.

Jackson SD, Tyning TF (1989) Effectiveness of drift fences and tunnels for moving spotted salamanders *Ambystoma maculatum* under roads. In:

Langton TES (ed) Amphibians and Roads: Proceedings of the toad tunnel conference. ACO Polymer Products, Shefford, UK, pp 93-99.

Jenkins CL, McGarigal K, Timm BC (2006) Orientation of movements and habitat selection in a spatially structures population of marbled salamanders (*Ambystoma opacum*). Journal of Herpetology 40:240-248

Kline NC, Swann DE (1998) Quantifying wildlife road mortality in Saguaro National Park. In: Evink GL, Garrett P, Zeigler D, Berry J (eds) Proceedings of the International Conference on Wildlife Ecology and Transportation. Florida Department of Transportation, Tallahassee, FL, pp 23-31.

Land D, Lotz M (1996) Wildlife crossing designs and use by Florida panthers and other wildlife in southwest Florida. In: Evink GL, Zeigler D, Garrett P, Berry J (eds) Highways and movement of wildlife: improving habitat connections and wildlife passageways across highway corridors. Florida Department of Transportation, Tallahassee, FL, pp 323-328.

MacKenzie DI, Nichols JD, Royle KH, Pollock KH, Hines JE, Bailey LL (2005). Occupancy estimation and modeling:inferring patterns and dynamics of species occurrence. Elsevier, San Diego, CA.

Opdam PFM (1997) How to choose the right solution for the right fragmentation problem? In: Canters K (ed) Habitat Fragmentation and Infrastructure. Ministry of Transportation, Public Works and Water Management, Delft, Netherlands, pp 55-60.

Paton PW, Crouch WB III (2002) Using the phenology of pond-breeding amphibians to develop conservation strategies. Conservation Biology 16:194-204.

Pearson K (2002) Linnet Lake long-toed salamander (*Ambystoma macrodactylum*) roadkill prevention and population estimation summary report. Unpublished technical report. Parks Canada, Waterton Lakes National Park, Alberta, Canada, 32pp.

Pough FH, Smith EM, Rhodes DH, Collazo A (1987) The abundance of salamanders in forest stands with different histories of disturbance. Forest Ecology and Management 20:1-9.

van der Ree R, Heinze D, McCarthy M, Mansergh I (2009) Wildlife tunnel enhances population viability. Ecology and Society 14:7-16.

Rodriguez A, Crema G, Delibes M (1996) Use of non-wildlife passages across a high speed railway by terrestrial vertebrates. Journal of Applied Ecology 33:1527-1540.

Semlitsch RD (1985) Analysis of climatic factors influencing migrations of the salamander *Ambystoma talpoideum*. Copeia 1985:477-489.

Sexton OJ, Phillips C, Bramble JE (1990) The effects of temperature and precipitation on the breeding migration of the spotted salamander (*Ambystoma maculatum*). Copeia 1990:781-787.

Sheppard RF (1977) The ecology and home range movements of *Ambystoma macrodactylum krausei* (Amphibia: Urodela). MSc. thesis, University of Calgary, Calgary, AB.

Smith C, Pagnucco K, Johnston B, Paszkowski C, Scrimgeour. G. 2010. Using specialized tunnels to reduce highway mortality of amphibians. In: Wagner PJ, Nelson D, Murray E (eds) Proceedings of the International Conference on Ecology and Transportation, Duluth, MN: DECC, pp 583-591.

Sokal RR, Rohlf FJ (1981) Biometry, the principles and practice of statistics in biological research. W.H. Freeman and Company, New York, NY.

Todd BD, Winne CT (2006) Ontogenetic and interspecific variation in timings of movement and responses to climatic factors during migrations by pond-breeding amphibians. Canadian Journal of Zoology 84:715-722.

Werner EE (1986) Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. The American Naturalist 128:319-341.

Williams RN, Gopurenko D, Kemp KR, Williams B, DeWoody JA (2009) Breeding chronology, sexual dimorphism, and genetic diversity of congeneric ambystomid salamanders. Journal of Herpetology 43:438-449.

Table 2.1. Summary of a) logistic regression models and b) model-averaged coefficients (β), standard errors (SE), odds ratios and their 95% Confidence Intervals (CI) for variables from AIC_C models predicting tunnel use by long-toed salamanders based on pitfall trap captures in 2009.

a) Models	*k*	log L	AICc	Δi	*wi*	Rank
Distance[a] + Direction[b]	3	-55.428	116.952	0.000	0.948	1
Distance + Direction + Distance*Direction[c]	4	-58.840	125.841	8.889	0.011	2
Distance*Direction	2	-60.927	125.901	8.949	0.011	3
SVL[d] + Direction	3	-60.073	126.241	9.289	0.009	4
Direction + Distance*Direction	3	-60.275	126.645	9.693	0.007	5
Distance + Sex[e] + Distance*Direction	4	-59.702	127.564	10.612	0.005	6
SVL + Sex + Direction	4	-59.742	127.644	10.692	0.005	7
SVL + Distance + Direction	4	-59.831	127.823	10.871	0.004	8
SVL + Distance + Sex + Direction + Distance*Direction	6	-67.150	146.640	29.688	3.39E-07	9
SVL + Distance + Sex	4	-75.300	158.755	41.803	7.93E-10	10

				95% CI	
b) Variables	β	SE	Odds ratio	Lower	Upper
Direction	2.06	1.11	19.50	3.05	72.60
Sex	0.18	0.59	1.21	0.38	3.32
Distance*Direction	0.07	0.12	1.08	0.97	1.25
Distance	-0.11	0.11	0.90	0.76	1.04
SVL	-0.14	0.11	0.87	0.75	1.01

[a] Distance from nearest tunnel entrance (m)

[b] Immigrating to or emigrating from Linnet Lake; emigration was the reference condition

[c] Interaction between distance and direction

[d] Snout-vent-length (mm)

[e] Male was the reference condition

Note: log *L* = log likelihood; $\Delta i = AIC_{c,i} - AIC_c$; w_i = Akaike weight

Table 2.2. Mean (± SE) snout-vent-length (SVL; mm), total length (mm), and mass (g) of female and male long-toed salamanders captured in pitfall traps during immigration to and emigration from Linnet Lake in spring (2008 and 2009) and fall (2008).

Season	Year	Direction	Sex	n	SVL (mm)	TL (mm)	Mass (g)
Spring	2008	Immigration	Females	203	64.3 ± 0.28	123.1 ± 0.79	6.1 ± 0.08
			Males	196	60.9 ± 0.26	119.3 ± 0.70	5.1 ± 0.08
		Emigration	Females	95	65.3 ± 0.48	129.1 ± 0.15	5.6 ± 0.14
			Males	58	62.2 ± 0.41	124.2 ± 1.13	5.0 ± 0.13
	2009	Immigration	Females	125	65.2 ± 0.36	124.3 ± 0.97	5.5 ± 0.08
			Males	42	60.8 ± 0.57	122.6 ± 1.63	4.4 ± 0.12
		Emigration	Females	87	64.2 ± 0.50	119.3 ± 1.58	5.0 ± 0.11
			Males	106	61.1 ± 0.44	116.7 ± 1.12	4.7 ± 0.08
Fall	2008	Immigration	Females	10	65.2 ± 1.04	122.2 ± 4.56	6.2 ± 0.49
			Males	5	61.1 ± 0.84	120.3 ± 4.50	4.9 ± 0.22
		Emigration	Females	4	64.6 ± 2.76	125.1 ± 3.79	5.6 ± 0.61
			Males	12	62.4 ± 0.95	129.6 ± 2.05	5.6 ± 0.33

Table 2.3. Mean, standard error (SE), minimum (min) and maximum (max) values of habitat variables measured in 2008 at all pitfall trap locations on the west (n=24; traps used during immigration) and east (n=20; traps used during emigration) side of the Entrance Road, Waterton Lakes National Park, Alberta.

	Immigration				Emigration			
Habitat variable	Mean	SE	min	max	Mean	SE	min	max
Slope (°)	22.29	1.87	5	33	23.00	1.41	15	35
Canopy cover (%)	29.60	6.17	0.16	83.88	22.43	5.66	0.16	71.92
Leaf litter depth (mm)	27.83	0.32	0.00	57.54	20.39	0.39	0.00	68.06
Soil moisture content	17.66	1.49	10.19	38.24	13.47	1.23	6.22	30.86
Ground cover (%)								
Shrubs	18.46	5.76	0	60	10.70	4.85	0	41
Grass/forbs	41.71	7.28	0	95	64.15	7.43	2	94
Leaf litter	18.12	4.26	0	69	10.30	3.72	0	70
CWD	4.42	2.25	0	12	2.55	1.50	0	8
Bare soil	17.29	6.22	0	95	12.30	3.99	0	68

Table 2.4. Summary of *a priori* models explaining relations between pitfall trap captures of long-toed salamanders with habitat attributes along the Entrance Road during a) immigration and b) emigration in 2009.

a) Immigration models	k	log L	AIC_c	Δ_i	w_i	Rank
Leaf Litter Depth + Shrub Cover	4	6.502	-2.899	0.000	0.482	1
Canopy Cover + Shrub Cover	4	5.729	-1.353	1.546	0.222	2
Soil Moisture + Shrub Cover	4	5.226	-0.348	2.552	0.134	3
Soil Moisture + Canopy Cover + Shrub Cover	5	5.795	1.742	4.642	0.047	4
Canopy Cover + Shrub Cover + CWD Cover	5	5.746	1.841	4.740	0.045	5
Soil Moisture + Shrub Cover + CWD Cover	5	5.226	2.880	5.780	0.027	6
Leaf Litter Depth + Soil Moisture	4	3.007	4.091	6.990	0.015	7
Soil Moisture + CWD Cover	4	2.648	4.810	7.709	0.010	8
Soil Moisture + Canopy Cover	4	2.606	4.893	7.792	0.010	9
Slope + Leaf Litter Depth + Soil Moisture	5	3.996	5.341	8.240	0.008	10
Global Model[a]	8	7.571	10.459	13.358	0.001	11

b) Emigration models	k	log L	AIC_c	Δ_i	w_i	Rank
Soil Moisture + CWD Cover	4	10.902	-11.137	0.000	0.347	1
Soil Moisture + Canopy Cover	4	10.057	-9.447	1.691	0.149	2
Leaf Litter Depth + Soil Moisture	4	9.681	-8.696	2.442	0.102	3
Soil Moisture + Shrub Cover	4	9.681	-8.696	2.442	0.102	4
Soil Moisture + Shrub Cover + CWD Cover	5	11.150	-8.015	3.122	0.073	5
Leaf Litter Depth + Shrub Cover	4	9.223	-7.780	3.358	0.065	6
Canopy Cover + Shrub Cover	4	9.175	-7.684	3.453	0.062	7
Slope + Leaf Litter Depth + Soil Moisture	5	10.678	-7.071	4.066	0.045	8
Canopy Cover + Shrub Cover + CWD Cover	5	10.217	-6.147	4.990	0.029	9
Soil Moisture + Canopy Cover + Shrub Cover	5	10.061	-5.836	5.301	0.024	10
Global Model[a]	8	14.638	-0.186	10.952	0.001	11

[a] Slope + Leaf Litter Depth + Soil Moisture + Canopy Cover + Shrub Cover + CWD Cover

Note: log L = log likelihood; Δ_i=$AIC_{c,i}$ - AIC_c; w_i= Akaike weight

Table 2.5. Model-averaged coefficients (β), standard errors (SE), 95% Confidence Intervals (CI) for variables from AICc models predicting habitat use by long-toed salamanders during (a) immigration to and (b) emigration from Linnet Lake, based on pitfall trap captures in 2009.

(a) Immigration			95% CI			
Variable	β	SE	Lower	Upper	*w*	Rank
Shrub Cover	0.026	0.008	0.010	0.042	0.947	1
Leaf Litter Depth	-0.185	0.117	-0.427	0.057	0.421	2
Canopy Cover	-0.007	0.007	-0.021	0.008	0.235	3
Soil Moisture	3.330	4.182	-5.344	12.004	0.179	4
Slope	1.235	0.025	1.184	1.286	0.148	5
CWD Cover	0.002	0.017	-0.033	0.038	0.140	6

(b) Emigration			95% CI			
Variable	β	SE	Lower	Upper	*w*	Rank
Soil Moisture	4.981	3.222	-1.762	11.725	0.458	1
CWD Cover	0.035	0.024	-0.014	0.085	0.440	2
Leaf Litter Depth	0.127	0.145	-0.177	0.431	0.288	3
Slope	0.030	0.037	-0.047	0.107	0.245	4
Canopy Cover	-0.006	0.006	-0.019	0.007	0.234	5
Shrub Cover	0.004	0.012	-0.020	0.029	0.183	6

Note: SE = (unconditional variance)$^{1/2}$; w=relative importance of predictor variable, calculated as the sum of AICc weight from all models including that variable

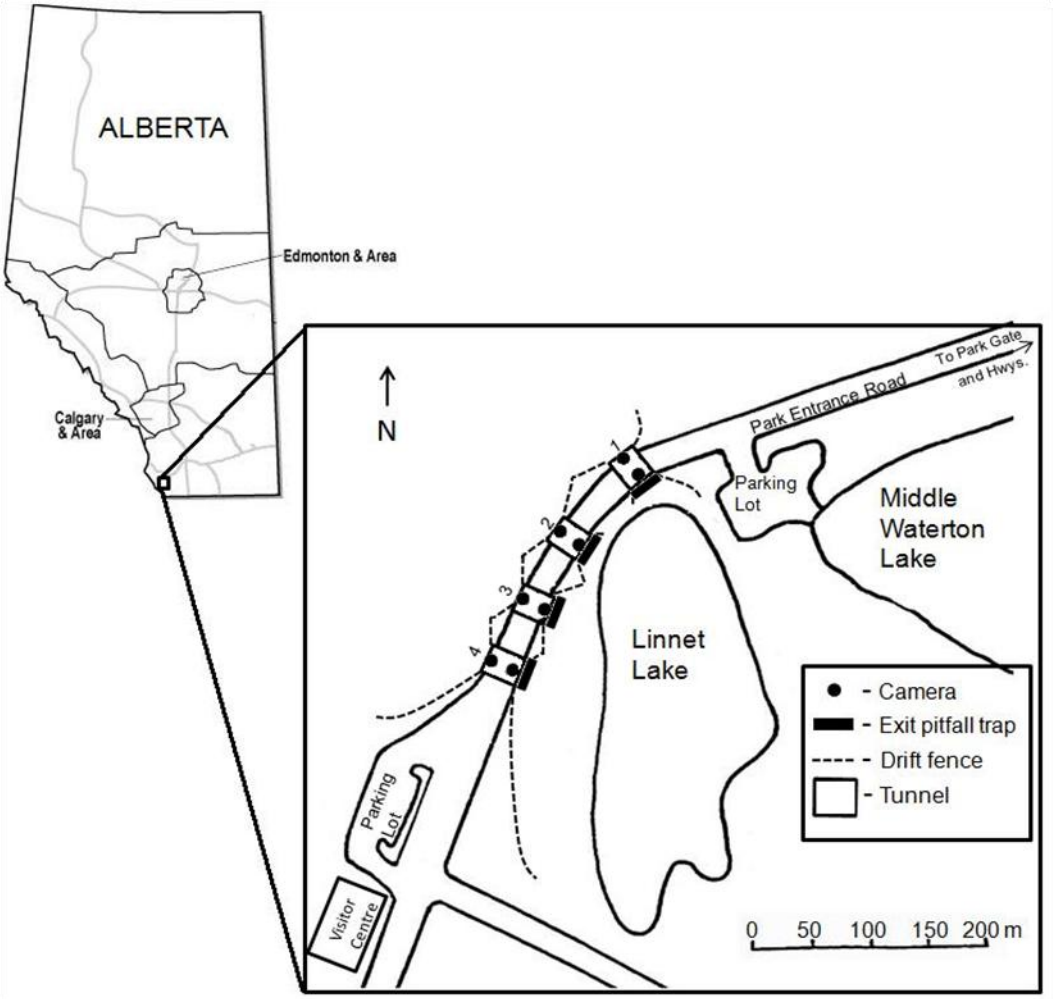

Figure 2.1. Map of Linnet Lake area in Waterton Lakes National Park, Alberta, including locations of under-road tunnels, exit pitfall traps, and drift fences in 2009. Adapted from Fukumoto (1995); Figure 3.

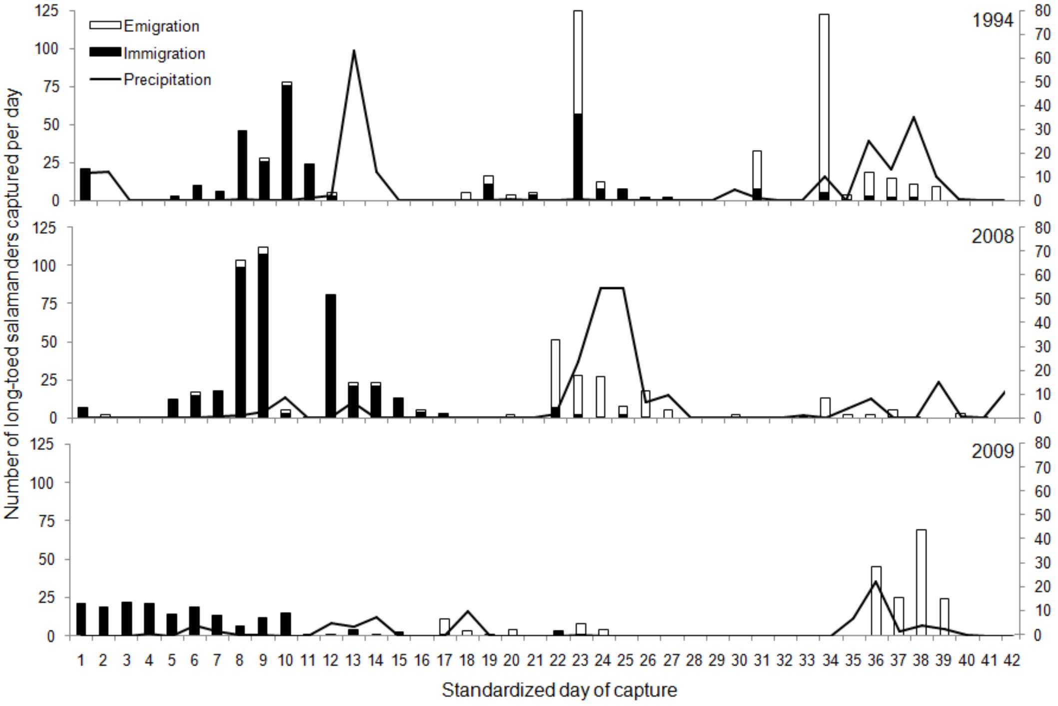

Figure 2.2. Movement phenology of long-toed salamanders at Linnet Lake in Waterton Lakes National Park. Shown is the number of individuals captured per day during peak immigration (from terrestrial habitat to Linnet Lake) and emigration (from Linnet Lake to terrestrial habitat) periods in 1994, 2008 and 2009, as well as the amount of precipitation (mm) on these days. Data from 1994 were acquired from Fukumoto (1995).

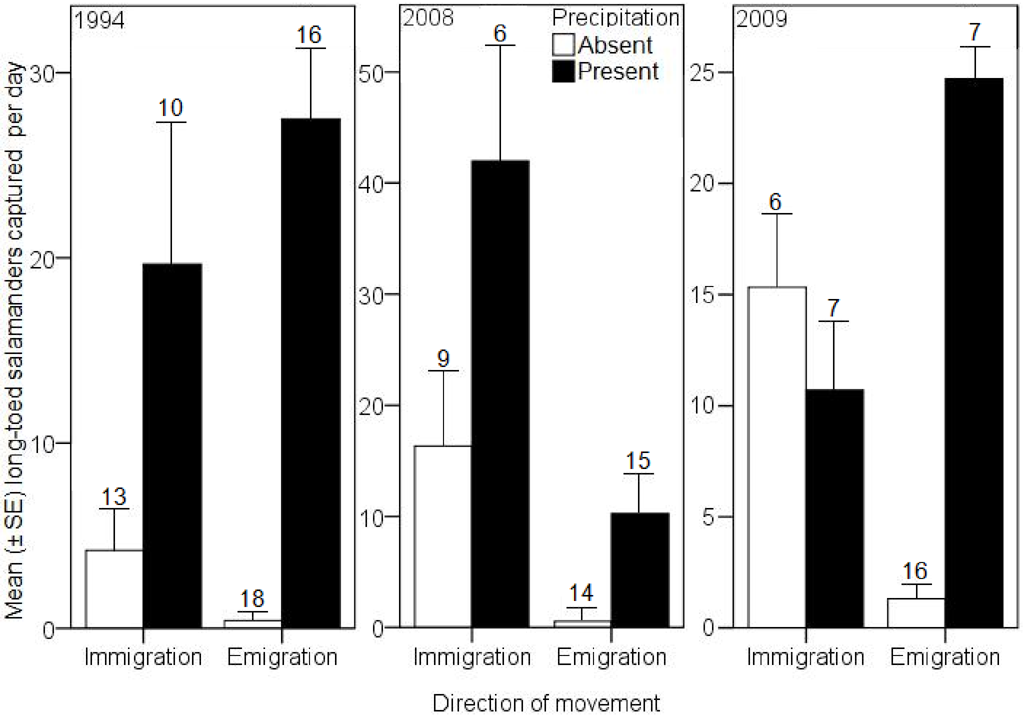

Figure 2.3. Effects of presence and absence of precipitation on mean (± SE) capture rates (number of long-toed salamanders captured per day in pitfall traps) during peak immigration and emigration periods in 1994, 2008, and 2009. Data from 1994 are from Fukumoto (1995). The number of days in each group are listed above each bar.

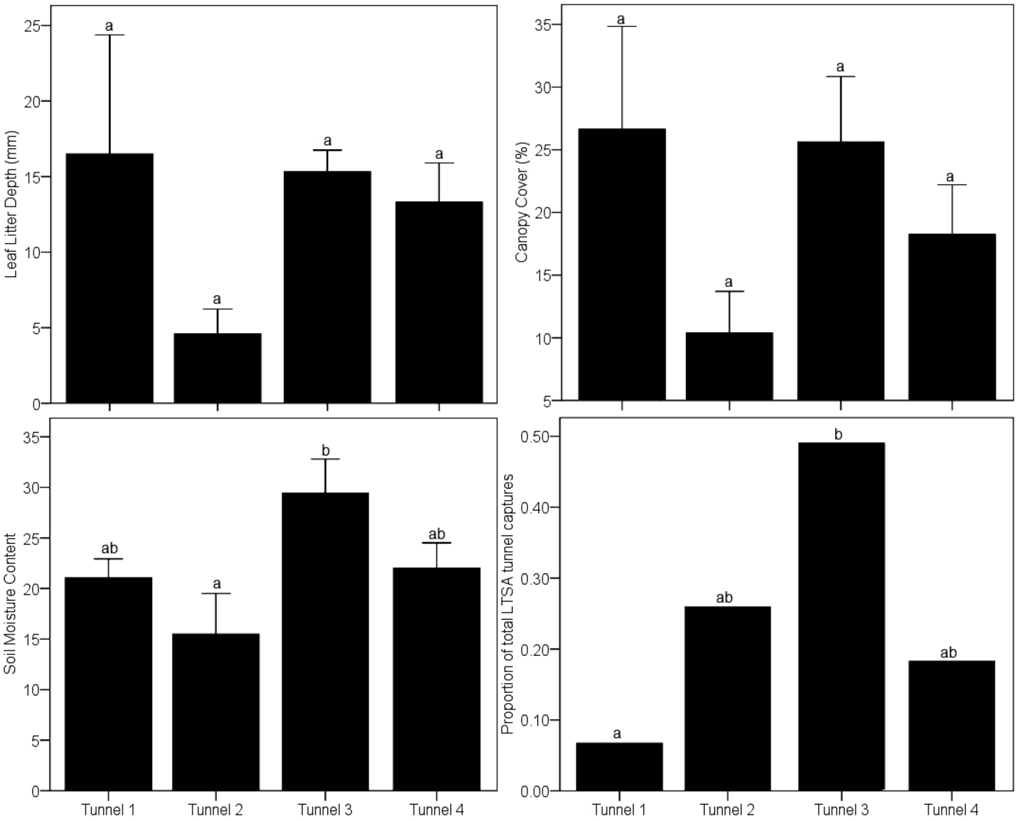

Figure 2.4. Mean (±SE) leaf litter depth (mm), canopy cover (%), and soil moisture content, as well as proportion of total long-toed salamanders (LTSA) captured in pitfall traps at each tunnel exit. Habitat variable were measured every 15 m along each fence leading to each tunnel (n=9 for tunnel 1; n=12 for tunnel 2; n=17 for tunnel 3; n=19 for tunnel 4). Bars with same letter are not significantly different from one another (Tukey's HSD post hoc test; *P*≥0.05).

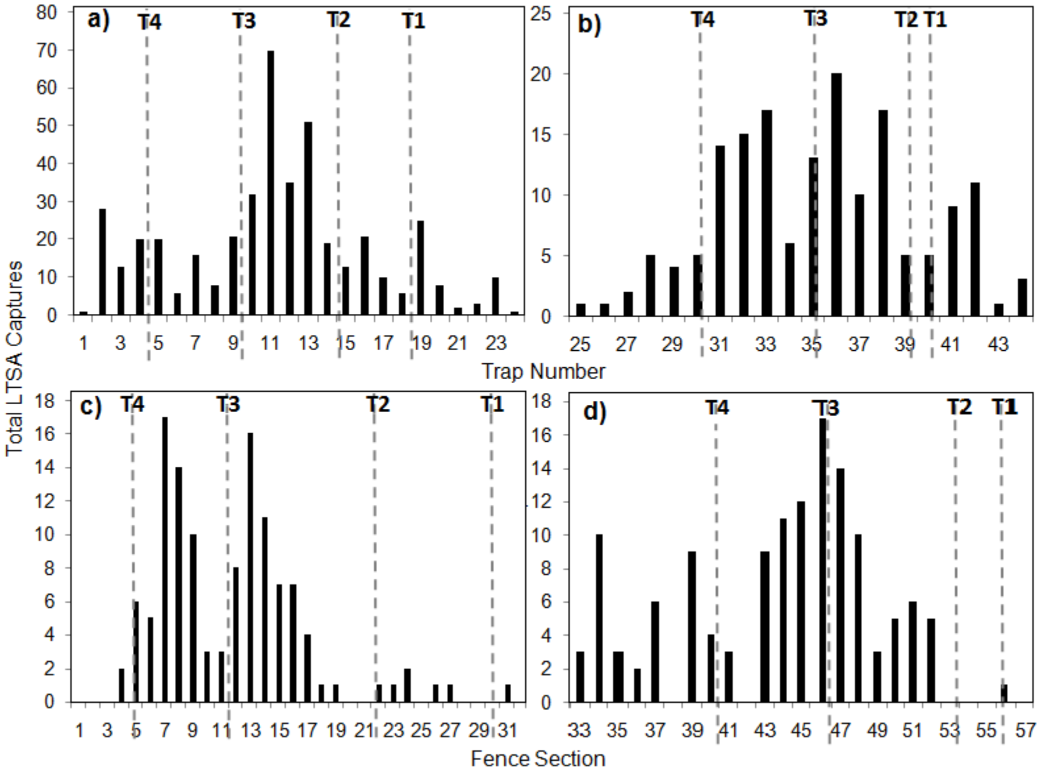

Figure 2.5. Total number of long-toed salamanders (LTSA) captured at roadside pitfall traps during (a) peak immigration in 2008, (b) peak emigration in 2008, (c) along fence sections during peak immigration in 2009, and (d) peak emigration in 2009. Tunnel locations relative to trap and fence locations are indicated by the dashed line (T1 = Tunnel 1, etc).

CHAPTER 3: USING CAMERAS TO MONITOR AMPHIBIAN TUNNEL USE: AN INFORMATIVE, COST-EFFICIENT TECHNIQUE

3.1 Introduction

The negative effects of roads on wildlife, which include increased mortality and decreased habitat connectivity, have been well-documented (Forman et al. 2003). As a result of these effects, crossing structures are being designed and incorporated into road construction and improvement projects throughout North America and Europe (Spellerberg 2002; Cain et al. 2003; Forman et al. 2003). Crossing structures include underpasses, overpasses, and under-road tunnels.

The success of road mortality mitigation projects may be measured by the extent that they reduce wildlife–vehicle collision rates or restore animal movement patterns. In order to assess the re-establishment of animal corridors, crossing structures must be monitored to determine species use, especially when wildlife population persistence and connectivity are primary concerns. Despite the inherent need to quantify the success of crossing structures, monitoring programs are rarely implemented.

I conducted a literature review of papers published between 1989-2009 using the BIOSIS Previews™ search engine and the following key terms: wildlife crossing structure, underpass, overpass, or culvert. Using this method, I found 25 studies that described how various methods were used to monitor the use of crossing structures. I found an additional 19 studies that monitored crossing structures by conducting an informal search through a variety of databases and conference proceedings. Of the combined 44 studies that monitored crossing structures (Table 3.1), I found that almost half used track-plates, whereas 43% used remotely-triggered cameras.

While track-plates and cameras are effective at documenting crossing structure use by mammals, they have seldom been applied to recording use by other vertebrates, such as amphibians. Of the 44 studies examined, only 9 (20%) documented tunnel use by amphibians (Table 3.1). Of these, 4 studies used track-plates, but in each case, only a limited number of amphibian tracks were observed. The remaining 5 studies documented amphibian use by direct observation or deployment of either funnel traps or pitfall traps at tunnel exits. To my knowledge, no one has successfully used cameras to document tunnel use by amphibians. Given the increasing number of under-road tunnels being installed, evaluation of monitoring methods that document tunnel use is a conservation priority.

In May 2008, Parks Canada installed 4 concrete tunnels under the Entrance Road adjacent to Linnet Lake in Waterton Lakes National Park, Alberta, Canada. Structures were deployed to reduce road mortality for a declining population of long-toed salamanders (*Ambystoma macrodactylum*), and to improve connectivity of seasonal habitats. My overall goal was to determine if remote cameras could be used to monitor tunnel use by long-toed salamanders and other amphibians as an alternative to direct observation or pitfall traps. My specific objectives were three-fold. First, I determined whether motion-detection or timed-interval image capture was the most effective method at recording amphibians using tunnels. Second, I quantified diel patterns in tunnel use by long-toed salamanders and potential predators, as well as how quickly long-toed salamanders navigated the tunnels. These data represent information which trap data alone cannot reveal. Lastly, I compared data from cameras and traps in their ability to document: i) temporal patterns in use of tunnels by long-toed salamanders; ii) variance in body size and proportion of male and female long-toed salamanders immigrating to and emigrating from Linnet Lake, and; iii) differences between the 4 tunnels in terms of long-toed salamander use. Determining when and where most long-toed salamanders use tunnels will allow optimization of monitoring efforts. In addition, determining the size classes and sex ratios for salamanders recorded crossing tunnels will document whether only certain individuals will use these structures. For example, bias towards only smaller, non-reproductive long-toed salamanders using tunnels could result in decreased reproduction and recruitment, which could lead to population declines. If cameras document similar spatio-temporal and demographic patterns in tunnel use as compared to exit traps, then cameras would represent a viable alternative to more invasive monitoring, such as pitfall trapping.

3.2 Methods

3.2.1 Tunnel installation

Four concrete tunnels were installed under the Entrance Road that runs parallel to Linnet Lake in spring 2008, from 26 May – 4 June (Smith et al. 2010). Each tunnel is a “box culvert” 60 cm wide, 52 cm high, and 12 m long (AT500 Amphibian Tunnels, ACO Technologies, Shefford, UK). Each section has slots along the top that allow air, moisture and light into the tunnel. Segments were placed together to span the width of the road and a sidewalk that abuts Linnet Lake. Tunnels were placed 80-110 m apart (see Chapter 2, Figure 2.1).

3.2.2 Using pitfall traps to monitor tunnel use

In order to monitor tunnel use by long-toed salamanders in 2009, I installed directional fencing angled towards tunnel entrances, and a rectangular pitfall trap (76 cm in length, 20 cm in width, and 18 cm in depth) at the exit of each tunnel based on the dominant direction of salamander movement. From 22 April – 19 May, exit traps were located on the east side of the road to catch individuals immigrating to Linnet Lake to breed. Traps were moved to the west side of the road to catch salamanders emigrating from Linnet Lake to upland habitats from 20 May – 19 August. Exit traps were intended to capture all long-toed salamanders travelling through tunnels and thus assess the performance of cameras in documenting tunnel use. Traps were checked daily between 0600-1000 h, and captured long-toed salamanders were measured (snout-vent-length, total length, mass), marked using Visible Implant Elastomer (Northwest Marine, Shaw, WA), and released 2 m upland under cover (see Chapter 2 for details on measuring and marking procedures).

3.2.3 Using cameras to monitor tunnel use

I conducted a pilot study in 2008 to test the ability of the 2 sampling methods for capturing images, motion-detection and timed-intervals, to document tunnel use by amphibians. I installed an infrared, motion-detecting camera (Reconyx™, Holmen, WI) at each tunnel entrance on both sides of the road (8 cameras) to monitor tunnel use. Cameras were placed inside steel security enclosures, and installed on ceilings of tunnel entrances so that they would monitor tunnel floors. Inside enclosures, cameras were about 42 cm from tunnel floors. At this distance, the field-of-vision of cameras was 23 cm in length by 17 cm in width, which allowed them to monitor activity along about 40% of the width of each tunnel floor. Cameras captured images from 28 August – 3 November in 2008, and were set to take 3 images at 1 second intervals whenever movement was detected, regardless of the time-of-day (i.e. motion-triggered images). Motion-detectors were set at maximum sensitivity in an effort to capture movement of small, slow amphibians. Cameras were also programmed to take 1 image at 1 minute intervals from 2100 to 0600 h daily (i.e. timed-interval images). Because of the number of images being recorded (540 images/camera/night using the timed-interval image capturing method, in addition to any motion-triggered events), I used a high-capacity memory card (4 GB) in each camera, which could hold about 20,000 photos that were each 0.2 MB in size. In 2009, cameras were set to take images from 22 April – 14 October at each tunnel entrance, using the same methods as in 2008.

Images of a metric ruler were taken in order to calibrate the size of animals in tunnels. I then used a ruler to measure snout-vent-length (SVL, mm) and total length (TL, mm) of all long-toed salamanders in images on a computer screen. In cases where salamanders triggered motion-detectors and multiple images were taken of a single event, I was able to calculate speed as the distance travelled divided by the time that was recorded by the camera (m/min; Figure 3.1a,b).

3.2.4 Statistical analyses

Temporal patterns in the use of tunnels by long-toed salamanders based on data from camera images and exit trap captures during the peak migration period in 2009 were compared using a two-sample Kolmogorov-Smirnov test. I also tested the relationship between the number of long-toed salamander crossings detected by camera and trap data using linear regression, where the total number of images containing long-toed salamanders per day was the dependant variable, and the total number of trap captures per day was the independent variable.

I compared SVL and TL as measured by hand at exit traps versus measurements taken from images using t-tests. Male and female data were analyzed separately, for a total of 4 comparisons. For each of the 2 sampling methods (i.e., cameras and traps), chi-square tests were used to test for differences in the proportion of: i) male and female salamanders found immigrating and emigrating, and ii) salamanders using each of the 4 tunnels. All statistical tests were executed with SPSS v.16 (SPSS Inc., Chicago, IL) and were deemed to be statistically significant at $P < 0.05$.

3.3 Results

3.3.1 Quality of photographic images taken using cameras

Timed-interval and motion-detector image capture provided high resolution images during both low-light night conditions (Figure 3.1a,b), and day light conditions (Figure 3.1c.d). Images taken under night and low light conditions were augmented, as the cameras automatically provide flash lighting. Time-stamping of images can be used to quantify travel speeds of animals using tunnels.

3.3.2 Total images and trap captures

Including both motion-triggered and timed-interval methods, tunnel cameras took over 260,000 images from 28 August – 3 November 2008, and another 750,000 images from 22 April – 14 October 2009. A variety of small mammals and herptiles were photographed using the tunnels to travel safely between habitats (Table 3.2).

In 2009, a total of 104 adult long-toed salamanders, 4 grey tiger salamanders (*Ambystoma mavortium*) and 7 western toads (*Anaxyrys boreas*) were caught in exit pitfall traps after successfully travelling through tunnels. At least 26 of the salamanders that were documented using tunnels with cameras were not captured in exit traps. These images were recorded on days when no long-toed salamanders were captured in the corresponding traps. Given the population estimate of adult long-toed salamanders breeding at Linnet Lake, which was estimated at 1372 individuals in 2009 (see Chapter 4), traps documented migration of about 8% of the adult population and cameras documented another 2%.

3.3.3 Timed-interval vs. motion-detection

Of the 58 long-toed salamanders photographed using tunnels, 80% were recorded based on timed-interval images, while only 20% triggered motion-detectors. In contrast, all images of grey tiger salamanders, garter snakes, and western toads were captured through motion-detection.

3.3.4 Speed and timing of tunnel crossings and predation

In 9 cases where a salamander was photographed multiple times while moving through a tunnel, I was able to calculate travel speed at the tunnel entrance, which averaged 1.1 ± 0.1 m/min [mean (± SE)]. On 4 occasions, salamanders were photographed entering and exiting the tunnel, allowing me to calculate the average crossing time (2.9 ± 2.2 min) and speed of crossing (4.1 ± 1.5 m/min).

Almost all (95%) long-toed salamander tunnel crossings were recorded at night, between 2000-0600 h (Figure 3.2). Four of the 6 grey tiger salamanders used tunnels between 2200-0300 h, while all western toads were photographed using tunnels between 2100-0500 h. On 48 occasions, wandering garter snakes were photographed using tunnels between 1000-1800 h, whereas only 3 long-toed salamanders (5%) used tunnels during this time (Figure 3.2).

On 27 August 2009, cameras documented 2 juvenile grey tiger salamanders entering Tunnel 1, moving towards Linnet Lake at 1141 h. At 1218 h, cameras documented a wandering garter snake dragging a juvenile grey tiger salamander by its head out of the same tunnel, moving away from Linnet Lake. Presumably, this was one of the salamanders photographed entering that tunnel 37 minutes earlier (Figure 3.1c,d).

3.3.5 Comparing camera data and trap data: temporal and spatial patterns of movement, and size of long-toed salamanders

Temporal patterns of long-toed salamander movement did not differ between the 2 sampling methods of camera (Figure 3.3a) and trap data (KS test statistic = 0.56; P=0.92; Figure 3.3b). In addition, camera and trap data were highly-correlated with one another (linear regression, y= 0.003 + 0.864x, where y = camera data and x = trap data; $F_{1,37}$=74.0, P<0.001, r^2=0.67; Figure 3.3c). Given a slope value of 0.44, I was able to ascertain a method of conversion between the 2 monitoring methods: detectability using cameras was 44% assuming that exit traps had 100% detectability.

Of the 58 long-toed salamanders photographed using tunnels, I was able to measure salamander SVL from photographs in 36 cases, and I was able to measure TL in 27 cases. Measurements of SVL and TL taken from images did not differ significantly from the same measurements taken by hand of captured long-toed salamanders (Figure 3.4a). The 2 methods did not differ in resulting average SVL values (females: $t_{157,0.05}$=-0.56, P=0.58; males: $t_{132,0.05}$=-0.98, P=0.33; Figure 3.4a) or TL values (females: $t_{150,0.05}$=-0.93, P=0.35; males:

$t_{130,0.05}$=-0.90, *P*=0.17; Figure 3.4a). I was able to determine sex from images in 45 cases (78%), which allowed me to determine that sex ratios generated by the 2 sampling methods did not differ (χ^2=5.82, *P*=0.12, Figure 3.4b).

Most salamanders were photographed using Tunnel 3 (67%), followed by Tunnel 4 (17%), Tunnel 2 (10%) and Tunnel 1(5%; Figure 3.4c). Although only 15% of long-toed salamanders were photographed using either Tunnels 1 or 2, 53% of snake crossings occurred in these 2 tunnels. Comparable differences in use among the 4 tunnels was apparent based on camera or trap data (χ^2=4.22, *P*=0.24, Figure 3.4c).

3.4 Discussion

My results showed that digital cameras and pitfall traps provided complimentary information on spatial and temporal patterns of use of wildlife tunnels by amphibians. Although twice as many salamanders were documented moving through tunnels based on trap data, tunnel use determined by digital cameras was strongly correlated with that derived from manual trapping. In addition, use of digital cameras allowed me to determine diel patterns of tunnel use and crossing speed of long-toed salamanders, as well as document potential predators within tunnels, information that pitfall trapping cannot provide without frequent checks.

3.4.1 Long-toed salamanders too small to trigger motion-detectors

The lack of studies using cameras to monitor use of crossing structures by amphibians may stem from concerns that amphibians are too small to trigger motion-detectors found in most wildlife cameras (Jackson 1999; Fitzgibbon

2001). In the case of long-toed salamanders, this concern is valid since 80% of all crossings were photographed during timed-intervals when motion-detectors were not triggered. The smallest animal that triggered the motion-detectors was a long-toed salamander (total length in frame = 106.4 mm; maximum width, at hindlimbs = 49.2 mm). However, not all amphibians failed to trigger motion-detectors: all records of adult western toads and grey tiger salamanders were captured by motion-triggered images. Although 5 juvenile western toads were captured in exit traps, camera images did not document any of them using tunnels, suggesting that small toads did not trigger motion-detectors. In cases where the target species is of equal or smaller size than long-toed salamanders, it would be advisable to use timed-interval images. If the number and resolution of images precludes taking exceedingly large numbers of images, cameras could be programmed to be active during the time-of-day when animals are active. Alternatively, motion-detectors could be augmented through the installation of sensitive weight triggers or laser beam sensors. For larger amphibians, programming cameras to capture images when motion-detectors were triggered may be sufficient to document tunnel use.

The main disadvantage to using timed-interval images is the large number of “empty” images generated. High numbers of images result in shortened battery life and require large capacity memory cards, creating the need for more frequent maintenance of camera systems, as well as increased amounts of time required to assess images (i.e. in this study, an experienced researcher could review 1875 images/hr). However, despite having cameras set to capture an image every minute from 2100-0600 h, batteries only needed to be changed on a weekly basis in this study, and memory cards emptied on a monthly basis.

3.4.2 Comparisons of metrics of tunnel use derived from camera and trap data

My study showed that camera data and exit trap data produced equivalent patterns in several metrics, including descriptions of: i) temporal patterns in tunnel use; ii) body size and proportion of males and female long-toed salamanders immigrating to and emigrating from Linnet Lake, and; iii) spatial variation related to the use of the 4 tunnels. Information on spatio-temporal variation in tunnel use will inform Parks as to when and where tunnels should be monitored to maximize information obtained. Information on body size and sex ratios of long-toed salamanders moving through tunnels may be used to extrapolate whether certain components of populations are more or less likely to cross tunnels.

When comparing the ratio of images of salamanders to trap captures across sampling days, cameras documented about half as many crossings as did traps. By obtaining this detection probability (Mackenzie et al. 2005), future monitoring of these tunnels could use camera data to extrapolate how many salamanders actually crossed through tunnels. However, this approach assumes that all salamanders using tunnels are trapped, which is not true as 26 of the salamanders that were photographed using tunnels were not subsequently captured in exit traps. Alternatively, some of these long-toed salamanders may have been photographed entering tunnels, but then turned back and did not actually cross. Visual observation of tunnel exits during periods of peak migration would allow calibration between the absolute number of salamanders crossing tunnels, and the number detected by traps and cameras.

3.4.3 Diel patterns in tunnel use and crossing speed of long-toed salamanders

In contrast to manual trapping, cameras provide information on crossing speeds, exact time-of-day of crossings, and the occurrence of predation within tunnels. Long-toed salamanders moved at a slower rate at tunnel entrances (mean ± SE = 1.11 ± 0.13 m/min) than their average crossing speed (4.8 ± 1.5 m/min), indicating that long-toed salamanders may hesitate at tunnel entrances. Salamanders may have moved slowly along the bare concrete at entrances, but increased speeds once inside the sand-lined tunnel, suggesting that the addition of substrate at tunnel entrances may increase use by amphibians.

My estimates of the mean speed of long-toed salamanders at tunnel entrances and within tunnels were about 5 times higher than those reported for spotted salamanders (*Ambystoma maculatum*; mean= 0.9 m/min; Charney et al. 2009). These differences in speeds are surprising given that adult spotted salamanders are on average twice the size of long-toed salamanders (Blackwell 2003). Running speeds of adult ambystomid salamanders are positively-correlated with body size (Bennett et al. 1989). Travel speeds of long-toed salamanders are closer to burst speeds of the larger tiger salamander (*A. californiense*; body mass range, 7.3-30.3 g; mean burst speed (± SE), 8.7 ± 0.5 m/min; Austin and Shaffer 1992). Long-toed salamanders may have increased their speed above normal values when within tunnels in order to minimize their time spent in the tunnel, which represent an unfamiliar route or an area of unsuitable substrate. The high alkalinity of concrete has been shown to deter other amphibian species (Mougey 1996 [as cited by Glista et al. 2009]).

3.4.4 Tunnels as predator “traps”

Several previous studies have expressed the concern that crossing structures could be used by mammalian predators to capture prey, since structures reduce the ability of prey species to avoid detection or escape (Little et al. 2002; Taylor and Goldingay 2003; Clevenger and Waltho 2005). However, this is the first study that I know of that has actually documented a predation event occurring in a crossing structure (Figure 3.1c,d). Cameras documented one instance of predation on a juvenile grey tiger salamander by a wandering garter snake (Figure 3.1c,d). Predation happened in late morning, when wandering garter snakes were typically active. All other grey tiger salamanders crossings occurred at night. Tiger salamanders are typically nocturnal (Madison and Farrand 1998), thus the cameras successfully recorded a rare occurrence.

However, the tunnels considered in this study are not likely to act as significant predator traps for long-toed salamanders because tunnel use by salamanders and garter snakes was separated both temporally (seasonal and diel separation) and spatially. In terms of seasonal patterns of tunnel use, all wandering garter snakes were photographed in the late summer (from 24 June – 18 September; see also Yanes et al. 1995), when most long-toed salamanders had finished migrating across the road from Linnet Lake. As for diel patterns of tunnel use, only 3 long-toed salamanders (5%) used tunnels between 1000-1800 h, whereas all 48 images of wandering garter snakes in 2009 were captured during this time period. In addition, while only 15% of long-toed salamanders were photographed using either Tunnel 1 or 2, 53% of snake crossings occurred in these tunnels.

Long-toed salamanders crossed tunnels at night when potential mammalian predators, such as mice, shrews, and skunks, were also documented using tunnels. However, cameras did not document any instances of mammals predating long-toed salamanders or other amphibians.

3.4.5 Using cameras in other aspects of amphibian research

Cameras could be used as a new and non-invasive method of monitoring other aspects of amphibian behaviour. Although pitfall traps are commonly used to assess amphibian species richness and abundance, the ability of traps to capture amphibians varies appreciably among species (Corn 1994). Monitoring pitfall traps with cameras would document what proportions of animals that encounter the traps avoid them or escape after entering traps.

3.4.6 Conclusions

My results showed that: i) long-toed salamanders could be effectively detected crossing tunnels using timed-interval images; ii) camera data can be used to calculate speed and exact time-of-day of amphibian crossings as well as predation in tunnels, which are all data that trapping alone cannot provide, and; iii) camera data revealed the same patterns of temporal and spatial variation in movement through tunnels, and of salamander body size and sex ratios, as determined from trap data. Appreciable overlap in the types of information provided by traps and cameras, and the fact that cameras can provide additional information not provided by traps, suggests that cameras may represent a novel approach to monitoring tunnel use by amphibians. Additionally, the 2 monitoring methods were highly-correlated with one another; however, neither monitoring method detected all salamander crossings. Therefore, coupled with initial validation using direct observation of tunnel exits, cameras represent a valuable new tool for amphibian monitoring.

In addition to being less-invasive, the use of cameras to monitor tunnels is less labour intensive than trapping. Each pitfall trap took 1 hr to install. Checking traps and measuring captured individuals took 1 – 5 hr daily during my study. Alternatively, it took 1 hr to install all 8 cameras. Changing camera batteries on a weekly basis took 30 min, and exchanging memory cards on a monthly basis required an additional 5 min. Although cameras are relatively expensive (~450-750 USD each, depending on make and model), they are likely more cost-effective in the long-term than pitfall trapping as a means of monitoring tunnels, especially if coupled with customized image analysis software.

3.5 Literature cited

Allaback ML, Laabs DM (2003) Effectiveness of road tunnels for the Santa Cruz long-toed salamander. 2002-2003 Transactions of the Western Section of the Wildlife Society 38/39:5-8.

Aresco MJ (2005) Mitigation measures to reduce highway mortality of turtles and other herpetofauna at a north Florida lake. Journal of Wildlife Management 69:549-560.

Ascensão F, Mira A (2007) Factors affecting culvert use by vertebrates along two stretches of road in southern Portugal. Ecological Research 22:57-66.

Austin CC, Shaffer HB (1992) Short-, medium-, and long-term repeatability of locomotor performance in the tiger salamanders *Ambystoma californiense*. Functional Ecology 6:145-153.

Bennett AF, Garland T Jr, Else PL (1989) Individual correlation of morphology, muscle mechanics, and locomotion in a salamander. American Journal of Physiology 256:1200-1208.

Blackwell EA, Cline GR, Marion KR (2004) Annual variation in population estimators for a southern population of *Ambystoma maculatum*. Herpetologica 60:304-311.

Boarman WI, Beigel ML, Goodlett GC, Sazaki M (1998) A Passive Integrated Transponder system for tracking animal movements. Wildlife Society Bulletin 26:886-891.

Braden AW, Lopez RR, Roberts CW, Silvy NJ, Owen CB, Frank PA (2008) Florida Key deer *Odocoileus virginianus clavium* underpass use and movements along a highway corridor. Wildlife Biology 14:155-163.

Cain AT, Tuovila VR, Hewitt DG, Tewes ME (2003) Effects of a highway and mitigation projects on bobcats in Southern Texas. Biological Conservation 114:189-197.

Chan J (1993) Evaluation of methods to reduce road mortality of red-sided garter snakes at Narcisse Wildlife Management Area. Master's thesis, University of Manitoba, Winnipeg, MN.

Charney ND, Letcher BH, Harp A, Warren PS (2009) Terrestrial Passive Integrated Transponder antennae for tracking small animal movements. Journal of Wildlife Management 73:1245-1250.

Chruszcz B, Clevenger A, Gunson K, Gibeau M (2003) Relationships among grizzly bears, highways and habitat in the Banff-Bow Valley, Alberta, Canada. Canadian Journal of Zoology 81:1378-1391.

Clevenger AP, Chruszcz B, Gunson K (2001) Drainage culverts as habitat linkages and factors affecting passage by mammals. Journal of Applied Ecology 38:1340-1349.

Clevenger AP, Waltho N (2000) Factors influencing the effectiveness of wildlife underpasses in Banff National Park, Alberta, Canada. Conservation Biology 14:47-56.

Clevenger AP, Waltho N (2003) Long-term, year-round monitoring of wildlife crossing structures and the importance of temporal and spatial variability in performance studies. In: Proceedings of the International Conference on Ecology and Transportation. Center for Transportation and the Environment, North Carolina State University, Raleigh, NC, pp 293-302.

Clevenger AP, Waltho N (2005) Performance indices to identify attributes of highway crossing structures facilitating movement of large mammals. Biological Conservation 121:453-464.

Corn PS (1994) Straight-line drift fences and pitfall traps. In: Heyer WR, Donnelly MA, McDiarmid RW, Heyek L-AC, Foster MS (eds) Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Smithsonian Institution Press, Washington, D.C., pp 109-117.

Dodd CK Jr, Barichivich WJ, Smith LL (2004) Effectiveness of a barrier wall and culverts in reducing wildlife mortality on a heavily travelled highway in Florida. Biological Conservation 118:619-631.

Dodd NL, Gagnon JW, Manzo AL, Schweinsburg RE (2007) Video surveillance to assess highway underpass use by elk in Arizona. Journal of Wildlife Management 71:637-645.

Donaldson BM (2005) Use of highway underpasses by large mammals and other wildlife in Virginia and factors influencing their effectiveness. In: Proceedings of the International Conference of Ecology and Transportation, San Diego, CA, pp 433-441.

Fitzgibbon KR (2001) An evaluation of corrugated steel culverts as transit corridors for amphibians and small mammals at two Vancouver Island wetlands and comparative culvert trials. Master’s thesis, Royal Roads University, Vancouver, BC.

Ford AT, Clevenger AP, Bennett A (2009) Comparison of methods of monitoring wildlife crossing-structures on highways. Journal of Wildlife Management 73:1213-1222.

Foresman KR (2001) Small mammal use of modified culverts on the Lolo South project of Western Montana. In: Proceedings of the International Conference of Ecology and Transportation, Keystone, CO., pp 342-343.

Foresman KR, Pearson DE (1998) Comparison of proposed survey procedures for detection of forest carnivores. Journal of Wildlife Management 62:1217-1226.

Forman RTT, Sperling D, Bissonette J, Clevenger A, Cutshall C, Dale V, Fahrig L, France R, Goldman C, Heanue K, Jones J, Swanson F, Turrentine T, Winter T (2003) Road ecology: science and solutions. Island Press, Washington, D.C.

Foster ML, Humphrey SR (1995) Use of highway underpasses by Florida panthers and other wildlife. Wildlife Society Bulletin 23:95-100.

Gagnon JW, Theimer TC, Dodd NL, Manzo AL, Schweinsburg RE (2007) Effects of traffic on elk use of wildlife underpasses in Arizona. Journal of Wildlife Management 71:2324-2328.

Gartshore RG, Purchase M, Rook RI, Scott L (2006) Bayview Avenue extension, Richmond Hill, Ontario, Canada habitat creation and wildlife crossings in a contentious climatic setting: a case study. In: Irwin CL, Garrett P, McDermott K (eds) Proceedings of the International Conference on Ecology and Transportation. Center for Transportation and the Environment, North Carolina State University, Raleigh, NC, pp 55-76.

Glista DJ, DeVault TL, DeWoody JA (2009) A review of mitigation measures for reducing wildlife mortality on roadways. Landscape and Urban Planning 91:1-7.

Gloyne CC, Clevenger AP (2001) Cougar *Puma concolor* use of wildlife crossing structures on the Trans-Canada highway in Banff National Park, Alberta. Wildlife Biology 7:117-124.

Grilo C, Bissonette JA, Santos-Reis M (2008) Response of carnivores to existing highway culverts and underpasses: implications for road planning and mitigation. Biodiversity and Conservation 17:1685-1699.

Hardy A, Clevenger AP, Huijser M, Neale G (2003) An overview of methods and approaches for evaluation the effectiveness of wildlife crossing structures: Emphasizing the science in applied science. In: Irwin CL, Garrett P, McDermott K (eds) Proceedings of the International Conference on Ecology and Transportation. Center for Transportation and the Environment, North Carolina State University, Raleigh, NC, pp 319-330.

Harris IM, Mills HR, Bencini R (2010) Multiple individual southern brown bandicoots (*Isoodon obesulus fusciventer*) and foxes (*Vulpes vulpes*) use underpasses installed at a new highway in Perth, Western Australia. Wildlife Research 37:127-133.

Jackson SD, Tyning TF (1989) Effectiveness of drift fences and tunnels for moving spotted salamanders *Ambystoma maculatum* under roads. In: Langton TES (ed) Amphibians and Roads, Proceedings of the toad tunnel conference. ACO Polymer Products, Shefford, UK, pp 93-99.

Jackson SD (1999) Overview of transportation related wildlife problems. In: Evink GL, Garrett P, Zeigler D (eds) Proceedings of the International Conference on Wildlife Ecology and Transportation. Florida Department of Transportation, Tallahassee, FL, pp 1-4.

Jacobson HA, Kroll JC, Browning RW, Koerth BH, Conway MH (1997) Infrared-triggered cameras for censusing white-tailed deer. Wildlife Society Bulletin 25:547-556.

Johnson TR (2000) The amphibians and reptiles of Missouri. Missouri Department of Conservation, Jefferson City, MO.

Kaye DR, Walsh KM, Ross CC (2005) Spotted turtle use of a culvert under relocated Route 44 in Carver, Massachusetts. In: Proceedings of the International Conference of Ecology and Transportation, San Diego, CA, pp 426-432.

Klar N, Herrmann M, Kramer-Schadt S (2009) Effects and mitigation of road impacts on individual movement behavior of wildcats. Journal of Wildlife Management 73:631-638.

Kleist AM, Lancia RA, Doerr PD (2007) Using video surveillance to estimate wildlife use of a highway underpass. Journal of Wildlife Management 71:2792-2800.

Kusak J, Huber D, Gomerčić T, Schwaderer G, Gužvica G (2009) The permeability of highway in Gorski kotar (Croatia) for large mammals. European Journal of Wildlife Research 55:7-21.

LaPoint SD, Kays RW, Ray JC (2003) Animals crossing the Northway: are existing culverts useful? Adirondack Journal of Environmental Studies Spring/Summer:11-17.

Little SJ, Harcourt RG, Clevenger AP (2002) Do wildlife passages act as prey-traps? Biological Conservation 107:135-145.

Locke SL, Cline MD, Wetzel DL, Pittman MT, Brewer CE, Harveson LA (2005) From the field: a web-based digital camera for monitoring remote wildlife. Wildlife Society Bulletin 33:761-765.

Madison DM, Farrand L III (1998) Habitat use during breeding and emigration in radio-implanted tiger salamanders, *Ambystoma tigrinum*. Copeia 1998:402-410.

Mata C, Hervás I, Herranz J, Suárez F, Malo JE (2005) Complementary use by vertebrates of crossing structures along a fenced Spanish motorway. Biological Conservation 124:397-405.

Mata C, Hervás I, Herranz J, Suárez F, Malo JE (2008) Are motorway wildlife passages worth building? Vertebrate use of road-crossing structures on a Spanish motorway. Journal of Environmental Management 88:407-415.

Mougey T (1996) Des tunnels pour batraciens. Le courier de la nature 155:22-28.

Ng SJ, Dale JW, Sauvajot RM, Riley SPD, Valone T (2004) Use of highway undercrossings by wildlife in Southern California. Biological Conservation 115:499-507.

Olsson MPO, Widén P, Larkin JL (2008) Effectiveness of a highway overpass to promote landscape connectivity and movement of moose and roe deer in Sweden. Landscape and Urban Planning 85:133-139.

Parker ID, Braden AW, Lopez RR, Silvy NJ, Davis DS, Owen CB (2008) Effects of US 1 project on Florida Key deer mortality. Journal of Wildlife Management 72:354-359.

van der Ree R, Heinze D, McCarthy M, Mansergh I (2009) Wildlife tunnel enhances population viability. Ecology and Society 14:7.

Rodriguez A, Crema G, Delibes M (1996) Use of non-wildlife passages across a high speed railway by terrestrial vertebrates. Journal of Applied Ecology 33:1527-1540.

Rothermel BB, Semlitsch RD (2002) An experimental investigation of landscape resistance of forest versus old-field habitats to emigrating juvenile amphibians. Conservation Biology 16:1324-1332

Smith C, Pagnucco K, Johnston B, Paszkowski C, Scrimgeour. G. 2010. Using specialized tunnels to reduce highway mortality of amphibians. In: Wagner PJ, Nelson D, Murray E (eds) Proceedings of the International Conference on Ecology and Transportation, Duluth, MN: DECC, pp 583-591..

Spellerberg IF (2002) Ecological Effects of Roads. Science Publisher Inc., Plymouth, UK.

Spight TM (1968) The water economy of salamanders: evaporative water loss. Physiological Zoology 41:195-203

Spotila JR (1972) Role of temperature and water in the ecology of lungless salamanders. Ecological Monographs 42:95-125

Taylor BD, Goldingay RL (2003) Cutting the carnage: wildlife usage of road culverts in north-eastern New South Wales. Wildlife Research 30:529-537.

van Wieren SE, Worm PB (2001) The use of a motorway wildlife overpass by large mammals. Netherlands Journal of Zoology 51:97-105.

Windmiller BS (1996) The pond, the forest, and the city: spotted salamander ecology and conservation in a human-dominated landscape. PhD dissertation, Tufts University, Medford, MA.

Wolf KN, Elvinger F, Pilcicki JL (2003) Infrared-triggered photography and tracking plates to monitor oral rabies vaccine bait contact by raccoons in culverts. Wildlife Society Bulletin 31:387-391.

Xia L, Yang Q, Li Z, Wu Y, Feng Z (2007) The effect of the Qinghai-Tibet railway on the migration of Tibetan antelope *Pantholops hodgsonii* in Hoh-xil National Nature Reserve, China. Oryx 41:352-357.

Yanes M, Velasco J, Suárez F (1995) Permeability of roads and railways to vertebrates: the importance of culverts. Biological Conservation 71:217-22

Table 3.1. Details of studies that monitored crossing structures published between 1989-2009. The presence of specific monitoring methods, detection of study taxa, and duration of study (years) are indicated with an asterisk. Cameras included infrared motion-detecting cameras, still cameras, video cameras, and game counters. Track-plates included substrate made with sand, soot, or dust. Large mammals included any species equal to or larger than coyote (*Canis latrans*).

	Method of monitoring					Taxa observed			
Paper	Camera	Track-plates	Trap	Tags	Visual	Amphibians	Reptiles	Small mammals	Large mammals
Allaback and Laabs 2003			*			*			
Aresco 2005		*			*		*		
Ascensão and Mira 2007		*				*	*	*	*
Boarman et al. 1998				*			*		
Braden et al. 2008	*								*
Brehm 1989			*			*			
Chan 1993			*				*		
Chruszcz et al. 2003			*	*					*
Clevenger and Waltho 2000		*							*
Clevenger and Waltho 2005		*							*
Clevenger et al. 2001		*						*	
Dodd et al. 2004	*	*	*			*	*	*	
Dodd et al. 2007	*								*
Donaldson 2005	*								*
Fitzgibbon 2001		*						*	
Ford et al. 2009	*	*							*
Foresman and Pearson 1998	*	*						*	
Foresman 2001	*							*	
Foster and Humphrey 1995	*						*	*	*
Gagnon et al. 2007	*								*
Gartshore et al. 2006			*			*		*	
Gloyne and Clevenger 2001	*	*							*
Grilo et al. 2008		*						*	
Harris et al. 2010		*		*				*	
Jackson and Tyning 1989					*	*			
Jacobson et al. 1997	*							*	
Kaye et al. 2005				*	*		*		
Klar et al. 2009				*					*
Kleist et al. 2007	*							*	*
Kusak et al. 2009									
LaPoint et al. 2003	*	*						*	
Locke et al. 2005	*							*	*
Mata et al. 2005	*	*				*	*	*	*
Mata et al. 2008		*					*	*	
Ng et al. 2004	*	*						*	*
Olsson et al. 2008				*					*
Parker et al. 2008	*								*
van der Ree 2009				*				*	
Rodriguez et al. 1996		*					*	*	*
Taylor and Goldingray 2003		*				*	*	*	*
van Wieren and Worm 2001		*						*	*
Wolf et al. 2003	*	*						*	*
Xia et al. 2007	*				*				*
Yanes et al. 1995		*				*	*	*	*

Table 3.2. Summary of the number of mammals and herptiles identified using the road tunnels using camera data in 2008 (28 August – 3 November) and 2009 (22 April – 14 October) in Waterton Lakes National Park, Alberta, Canada.

			Number of events	
	Common name	Scientific name	2008	2009
Herptiles	long-toed salamander	*Ambystoma macrodactylum*	2	58
	grey tiger salamander	*Ambystoma mavortium*	1	6
	western toad	*Anaxyrus boreas*	0	5
	wandering garter snake	*Thamnophis elegans vagrans*	5	48
Mammals	deer mouse/shrews/voles	*Peromyscus maniculatus*; *Sorex* spp.; *Arivicola richarsonii*, Clethrionomys gapperi*,* *Microtus* spp., *Phenacomys intermedius**	110	533
	ground squirrels	*Spermophilus* spp. (*S. columbianus*)[#]	101	317
	red squirrel	*Tamiasciurus hudsonicus*	109	35
	chipmunks	*Tamias* spp. (*T. minimus*)[#]	44	280
	snowshoe hare	*Lepus americanus*	113	68
	striped skunk	*Mephitis mephitis*	10	2
	raccoon	*Procyon lotor*	12	0
		TOTAL	**507**	**1352**

* indicates species known to occur in Waterton Lakes National Park that could not be positively identified in images.

[#] indicates species commonly observed in area.

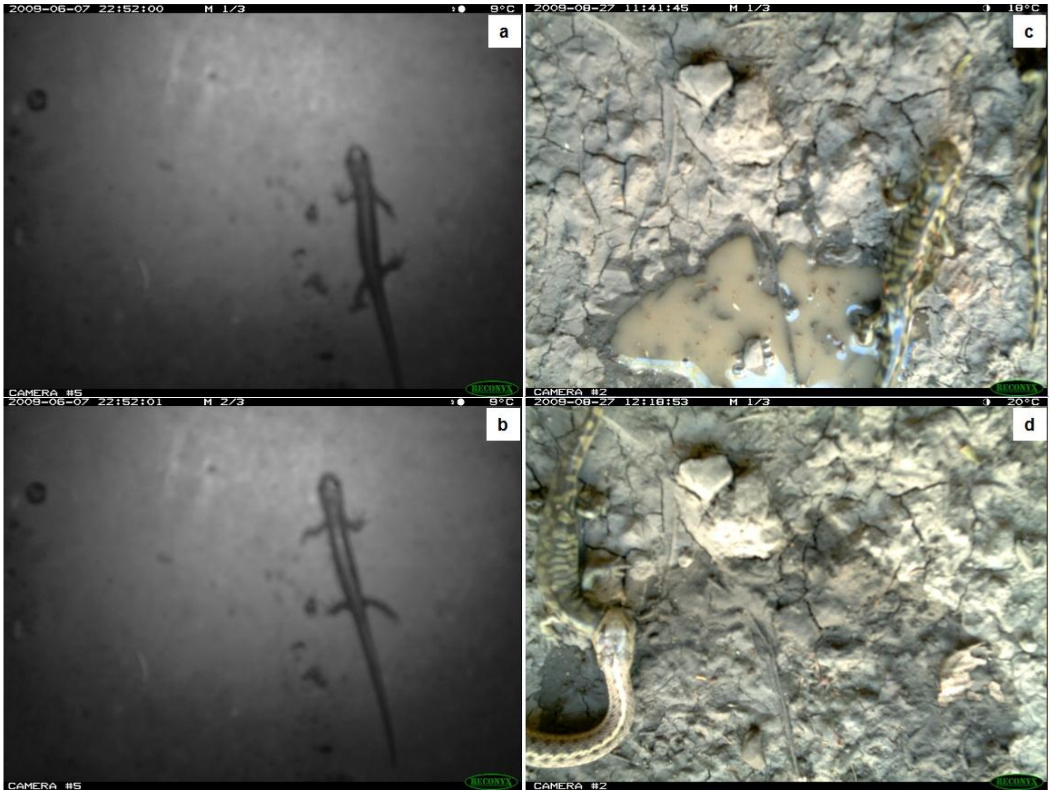

Figure 3.1. Motion-triggered images of a long-toed salamander entering a tunnel at night (a,b), and a predation event documented by tunnel cameras during the day (c,d). Two juvenile grey tiger salamanders enter a tunnel (c), and 37 minutes later, a wandering garter snake drags a juvenile salamander out of the same tunnel (d). Images were time-stamped, making it possible to calculate speed at entrances and crossing speed when an individual was photographed entering and exiting a tunnel.

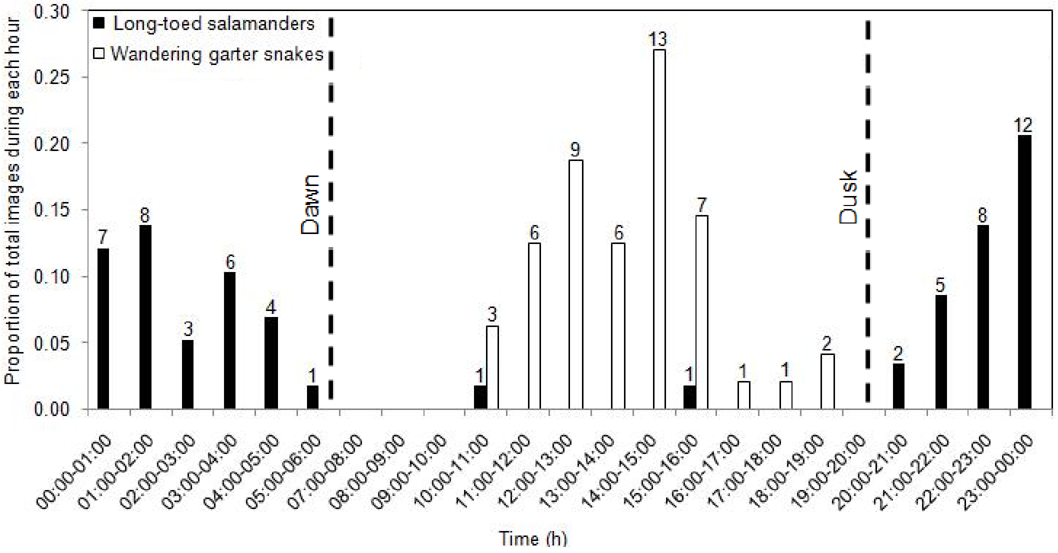

Figure 3.2. Proportion of long-toed salamanders (n=58) and wandering garter snakes (n=48) photographed entering or exiting tunnels at different hours of the day from 2 May – 14 October 2009. Dawn and dusk are indicated by dashed lines. Sample sizes for each species during each hour given above bars.

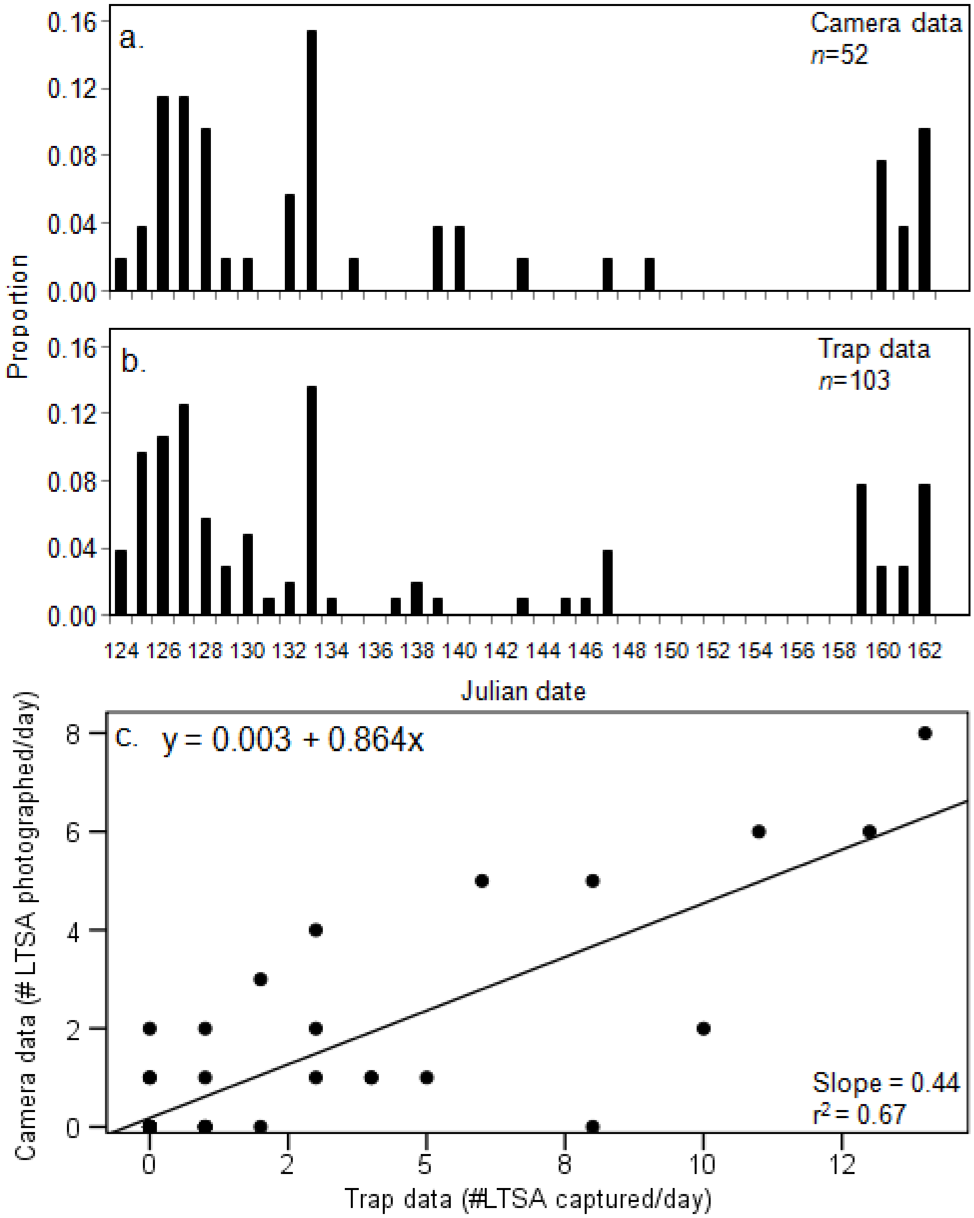

Figure 3.3. Comparison of proportions of long-toed salamanders (LTSA) crossing tunnels in 2009, documented with cameras (a) and exit pitfall traps (b). Linear regressions revealed that measures derived using cameras are highly-correlated to measures derived from trapping (c). Data are restricted to the peak period of migration to and from Linnet Lake (3 May – 10 June).

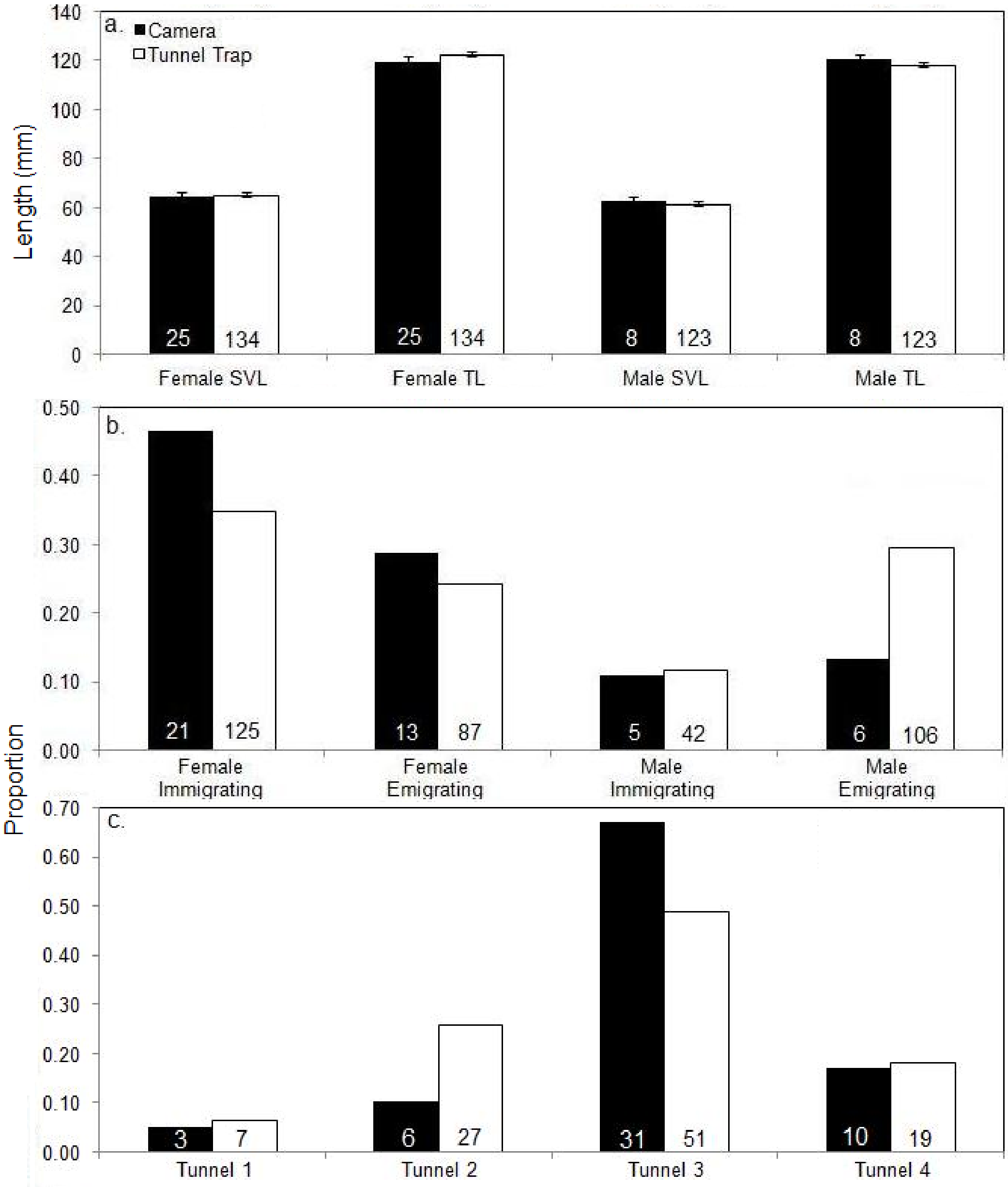

Figure 3.4. Comparisons of (a) mean (± SE) lengths (mm) of long-toed salamanders, (b) sex ratios during immigration and emigration from Linnet Lake, and (c) total individuals using the four tunnels. Data were derived from digital cameras and pitfall traps from 22 April – 19 August 2009. SVL = snout-vent-length; TL = total length. Sample sizes for each group are given at the base of each bar.

CHAPTER 4: EFFECTS OF PREDATION BY SMALL-BODIED FISH ON SURVIVAL AND BEHAVIOUR OF SALAMANDER LARVAE

4.1 Introduction

Fish can affect amphibian larvae directly through predation (Leu et al. 2009), competition (Finlay and Vredenburg 2007), and disease transmission (Kiesecker et al. 2001), and indirectly by reducing activity (Pearson and Goater 2009), increasing refuge use (Sih et al. 1988), or by shifting diel activity patterns (Taylor 1983). These antipredator defences incur costs, including decreased growth, extended larval periods, and reduced size at metamorphosis, which may ultimately affect fitness. Despite the estimation that 93% of Canadian freshwater fish species are capable of consuming amphibian larvae (Scott and Crossman 1973), most research has focused on large, piscivorous fish, such as trout, which are well-established predators of amphibians. The introduction of nonpiscivorous fishes to amphibian breeding sites is also common, however, it is often assumed that small-bodied, gape-limited fish are not key amphibian predators.

The long-toed salamander (*Ambystoma macrodactylum*) occurs in two Canadian provinces, Alberta and British Columbia. In Alberta, it has legal protection as a "species of special concern" (Government of Alberta 2009) because of its limited distribution. Throughout its range, the species shows an allopatric distribution with fish (Funk and Dunlap 1999). Pearson (2003) found that 100% of wetlands in the Castle and Waterton River drainages in southwestern Alberta that contained long-toed salamanders lacked trout and 94% lacked minnows. Under experimental settings, fathead minnows (*Pimephales promelas*) reduced survival of larval salamanders to the same extent as rainbow trout (*Oncorhynchus mykiss*). However, Pearson and Goater (2009) concluded that, unlike trout, minnows likely reduced salamander survival through competition for food rather than direct consumption. As a result of fish predation, many amphibians including long-toed salamanders usually inhabit ephemeral ponds that lack fish (Semlitsch 1987a). However, amphibians utilizing ephemeral ponds must contend with increasing rates of intraspecific competition and cannibalism as resources dwindle (Walls et al. 1993; Wildy et al. 1998), as well as pond drying as a direct source of mortality (Semlitsch 1987b).

Linnet Lake, located in Waterton Lakes National Park (WLNP), represents the rare situation where long-toed salamanders seemingly coexist with fish, in this case, lake chub (*Couesius plumbeus*) and white sucker (*Catostomus commersonii*). The average adult lake chub (the main fish species of interest, see below) is 100 mm in fork length; the largest recorded length for this species in Alberta is 160 mm (Nelson and Paetz 1992). Despite apparent coexistence with fish, this population of long-toed salamanders has declined since 1994 (Fukumoto 1995; Pearson 2002). This decline has usually been attributed to mortality of breeding adult salamanders as they cross a road that separates Linnet Lake from their terrestrial habitat. In 2008, four under-road tunnels were installed by Parks Canada to mitigate road mortalities (Smith et al. 2010). However, if fish predation in Linnet Lake reduces survival of salamander larvae to metamorphosis, the population may still be at risk of extirpation.

My primary objective was to assess the potential effects of a small-bodied fish on a co-occurring population of salamanders using the Linnet Lake system. I combined field observations with laboratory experiments to determine if lake chub had direct (consumptive) and/or indirect (non-consumptive) effects on the resident long-toed salamander population. I estimated the population size and reproductive activity of adult long-toed salamanders at Linnet Lake by conducting surveys and employing standard mark-recapture methods in 2008 and 2009. I also estimated the population size and size distribution of lake chub in 2009 in order to determine the level of predation threat that lake chub pose to larval long-toed salamanders in Linnet Lake. Survival experiments were conducted to determine the capacity of lake chub to consume long-toed salamander larvae. I hypothesized that lake chub were capable of consuming salamander larvae and that survival of larvae in the presence of fish would be negatively-correlated with lake chub size and positively-correlated with larva size. Antipredator behaviour experiments were conducted in aquaria to determine non-consumptive effects of lake chub on long-toed salamander larvae. I hypothesized that salamander larvae would perceive lake chub as predators, and that larval salamanders would reduce activity, increase refuge use, and alter their position in aquaria (horizontally and vertically) in the presence of lake chub.

4.2 Methods

4.2.1 Study sites

Field work was conducted at three sites within Waterton Lakes National Park: Linnet Lake (49°04'N, 113°54'W), and 2 reference sites: Stable Pond (49°04'N, 113°53'W) and Red Rock Roadside Pond (49°06'N, 113°58'W). Linnet Lake, the primary study site (Figure 4.1), is a small (3.5 ha), shallow (max depth = 5 m) water body situated between Middle and Upper Waterton Lakes. Stable Pond, located 1.4 km northeast of Linnet Lake, is a fishless, ephemeral pond (0.06 ha) that can dry completely by mid-July, as was the case in 2009 (K. Pagnucco, personal observation). Red Rock Roadside Pond is a fishless, permanent pond (0.1 ha), located 7.4 km northwest of Linnet Lake.

4.2.2 Field observations

4.2.2.1 Long-toed salamander population size

To collect data on abundance, size-frequency distribution and reproductive output of long-toed salamanders, I designed a drift fence and pitfall trap system to intercept animals migrating to and from Linnet Lake. I installed drift fencing on both sides of a 500 m section of the Entrance Road that parallels Linnet Lake (Figure 4.1). Fences consisted of 1 m high silt fencing buried 15 cm to prevent salamanders from digging beneath. Pitfall traps were 8 L plastic buckets (25 cm in diameter, 25 cm in height) buried flush with the soil surface. In 2008, 44 pitfall traps were installed along the fences (Figure 4.1), and were checked daily from 14 April - 14 October. In 2009, traps were removed and I collected salamanders during nightly searches from 3 May – 16 June by patrolling the fences. In both years, I determined the age-class, sex, snout-vent-length (SVL; mm), total length (TL; mm) and mass (g ± 0.1 g) of each captured salamander before marking and releasing it. I used Visible Implant Elastomer (VIE; Northwest Marine, Shaw, WA) to give each adult salamander a unique mark. Immediately after measuring and marking, salamanders were released on the opposite side of the road, in the direction that they were headed.

4.2.2.2 Lake chub population size and size frequency distribution

Minnow traps (42 cm long by 22.5 cm in diameter, 2.5 cm diameter opening, and 6 mm wire mesh) were used to capture larval amphibians and fish in Linnet Lake. In addition to lake chub, traps also caught smaller numbers of white suckers. Unlike lake chub, suckers are unable to reproduce in Linnet Lake and larger individuals are highly susceptible to low winter oxygen conditions (K. Pagnucco, personal observation), thus I chose to focus on interactions between lake chub and salamanders. Minnow traps were set for 55 d in 2008 (16 June-14 August), and 27 d in 2009 (22 May-29 July 2009). Ten minnow traps were distributed along the perimeter of Linnet Lake, at 100 m intervals (Adams et al. 1997) and checked every 24 h to ensure unbiased sampling of organisms with diurnal and nocturnal activity patterns. I identified and counted all fish captured in each trap and measured fork length of 10 randomly chosen lake chub from each trap. Fish were released at the site of capture. In 2009, all lake chub were marked with a single fin clip to the caudal fin to estimate population size.

4.2.2.3 Evidence of long-toed salamander recruitment

Visual encounter surveys were conducted in 2008 and 2009 to assess long-toed salamander reproductive activity at Linnet Lake and the 2 fishless reference sites. At each site, I conducted surveys 3 times during the egg-laying period (mid-April through June), and 3 more times in the summer (June to August) to monitor larvae. During each survey, 2 observers walked the shoreline of the water body searching for eggs or larval long-toed salamanders. Egg mass locations were marked and I determined % egg survival 48 h after each survey. I counted and recorded the post-hatching developmental stage (Watson and Russell 2000) of all larval long-toed salamanders encountered. Larval surveys were followed by sampling with minnow traps for one 24 h period. Ten minnow traps were distributed around Linnet Lake in the same manner as for fish sampling, whereas three minnow traps were set along the perimeter of Stable Pond and Red Rock Roadside Pond at equal intervals.

4.2.3 Laboratory experiments

4.2.3.1 Larval salamander survival experiments

I conducted 140 laboratory trials to evaluate larval long-toed salamander survival when exposed to a lake chub or a conspecific larva. These experiments examined 2 main factors: predator identity and larval salamander size. Predator identity was comprised of 5 treatments that paired a single long-toed salamander larva with: i) a large non-predatory invertebrate, tadpole shrimp (*Lepidurus couesii*; 17-23 mm; n=28), ii) a similar-sized conspecific larva, or a iii) small (range, 69-73 mm; n=28), iv) medium (80-84 mm, n=28), or v) large (90-96 mm, n=28) lake chub. Small lake chub likely represented 1-yr-old fish, whereas medium and large lake chub were 2-yr or older (Becker 1983). The tadpole shrimp treatment acted as a “control” to provide a baseline of larval salamander survival in the absence of predation, which I used to compare survival in the conspecific larva and fish treatments. Larval long-toed salamanders have been shown to be cannibalistic under both natural and laboratory conditions (Walls et al. 1993). Therefore, I considered the “conspecific larva treatment” as exposure to a familiar potential predator. During my field work in 2008 and 2009, I did not encounter any larval long-toed salamanders displaying the cannibalistic morphologies described by Walls et al. (1993), such as longer and wider heads and larger vomerine teeth. Thus, only the typical morph (i.e. non-cannibalistic) of long-toed salamander larvae were used in experiments. Larval salamanders were divided into 4 size classes based on total length: 10-19 mm (n=10), 20-29 mm (n=54), 30-39 mm (n=37), and 40-49 mm (n=39). Sample sizes for various larval sizes varied based on availability at the source site.

I used dipnets and minnow traps to collect larvae from Red Rock Roadside Pond in the morning prior to the start of each set of experiments in

2009 (22, 26 June; 6, 12, 17, 29 July; 5 August). Twelve lake chub were obtained from Linnet Lake and 4 tadpole shrimp were collected from Stable Pond on each date. All fish, tadpole shrimp and salamander larvae were maintained in aerated, 20-L aquaria (40 cm x 20 cm x 25 cm) filled with dechlorinated tap water at 14°C prior to commencement of trails. For each trial, treatments were conducted simultaneously in a random spatial arrangement of 20 8-L buckets on a laboratory bench. Each treatment was replicated 4 times during each set of trials. Buckets were filled with dechlorinated tap water at 14°C and were subjected to a 12:12 h photoperiod. One larval salamander was added to each bucket at 1600 h. At 1800 h, a tadpole shrimp, conspecific larva, or lake chub was added to each bucket. Buckets were monitored every 6 h for larval survival. At 96 h, fish and tadpole shrimp were removed and survival of larval salamanders was evaluated. Larvae remaining at the end of trials were given a “survival period” of 96 h. Individual larvae and fish were used only once.

4.2.3.2 Larval salamander antipredator behaviour experiments

These experiments were designed to test the effects of time-of-day, food availability, and the presence of a potential predator on long-toed salamander behaviour in a 2 x 2 x 4 factorial design. There were 2 time-of-day treatments (day and night) and 2 food availability treatments (unfed and fed). Predator identity comprised 4 treatments that paired a single long-toed salamander larva with: i) a tadpole shrimp, ii) a similar-sized conspecific larva, iii) a lake chub, or iv) no added individuals (control). Each time-of-day, food, and predator identity treatment combination was replicated 8 times, for a total of 128 trials. Each organism was used once.

I used dipnets and minnow traps to collect salamander larvae (total length: mean ± SE, 28.7 ± 0.10 mm; n=160) and tadpole shrimp (22.6 ± 1.71 mm; n=32) from Stable Pond. The sizes of larvae used in these experiments were based on those available in Stable Pond. I used average-sized lake chub (82.1 ± 0.10 mm; n=32), based on fish caught with minnow traps in Linnet Lake from 1-6 June 2009 (82.0 ± 0.08 mm; n=270). Trials were conducted in 8 aerated, 20-L aquaria (40 cm x 20 cm x 25 cm) filled with dechlorinated tap water at 14°C. Each aquarium was divided into a prey and a predator section. A divider made of 2 mm aluminum mesh was installed lengthwise in each aquarium to separate two-thirds of the tank (prey section) from the other third (predator section). A plastic aquarium plant (15 cm in height) was added to the center of the prey section to act as a refuge for the larva. A cover of 2 mm aluminum mesh was attached to the top of aquaria to prevent fish from escaping.

Larvae were added to aquaria 12 h prior to the beginning of each trial in order to allow acclimation and resumption of normal behaviour. Trials lasted 12 h (day treatment: 0600–1800 h; night treatment: 1800–0600 h). Overhead fluorescent lights were turned on for the duration of day trials. During night trials, artificial lighting was turned off, and windows were covered with black sheets to block out natural light. Relative locations of treatment and aquaria on the laboratory bench were randomized for each trial to eliminate bias. Trials for the day treatment were conducted 16-23 June 2009, and trials for the night treatment were conducted 24 June-1 July 2009. At the beginning of each trial, potential "predators" were added to the predator section (which remained empty in control treatments), and tubifex worms were added *ad libitum* to both the prey and predator sections of aquaria during "fed" treatments. A 4 x 4 cm grid (50 squares) drawn onto each aquarium cover was used to describe the horizontal position of each larva and potential “predator”. The refuge was positioned in the center of the prey section and occupied 2 grid squares. A larva was considered to be “in refuge” if it was located in either of these 2 squares. A larva was considered to be “adjacent to the predator enclosure” if it was located in any grid square abutting the partition. The vertical position of each larva and potential “predator” was estimated by the observer as being either in the lower-, middle-, or upper-third portion of the water column. Observations were made every 2 h, beginning 2 h after the potential “predator” was added, for a total of 6 observation periods per trial. During each period, point observations were made at each tank every minute for 4 minutes, for a total of 24 point observations per trial. Observations during night trials were made using a headlamp with an infrared light. During each point observation, the observer recorded: larva activity (still or swimming), horizontal position of larva and predator (grid square), vertical position of larva and predator (lower, middle, or upper). Using these data, I created four response variables based on the proportion of time each larva spent: i) swimming, ii) in the refuge, iii) adjacent to the predator enclosure, and iv) at the bottom of the aquarium. The 4 response variables were not mutually exclusive.

4.2.4 Statistical analyses

Estimates of population size for lake chub and long-toed salamanders were made using the Schnabel estimator, a multiple-occasion adaptation of the Lincoln Index (Krebs 1999).

I tested for differences in proportion survival among predator identity treatments and among larval size-classes, as well as the interactions between these factors, with the G-test of independence (Sokal and Rohlf 1981). A second

G-test of independence was performed excluding control and conspecific larva treatments to compare the 3 fish-size treatments. Two-way analysis of variance (ANOVA) using a General Linear Model (GLM) was used to evaluate whether length of larval salamander survival period (h) varied as a function of predator identity and/or larva size.

Point observations from the antipredator behaviour experiments were combined into 2 within-subjects factors: short exposure (observations from the first 6 h of the trial) and long exposure (observations from the last 6 h of the trial). The effects of exposure duration (short exposure/long exposure), time-of-day (day/night), food availability (unfed/fed) and predator identity (control/tadpole shrimp/conspecific larva/lake chub) on larval salamander behaviour were examined using repeated-measures MANOVA using a GLM. To meet the assumptions of parametric tests, proportion data were arcsine square-root transformed prior to analyses (Zar 1999). Wilks' λ was used to determine statistical significance in MANOVA. Tukey's HSD was used to determine differences between pairs of means. Exposure time and food availability had no significant effects on any of the response variables and were therefore not included as factors in the final models. All statistical tests were executed with SPSS v.16 (SPSS Inc., Chicago, USA) and were deemed to be statistically significant at $P < 0.05$.

4.3 Results

4.3.1 Long-toed salamander population size

In 2008, I captured 445 adult long-toed salamanders migrating to Linnet Lake, and 172 returning to their terrestrial habitat (102 of which were previously marked). In 2009, I captured 91 salamanders migrating to Linnet Lake, and 128 returning (40 of which were previously marked). Using the Schnabel Method, I estimated the breeding population of salamanders to be 1492 (95%CI: 1243 – 1865) in 2008, and 1372 (95%CI: 1045 – 2001) in 2009. Sexual maturity occurs when long-toed salamanders are between 43-50 mm in SVL (Russell et al. 1996), thus I considered salamanders <40 mm in SVL to be juveniles. I captured no juvenile salamanders in 2008, and 1 individual in 2009 (SVL, 36.3 mm) which was likely 1-2 yrs old.

4.3.2 Lake chub population size and size frequency distribution

In 2008, I captured 8394 lake chub over 55 d, 10 traps set per day to generate a CPUE (number of individuals x $d^{-1}trap^{-1}$) value of 15.26. In 2009, I captured 3436 lake chub over 27 d, generating a CPUE value of 12.73. Using the Schnabel Method and mark-recapture data collected in 2009, the estimated population size for lake chub vulnerable to trapping was 4148 individuals (95% CI: 3795 – 4572). Length of trapped lake chub over the 2 yrs ranged from 38.9 mm to 132.0 mm. Lake chub were on average larger in 2008 than in 2009. Mean fork length (mm) of lake chub was 87.5 ± 0.01 (n=2607) in 2008 and 79.1 ± 0.02 (n=1721) in 2009.

4.3.3 Evidence of long-toed salamander recruitment

In 25 h of visual encounter surveys at Linnet Lake, I found 4 long-toed salamander egg masses (50 eggs) in 2008 and 4 egg masses (121 eggs) in 2009. In 2009, I found 78 egg masses (992 eggs) in Stable Pond during 3 h of surveying and 3 egg masses (81 eggs) in Red Rock Roadside Pond during 3 h of surveying. No eggs found at Linnet Lake remained after 48 h in either year. Egg survival after 48 h was 59.1 ± 0.8% at Stable Pond and 72.8 ± 0.4% at Red Rock

Roadside Pond. Egg mortality at Stable Pond and Red Rock Roadside Pond was likely due to infection. In all cases of egg mass mortality at Linnet Lake, traces of eggs were completely absent; neither outer jelly nor embryos remained. During an additional 20 h of visual encounter surveys and 82 d of minnow trapping in both 2008 and 2009, no long-toed salamander larvae were found in Linnet Lake. In 2009, 3 h of surveying yielded 93 salamander larvae in Stable Pond and 198 larvae in Red Rock Roadside Pond.

4.3.4 Larval salamander survival experiments

Larval size affected the degree of association between predator identity and survival (G-test of independence, χ^2=98.3, df = 31, P<0.001). Survival tended to decrease with increasing larval size in the conspecific treatment, and increase with increasing larval size across all three fish treatments (Figure 4.2). While larval salamanders between 40-49 mm cannibalized 88% of equal-sized conspecific larvae, cannibalism was not seen for larvae <20 mm. While 100% of larvae between 40-49 mm in length survived in fish treatments, 100% of larvae in the smallest size class were consumed by fish within 54 h (Figure 4.2a). Overall larval survival decreased with increasing lake chub size (G-test, χ^2= 7.8, df=2, P<0.05; Figure 2a). Small fish only consumed 21% of larvae, whereas medium and large fish consumed 43% and 57% of larvae, respectively. All larvae in control tanks survived to the end of the trials.

Survival duration (h) differed significantly among predator identity treatments (two-way ANOVA, F = 5.5, P<0.05) and larval sizes (F = 4.1, P<0.05). Medium fish (Tukey's HSD, mean ± SE, 24.5 ± 0.97 hrs, n=12, P<0.05) and large fish (26.6 ± 0.83 h, n=16, P<0.05) predated larvae more quickly than did small fish (60.0 ± 1.95 h, n=6; Figure 2b). Survival duration tended to increase with increasing larval size for all fish treatments, but decreased with increasing larval size for the conspecific treatment (Figure 2b). The interaction between predator identity and larval size was not significant for survival duration (*F*=0.4, *P* >0.05).

4.3.5 Larval salamander antipredator behaviour experiments

There was a significant effect of time-of-day and predator identity on larval behaviour (Table 4.1). Salamander larvae were more active and spent more time in refugia during the day than at night (Table 4.1; Figure 4.3a,b). Even when alone in the aquaria, larvae swam 10% of the time in the day and 7% at night. Larvae spent more time immediately adjacent to the predator enclosure at night (29%) than during the day (19%, Table 4.1; Figure 4.3c). There was no overall effect of time-of-day on the proportion of time larvae spent on the bottom of aquaria (Table 4.1, Figure 4.3d).

The effects of predator identity on larval salamander behaviour were driven by the lake chub treatment. When lake chub were present in the tank, larvae spent significantly less time swimming and increased refuge use relative to all other treatments (Figure 4.3a,b). Larvae spent significantly less time adjacent to the predator enclosure and at the bottom of aquaria when a lake chub was present (Figure 4.3c,d).

The significant interaction between time-of-day and predator identity (Table 4.1) arose because larvae used refugia disproportionately more in the day compared to the night in the lake chub treatment. In the presence of lake chub, salamander larvae spent 65% of their time in refugia in the day compared to 35% of their time at night (Figure 4.3b). Larvae also spent disproportionately more time at the bottom of aquaria when a lake chub was present in the night compared to the day (Figure 4.3d).

Multiple pairwise comparisons using Tukey’s HSD showed that the behaviour of larval salamanders paired with conspecifics did not differ from the behaviour of larvae that were alone or paired with non-predatory invertebrates (Figure 4.3).

4.4 Discussion

Previous studies suggest that many salamanders are unable to coexist with large-bodied, piscivorous fishes due to predation, or with small-bodied fish due to competition (Pilliod and Peterson 2001; Pearson and Goater 2009). Linnet Lake represents a rare example of coexistence between long-toed salamanders and fish. In my study, I quantified size and size structure of lake chub and long-toed salamander populations resident to Linnet Lake. I then conducted laboratory experiments to determine: i) the capacity of lake chub to consume long-toed salamander larvae, and; ii) the non-consumptive effects of lake chub on long-toed salamander larvae.

4.4.1 Field observations of a declining salamander population with limited juvenile recruitment

Fukumoto (1995) estimated the population size of adult long-toed salamanders breeding at Linnet Lake to be 3856 (95%CI: 3274 – 4690) in 1994, indicating a possible population decrease of more than 60% during the past 14 years. Consistent with a declining population of long-toed salamanders in Linnet Lake that suffered from low recruitment, I found very few egg masses, and no larval long-toed salamanders despite extensive sampling in 2008 and 2009. In contrast, I encountered large numbers of egg masses and larvae during surveys at other sites. In Linnet Lake, all egg masses I found disappeared within 48 h.

Long-toed salamander egg masses are typically attached to emergent vegetation (Graham and Powell 1999) and should persist at a site in the absence of predation. The disappearance of entire egg masses (including the indigestible gelatinous outer membrane) suggests predation by fish, as they typically ingest entire eggs and either expel egg jelly during mastication or pass it through the digestive system (Monello and Wright 2001). In contrast, invertebrates access amphibian embryos by scraping away the egg membrane, leaving the egg jelly behind (Gamradt and Kats 1996). Most salamander egg mortality at Stable Pond and Red Rock Roadside Pond was due to infection, likely the water mould, *Saprolegnia ferax* (Blaustein 1994). During my 2-yr study I only caught 1 juvenile long-toed salamander at Linnet Lake in June 2009. Using similar but less intensive sampling methods, Fukumoto (1995) captured 52 newly-transformed juvenile long-toed salamanders in Linnet Lake in 1993-1994, indicating that I should have encountered young animals if they were present. Other studies have shown that the introduction of fish is typically accompanied by reduced abundance of all life stages of long-toed salamanders (Tyler et al*.* 1998; Knapp and Matthews 2000).

4.4.2 Direct predation by lake chub on salamander larvae

The effects of small-bodied fish on the survival of larval salamanders have been largely unexplored and few studies have documented the ability of smaller fish species to consume larval amphibians. However, small goldfish (*Carassius auratus*; Monello and Wright 2001), threespine stickleback (*Gasterosteus aculeatus*; Laurila et al. 2006), sunbleak (*Leucaspius delinieatus*; Leu et al. 2009), golden topminnow (*Fundulus chyrosotus*), and mosquitofish (*Gambusia* spp.; Baber and Babbitt 2003) have all been shown to directly predate larval amphibian species, occasionally leading to population reductions (Kats and Ferrer 2003). My laboratory trials showed that lake chub between 70-100 mm in length are capable of consuming larval salamanders up to 40 mm SVL. Metamorphosis from larval to adult body form typically occurs when long-toed salamander larvae are approximately 50 mm in total body length (Sheppard 1977). Assuming that some lake chub in Linnet Lake were able to reach the maximum reported size for the species in Alberta (>160 mm; Nelson and Paetz 1992), and were not sampled because they were too large to be caught by minnow traps, larval salamanders likely never reach a body size that exceeds the "predation window," and experience predation by lake chub throughout their entire larval period. Several studies have shown that due to gape-limitation, consumption rates of amphibian larvae by various invertebrate and fish predators decrease with increasing larval size and increase with increasing predator size (Semlitsch 1987a; Laurila et al*.* 2006; Leu et al*.* 2009), as I also documented.

The abundance of lake chub between 70 – 100 mm (the size range of lake chub demonstrated to be capable of consuming larvae) in Linnet Lake suggests that the species presents a real threat to long-toed salamander larvae. Other studies have demonstrated that direct predation by fish limits juvenile recruitment and contributes to declines in amphibian populations (Gamradt and Kats 1996; Eaton et al. 2005). Benthic-feeding white suckers may also predate on long-toed salamanders during early life stages, particularly salamander eggs.

4.4.3 Effects of lake chub on larval salamander behaviour

One of the most widespread behaviours displayed by prey to avoid predators is the use of refugia (Sih et al*.* 1988). Increased use of refugia is associated with reductions in foraging time and subsequent food acquisition, which can lead to decreased growth, slower development and ultimately, lower fitness (Orizaola and Braña 2003). As expected, larval salamanders altered their behaviour in the presence of lake chub by reducing their activity levels and by occupying areas of aquaria that were located at greater distances from these potential predators. Larvae in my study were naïve to fish prior to experimentation. Therefore, salamander larvae may have altered behaviour either in response to perceived predator risk, or simply in response to an unknown stimulus. Further experimentation involving a treatment that pairs long-toed salamander larva with a non-predatory fish that the larva has not previously encountered is required in order to examine the possibility that long-toed salamander larvae only express avoidance behaviours in the presence of predatory fish.

By using live lake chub in a system allowing visual contact and water flow, my experiment was not designed to determine which senses long-toed salamanders use to perceive potential predators; other amphibian species have been shown to respond to both visual and chemical cues (Chivers 1997; Wildy and Blaustein 2001). Stangel and Semlitsch (1987) demonstrated that smallmouth salamander (*Ambystoma texanum*) larvae responded to visual and/or chemical cues from predatory fish in aquaria within hours of initial exposure, despite having never encountered fish prior to experiments. In addition, ambystomid larvae can detect movement in surrounding water using the lateral line system (Parichy 1996). The ability of long-toed salamander larvae to detect and respond to the presence of fish using this sensory mode may explain the change in larval behaviour during the night trials, when lake chub were most active, but visual cues were limited. Being able to recognize novel predators and respond accordingly is particularly advantageous for organisms, such as ambystomid larvae, whose environments contain a wide range of predators (Gonzalo et al*.* 2007).

Assuming that both foraging and predation rates are higher in well-illuminated waters during the day (Nicieza 2000), salamander larvae must allocate daytime activity to balance the benefits of increased foraging rates with the costs of greater predation risk. In many amphibians, foraging is greatly reduced when the risk of predation is perceived to be high (Holomuzki 1989). In my study, salamander larvae were more active during the day than at night in all predator identity treatments, including the control, a solitary larva. This contradicts both my hypothesis and the literature, which indicate that larval ambystomids are typically more active at night (Branch and Altig 1981). However, larvae in my experiments spent very little time swimming in general (time spent swimming never exceeded 11%). Larvae used in this study were native to a small ephemeral pond where the costs of reduced foraging bouts are exacerbated because the pond dries by mid-summer. A greater cost for reduced diurnal foraging activity compared to increased predation risk has been demonstrated by Petranka (1983) for the smallmouth salamander.

4.4.4 Cannibalism increased with increasing larval size

Cannibalism by larval long-toed salamanders has been well-documented (Walls et al*.* 1993; Chivers et al*.* 1997; Wildy et al*.* 1998), and is often accompanied by changes in morphology (Walls et al*.* 1993). In my study, both the proportion of cannibalized larvae and the rate that larvae were cannibalized increased with increasing larval size. Large amphibian larvae have been shown to be more aggressive towards conspecific larvae than smaller larvae in contests over limited resources, presumably in an effort to offset higher overall metabolic demands linked to increased body size (Polis 1988). Larval salamanders did not display antipredator behaviours in the presence of a conspecific animal (i.e. their behaviour did not differ significantly than from either the control or tadpole shrimp treatments), despite the fact that cannibalism resulted in mortality equal to that imposed by medium-sized fish in my study. Wildy and Blaustein (2001) demonstrated that larval long-toed salamanders naïve to cannibalism fail to display antipredator behavioural responses in the presence of injured or cannibalistic conspecific animals. Since amphibian larvae frequently encounter members of their own species in breeding ponds, larvae that attempted to avoid all conspecific animals would spend most of their time immobile or in refuges and subsequently experience reduced foraging opportunities.

4.4.5 Coexistence of long-toed salamanders and lake chub

I propose that the persistence of the long-toed salamander population at Linnet Lake relies on a low-level of successful reproduction when fish are present, and occasional episodes of high recruitment when fish populations are absent or low because of winterkills. Lake chub, as well as other fish species, have the opportunity to invade Linnet Lake during periodic, likely decadal, conditions of high spring water levels when flooding connects Linnet Lake with Middle Waterton Lake. Bouts of winter hypoxia at Linnet Lake, triggered by extended winter conditions (i.e. increased duration of ice cover and accumulation of snow) and/or an episode of high primary production during the growing seasons, also likely result in winterkills that either reduce or eliminate fish populations (Danylchuk and Tonn 2003). The lake chub population can eventually recover from winterkill through reproduction of small individuals that were able to survive winter hypoxia, or through re-colonization during future flooding events. However, if most of the larger, sexually-mature lake chub winterkilled, several years of decreased predation risk for salamander eggs and larvae from lake chub may then permit higher rates of survival to metamorphosis and recruitment of juvenile salamanders into the Linnet Lake population (Eaton et al 2005). Given that the maximum lifespan of long-toed salamanders is 10 yrs (Russell et al. 1996), populations may persist for a modest length of time without successful reproduction or immigration. However, if fish periodically extirpate long-toed salamanders from Linnet Lake, the site may be re-colonized by dispersers from other ponds (see Funk and Dunlap 1999); Stable Pond, for example, is <2 km away. Ultimately, lake chub and long-toed salamanders may coexist in Linnet Lake because of a dynamic interplay between extirpation, driven by either abiotic or biotic factors, punctuated by episodes of re-colonization and/or strong local recruitment.

4.5 Literature cited

Adams MJ, Richter KO, Leonard WP (1997) Surveying and monitoring amphibians using aquatic funnel traps. In: Olson DH, Leonard WP, Bury RB (eds) Sampling amphibians in lentic habitats: methods and approaches for the Pacific Northwest. Society for the Northwestern Vertebrate Biology, Olympia, WA, pp 47-54.

Baber MJ, Babbitt KJ (2003) The relative impacts of native and introduced predatory fish on a temporary wetland tadpole assemblage. Oecologia 136:289-295.

Becker GC (1983) Fishes of Wisconsin. University of Wisconsin Press, Madison, WI.

Binckley CA, Resetarits WJ Jr (2002) Reproductive decisions under threat of predation: squirrel treefrog (*Hyla squirella*) responses to banded sunfish (*Enneacanthus obesus*). Oecologia 130:157-161.

Blaustein AR, Hokit DG, O'Hara RK, Holt RA (1994) Pathogenic fungus contributes to amphibian losses in the Pacific Northwest. Biological Conservation 67:251-254.

Branch LC, Altig R (1981) Nocturnal stratification of three species of *Ambystoma* larvae. Copeia 1981:870-873.

Chivers DP, Kiesecker JM, Wildy EL, Anderson MT, Blaustein AR (1997) Chemical alarm signalling in terrestrial salamanders: intra- and interspecific responses. Ethology 103:599-613.

Danylchuk AJ, Tonn WM (2003) Natural disturbances and fish: local and regional influences on winterkill of fathead minnows in boreal lakes. Transactions of the American Fishery Society 132:289-298.

Eaton BR, Tonn WM, Paszkowski CA, Danylchuk AJ, Boss SM (2005) Indirect effects of fish winterkills on amphibian populations in boreal lakes. Canadian Journal of Zoology 83:1532-1539.

Finlay JC, Vredenburg VT (2007) Introduced trout sever trophic connections in watersheds: consequences for a declining amphibian. Ecology 88:2187-2198.

Fukumoto J (1995) Long-toed salamander (*Ambystoma macrodactylum*) ecology and management in Waterton Lakes National Park. MSc. thesis, University of Calgary, Calgary, AB.

Funk WC, Dunlap WW (1999) Colonization of high-elevation lakes by long-toed salamanders (*Ambystoma macrodactylum*) after the extinction of introduced trout populations. Canadian Journal of Zoology 77:1759-1767.

Gamradt SC, Kats LB (1996) Effect of introduced crayfish and mosquitofish on California newts. Conservation Biology 10:1155-1162.

Gonzalo A, López P, Martín J (2007) Iberian green frog tadpoles may learn to recognize novel predators from chemical alarm cues of conspecifics. Animal Behaviour 74:447-453.

Government of Alberta (2009) Species assessed by Alberta's Endangered Species Conservation Committee: short list. Available online: http://www.srd.alberta.ca/BioDiversityStewardship/SpeciesAtRisk/SpeciesSummaries/documents/SpeciesAssessedbyESCC-Short%20List-Dec03-2009.pdf.

Graham KL, Powell GL (1999) Status of the long-toed salamander (*Ambystoma macrodactylum*) in Alberta. Alberta Wildlife Status Report No. 22. Alberta Environmental Protection, Fisheries and Wildlife Management Division, and Alberta Conservation Association, Edmonton, Alberta, Canada.

Holomuzki JR (1989) Predation risk and macroalga use by the stream-dwelling salamander *Ambystoma texanum*. Copeia 1989:22-28.

Kats LB, Ferrer RP (2003) Alien predators and amphibian declines: review of two decades of science and the transition to conservation. Diversity and Distributions 9:99-110.

Kiesecker JM, Blaustein AR, Miller CL (2001) The transfer of a pathogen from fish to amphibians. Conservation Biology 15:1064-1070.

Knapp RA, Matthews KR (2000) Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. Conservation Biology 14:428-438.

Krebs CJ (1999) Ecological methodology. 2nd edn. Benjamin Cummings, Menlo Park, CA.

Laurila A, Pakkasmaa S, Merilä J (2006) Population divergence in growth rate and antipredator defences in *Rana arvalis*. Oecologia 147:585-595.

Leu T, Lüscher B, Zumbach S, Schmidt BR (2009) Small fish (*Leucaspius delineates*) that are often released into garden ponds and amphibian breeding sites prey on eggs and tadpoles of the common frog (*Rana temporaria*). Amphibia-Reptilia 30:290-293.

Monello RJ, Wright RG (2001) Predation by goldfish (*Carassius auratus*) on eggs and larvae of the eastern long-toed salamander (*Ambystoma macrodactylum columbianum*). Journal of Herpetology 35:350-353.

Nelson JS, Paetz MJ (1992) The fishes of Alberta. 2nd edn. University of Alberta Press, Edmonton, Alberta.

Nicieza AG (2000) Interacting effects of predation risk and food availability on larval anuran behaviour and development. Oecologia 123:497-505.

Orizaola G, Braña F (2003) Response of predator-naïve newt larvae to food and predator presence. Canadian Journal of Zoology 81:1845:1850.

Parichy DM (1996) Pigment patterns of larval salamanders (Ambystomatidae, Salamandridae): the role of the lateral line sensory system and the evolution of pattern-forming mechanisms. Developmental Biology 175:265-282.

Pearson KJ (2002) Linnet Lake long-toed salamander (*Ambystoma macrodactylum*) roadkill prevention and population estimation project summary report, Waterton Lakes National Park, 2001. Unpublished technical report. Parks Canada Agency, Waterton Lakes Nationbal Park, AB.

Pearson KJ (2003) Distribution and habitat associations of the long-toed salamanders (*Ambystoma macrodactylum*) in the Oldman River Drainage. Alberta Sustainable Resource Development, Fish and Wildlife Division, Alberta Species at Risk Report No. 75, Edmonton, AB, 25 pp.

Pearson KJ, Goater CP (2009) Effects of predaceous and nonpredaceous introduced fish on the survival, growth, and antipredation behaviours of long-toed salamanders. Canadian Journal of Zoology 87:948-955.

Petranka JW (1983) Fish predation: a factor affecting the spatial distribution of a stream-breeding salamander. Copeia 1983:624-628.

Pilliod DS, Peterson CR (2001) Local and landscape effects of introduced trout on amphibians in historically fishless watersheds. Ecosystems 4:322-333.

Polis GA (1988) Exploitation competition and the evolution of interference, cannibalism, and intraguild predation in age/size-structured populations. In: Ebenman B, Persson L (eds) Size-structured populations. Springer, Berlin, pp 185–202.

Russell AP, Powell GL, Hall DR (1996) Growth and age in Alberta long-toed salamanders (*Ambystoma macrodactylum krausei*): a comparison of two methods of estimation. Canadian Journal of Zoology 74:397-412.

Scott WB, Crossman EJ (1973) Freshwater fishes of Canada. Bulletin of the Fisheries Research Board of Canada No. 184.

Semlitsch RD (1987a) Interactions between fish and salamander larvae. Costs of predator avoidance or competition? Oecologia 72:481-486.

Semlitsch RD (1987b) Relationship of pond drying to the reproductive success of the salamander *Ambystoma talpoideum*. Copeia 1987:61-69.

Sheppard RF (1977) The ecology and home range movements of *Ambystoma macrodactylum krausei* (Amphibia: Urodela). MSc. thesis, University of Calgary, Calgary, AB.

Sih AJ, Petranka W, Kats LB (1988) The dynamics of prey refuge use: a model and tests with sunfish and salamander larvae. American Naturalist 132:463-483.

Smith C, Pagnucco K, Johnston B, Paszkowski C, Scrimgeour. G . 2010. Using specialized tunnels to reduce highway mortality of amphibians. In: Proceedings of the International Conference on Ecology and Transportation, Duluth, MN, pp 583-591.

Sokal RR, Rohlf FJ (1981) Biometry, the principles and practice of statistics in biological research. W.H. Freeman and Company, New York, NY.

Stangel PW, Semlitsch RD. 1987. Experimental analysis of predation on the diel vertical migrations of a larval salamander. Canadian Journal of Zoology 65:1554-1558.

Taylor JT (1983) Orientation and flight behaviour of a neotenic salamander (*Ambystoma gracile*) in Oregon. American Midland Naturalist 109:40-49.

Tyler TJ, Liss WT, Ganio LM, Larson GL, Hoffman R, Deimling E, Lomnicky G (1998) Interaction between introduced trout and larval salamanders (*Ambystoma macrodactylum*) in high-elevation lakes. Conservation Biology 12:94-105.

Walls SC, Belanger S, Blaustein AR (1993) Morphological variation in a larval salamander: dietary induction of plasticity in head shape. Oecologia 96:162-168.

Watson S, Russell AP (2000) A posthatching developmental staging table for the long-toed salamander, *Ambystoma macrodactylum krausei*. Amphibia-Reptilia 21:143-154.

Wildy EL, Blaustein AR (2001) Learned recognition of intraspecific predators in larval long-toed salamanders *Ambystoma macrodactylum*. Ethology 107:479-493.

Wildy EL, Chivers DP, Kiesecker JM, Blaustein AR (1998) Cannibalism enhances growth in larval long-toed salamanders (*Ambystoma macrodactylum*). Journal of Herpetology 32:286-289.

Zar JH (1999) Biostatistical Analysis. 4th edn. Prentice Hall, Upper Saddle River, NJ.

Table 4.1. Results of MANOVA and individual ANOVAs for effects of predator identity ("predator"), and time-of-day ("time") on behaviour of larval long-toed salamanders. The predator treatments were: (a) 1 larva; (b) 1 larva, 1 tadpole shrimp; (c) 2 larvae; (d) 1 larva, 1 lake chub. Variables of larval behaviour included the proportion of time larvae spent: swimming ("swimming"), in refugia ("refuge use"), adjacent to the predator enclosure ("predator enclosure"), and in the lower-third of the aquaria ("bottom"). Statistically non-significant main factors were excluded.

Variable	Source of variation	*df*	Wilk's λ	*F*	*P*
Multivariate analysis	Predator	12, 310	0.54	6.80	0.000***
	Time	4, 117	0.79	7.64	0.000***
	Predator x Time	12, 310	0.84	1.80	0.047*
		df	MS	*F*	*P*
Univariate analyses					
Swimming	Predator	3	0.25	6.15	0.001*
	Time	1	0.24	6.04	0.015*
	Predator x Time	3	0.04	0.93	0.428
	Error	120	0.04		
Refuge use	Predator	3	3.72	25.97	0.000***
	Time	1	2.32	16.21	0.000***
	Predator x Time	3	0.45	3.13	0.028*
	Error	120	0.14		
Predator enclosure	Predator	3	0.26	2.50	0.063
	Time	1	1.46	14.06	0.000***
	Predator x Time	3	0.06	0.59	0.625
	Error	120	0.10		
Bottom	Predator	3	0.17	1.86	0.140
	Time	1	0.37	2.72	0.101
	Predator x Time	3	0.43	3.18	0.026*
	Error	120	0.14		

* significant at alpha <0.05

***significant at alpha <0.0001

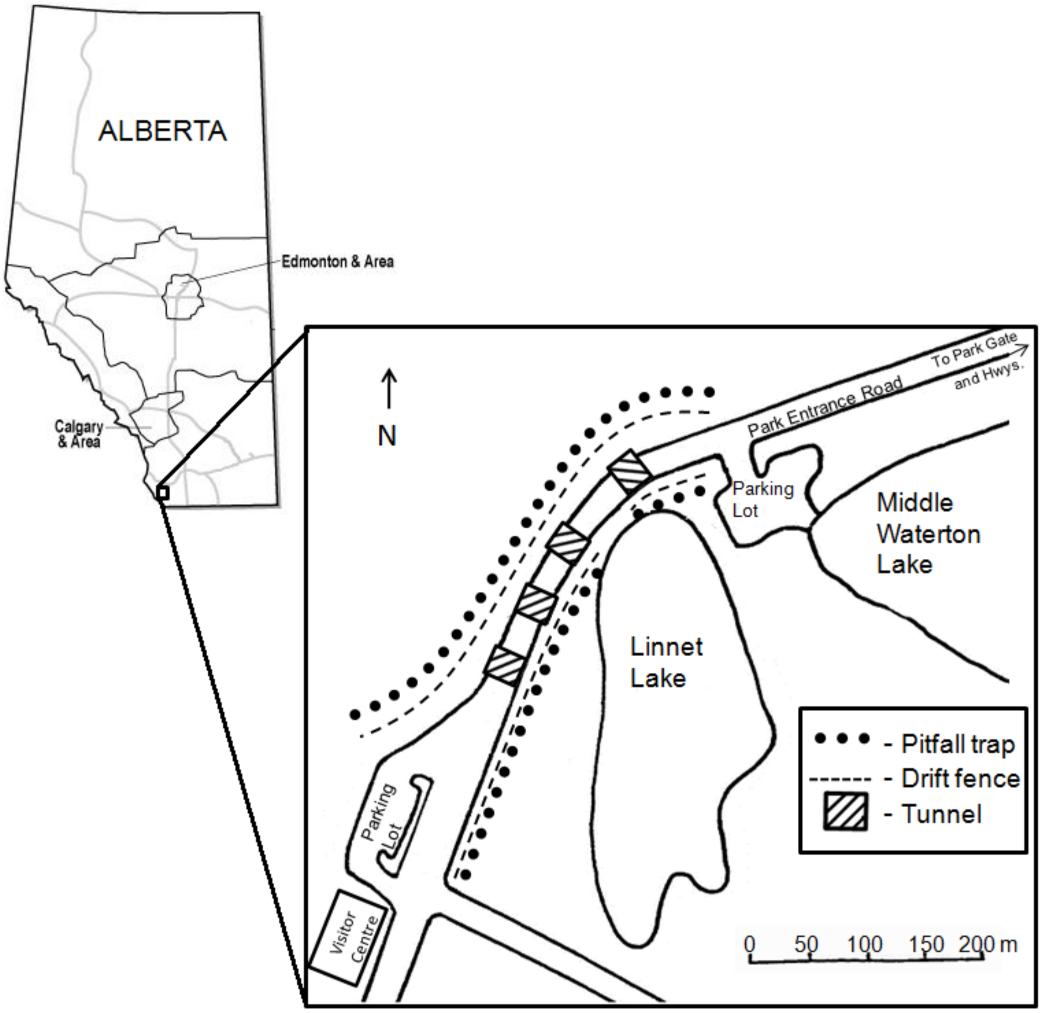

Figure 4.1. Map of Linnet Lake area including locations of under-road tunnels, pitfall traps, and drift fences in 2008. Adapted from Fukumoto (1995); Figure 3.

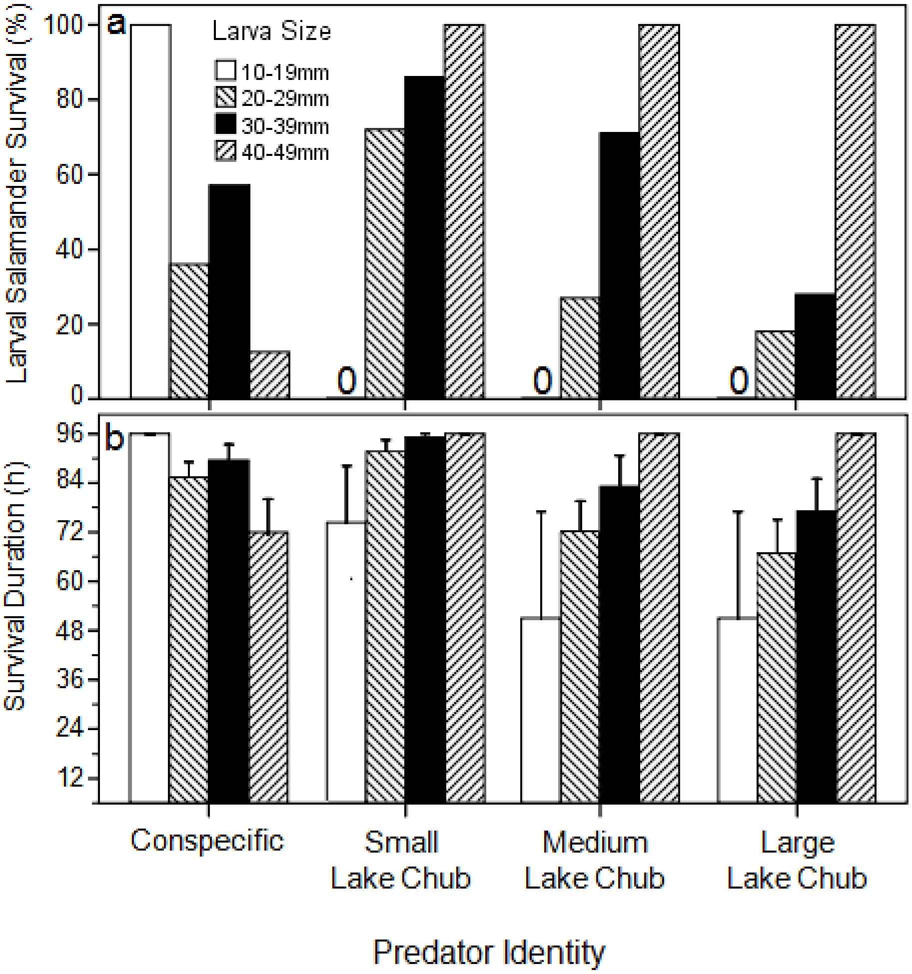

Figure 4.2. (a) Survival (%) and (b) mean (±SE) survival duration (h) for long-toed salamander larvae paired with an equal-sized conspecific (n=28), or a small (range, 69-73 mm, n=28), medium (80-84 mm, n=28), or a large lake chub (90-96 mm, n=28). All larvae in control treatments survived to the end of each trial.

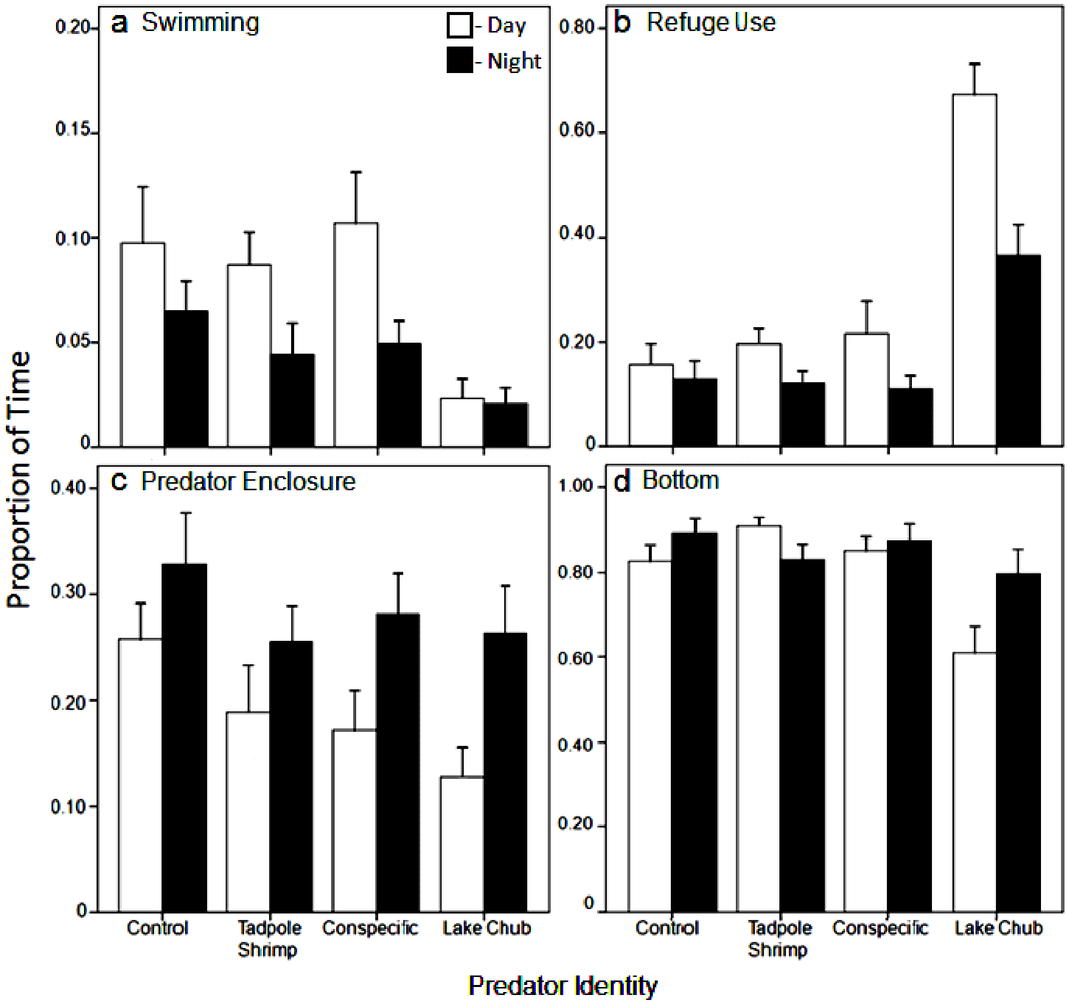

Figure 4.3. Mean (±SE) proportion of time long-toed salamander larvae spent: (a) swimming; (b) in refugia; (c) adjacent to predator enclosure; (d) at the bottom (lower-third) of aquaria during trials under different predator identity treatments during different times of day; n= 16 for each combination of treatment and time regime.

CHAPTER 5: SUMMARY AND MANAGEMENT RECOMMENDATIONS

5.1 Summary of main findings

Chapter 2 - Characterizing the spatio-temporal use of tunnels and movement patterns of long-toed salamanders in Waterton Lakes National Park

Installation of under-road tunnels along with directional drift fencing leading to each tunnel entrance resulted in appreciable reductions in vehicle-caused mortality of long-toed salamanders (*Ambystoma macrodactylum*). In both 2008 and 2009, less than 2% of the long-toed salamanders that attempted to cross the Entrance Road were killed by vehicles. This represents a reduction in salamander road mortality of 80% from a previous study in 1994, prior to installation of fences and tunnels (Fukumoto 1995).

Salamanders and other amphibians used the tunnels to a modest extent during the first full year following their installation. Combining camera and tunnel exit trap data, at least 130 long-toed salamanders used the tunnels when migrating between overwintering and breeding habitat in 2009. The four tunnels were not used equally: half of all salamanders caught in tunnel exit traps used Tunnel 3.

My model of the probability of individuals that crossed through tunnels showed that long-toed salamanders were almost 20 times more likely to use tunnels during immigration to Linnet Lake compared to emigration. My results showed that 23% (21 of 91) of immigrating and 1% (2 of 179) of emigrating long-toed salamanders that encountered drift fences were subsequently captured in exit traps after presumably moving through the tunnels. These values were similar to the rate of tunnel effectiveness reported for Santa Cruz long-toed salamanders (*A. m. croceum*; Allaback and Laabs 2003; 9%), but far lower than the tunnel effectiveness reported for spotted salamanders (*A.*

maculatum; Jackson and Tyning 1989; 68%). One explanation for differences in tunnel effectiveness may be the different monitoring techniques used. Both Allaback and Laabs (2003) and I used pitfall traps to monitor tunnel use, whereas Jackson and Tyning (1989) visually-monitored tunnel exits for salamanders. This latter method ensures that the vast majority of salamanders using tunnels are detected. In contrast, my tunnel cameras documented 26 salamanders using tunnels that were not subsequently captured in exit traps. This suggests that many long-toed salamanders using the tunnels escaped capture in pitfall traps.

An alternative explanation for why 20 times more salamanders used tunnels during immigration is that although breeding adult salamanders that overwinter west of the Entrance Road must cross the road to access Linnet Lake, crossing may not be necessary for animals to complete their annual cycles. My data suggest that some adult salamanders successfully overwinter in areas east of the road and do not annually migrate across the road.

Relations between spatial variability in salamander captures with habitat variables were much stronger during immigration from overwintering terrestrial habitats to Linnet Lake than during emigration from Linnet Lake to terrestrial habitats. Moving through shaded, moist habitats may be more important during immigration when precipitation is intermittent, as opposed to during emigration, when almost all salamander movement coincided with some precipitation, resulting in extensive areas of saturated soils. Consequently, variability in moisture among different habitat types may have been low during the late spring when the majority of emigration occurred.

Chapter 3 – Using cameras to monitor amphibian tunnel use: an informative, cost-efficient technique

Although they usually did not trigger the motion-detectors of digital cameras installed at tunnel entrances, long-toed salamanders were effectively detected moving through tunnels using cameras programmed for timed-interval image capture. However, not all amphibians failed to trigger motion-detectors. In fact, all records of adult western toads (*Anaxyrus boreas*) and grey tiger salamanders (*Ambystoma mavortium*) were captured by motion-triggered images, probably reflecting their larger size and higher movement speeds.

My results showed that camera data and exit trap data produced equivalent patterns in several metrics of abundance and activity, including descriptions of: i) temporal patterns in tunnel use; ii) body size and proportion of males and female long-toed salamanders immigrating to and emigrating from Linnet Lake, and; iii) spatial variation related to the use of the 4 tunnels. In addition, camera data were used to calculate speed and exact time-of-day of amphibian crossings as well as predation in tunnels, which trapping alone could not. Camera images suggested that tunnels are not likely to act as significant predator traps, as long-toed salamanders' use of tunnels is separated both temporally (seasonal and diel separation) and spatially from use by predatory wandering garter snakes (*Thamnophis elegans vagrans*).

Appreciable overlap in the types of information provided by traps and cameras, and the fact that cameras provide information, suggest that cameras represent a novel approach and effective method of monitoring tunnel use by amphibians. Additionally, while numbers of long-toed salamanders detected by cameras were lower than with traps, these monitoring methods were highly-correlated. Although cameras are moderately-expensive (~450-750 USD), they are more cost-effective and a less invasive technique in the long-term than pitfall trapping for monitoring tunnel use by amphibians.

Chapter 4 - Effects of a small-bodied fish on survival and behaviour of long-toed salamander larvae

Although tunnels may reduce salamander road mortalities, this was not the only additive source of mortality that the Linnet Lake population of long-toed salamander was facing. A substantial population of lake chub (*Couesius plumbeus*), a potential predator of long-toed salamander eggs and larvae, is also resident to Linnet Lake. I estimated the breeding population of salamanders to be 1492 (95%CI= 1243 – 1865) in 2008, indicating over a 60% decrease since 1994 (3856 individuals; 95%CI = 3274 – 4690; Fukumoto 1995). Consistent with a declining population of long-toed salamanders in Linnet Lake that suffered from low recruitment, I found very few egg masses, and no larval long-toed salamanders despite extensive sampling in 2008 and 2009. In contrast, I encountered large numbers of egg masses and larvae during surveys at other sites within Waterton Lakes National Park.

Survival experiments showed that lake chub can readily consume salamander larvae. The abundance of moderate-sized lake chub between 70 – 100 mm (the size range of lake chub we demonstrated were capable of consuming salamander larvae) in Linnet Lake suggests that they represent a real threat to the survival of long-toed salamander larvae.

Behaviour experiments showed that salamander larvae responded to lake chub presence by reducing activity and increasing refuge use. These antipredator defences may incur costs that may ultimately affect larval fitness. Larval salamanders did not display antipredator behaviours in the presence of conspecifics, despite the fact that cannibalism resulted in mortality equal to that imposed by medium-sized lake chub in my study. Salamander larvae in my study may not have perceived a threat from conspecific individuals and consequently did not alter their behaviour.

My study was one of few that have documented the ability of a native small-bodied fish to consume amphibian larvae, and establish a possible link between predation and population consequences in terms of young-of-the-year recruitment and reductions in the population size of long-toed salamanders. The coexistence of fish and salamanders in Linnet Lake may result from an interplay between periodic extirpation of fish by winter hypoxia and of salamanders by fish predation, punctuated by episodes of strong recruitment or re-colonization by long-toed salamanders.

5.2 Management implications

5.2.1. Population declines and the importance of continued monitoring of the Linnet Lake long-toed salamander population

Fukumoto (1995) estimated the population size of adult long-toed salamanders breeding at Linnet Lake to be 3856 (95%CI: 3274 – 4690) in 1994. In 2001, a short-term mark-recapture study resulted in a population estimate of 289 adults (Pearson 2002). I estimated the breeding population of salamanders to be 1492 (95%CI: 1243 – 1865) in 2008, and 1372 (95%CI: 1045 – 2001) in 2009, indicating a possible population decrease of about 65% during the past 15 y. Since past mark-recapture studies have been separated by several years where virtually no monitoring occurred, it is impossible to know whether the population has been: i) steadily decreasing, ii) decreased from 1994 to 2001, but has since been increasing, or iii) fluctuating from year to year due to a variety of interacting factors.

In order to reach a better understanding of the trajectory of this population, I recommend that Parks conduct mark-recapture studies of long-toed salamanders every 3 - 5 years to generate a robust estimate of population size, while monitoring with cameras could be used to generate estimates of the population’s relative abundance, age structure, and sex ratios on an annual or semi-annual basis.

5.2.2 Detection of salamanders in tunnels

Camera and trap data revealed that at least 130 long-toed salamanders passed through tunnels in 2009. Given the 2009 population estimate of 1372 individuals, only 10% of the estimated breeding adult population of long-toed salamanders were recorded using tunnels. This suggests that either: i) long-toed salamanders frequently passed through tunnels without being detected by either monitoring technique, or ii) many long-toed salamanders avoided passing through tunnels.

Tunnel exit traps were intended to capture all long-toed salamanders that travelled through tunnels. However, my analysis of camera data revealed that this did not happen: images documented 26 salamanders using tunnels that were not subsequently captured in exit traps. This represented 47% of all salamanders photographed in the tunnels and suggests that many long-toed salamanders using the tunnels escaped capture in exit traps.

Given these limitations, I would recommend visually-monitoring tunnel exits on 5-10 nights when migrations are expected (as per Jackson and Tyning 1989), which ensures that the vast majority of individuals moving through tunnels are documented. These data would allow Parks staff to calibrate how many animals escape detection by cameras and traps, and ultimately gain an improved understanding of relative abundance of a variety of species.

5.2.3 Reducing possible tunnel avoidance by long-toed salamanders

My study showed that since very few animals were found alive or dead along the Entrance Road, it is unlikely that the majority of breeding salamanders, which evaded detection by cameras and traps, were circumventing the tunnels and drift fences when migrating to and from Linnet Lake. In addition, my results showed that long-toed salamanders moved at a slower rate at tunnel entrances than their average crossing speed, suggesting that long-toed salamanders may hesitate at tunnel entrances. Salamanders may have moved slowly along the bare concrete at entrances, but increased speeds once inside the sand-lined tunnel.

I suggest that the addition of substrate (e.g. fine sand or organic matter such as leaf litter, wood chips or peat moss) at tunnel entrances may result in increased tunnel use by long-toed salamanders and other amphibians. Continued monitoring is needed to determine if tunnel use by long-toed salamanders increases through time, as individuals becomes increasingly familiar with these new structures, and tunnels accumulate organic matter and provide conditions that more closely resemble natural substrates.

5.2.4 Use of visual observations to evaluate possible avoidance of drift fences

My results indicated that long-toed salamanders could travel along drift fences up to 78 m to access and successfully cross tunnels. However my models of tunnel use revealed that long-toed salamanders were more likely to cross tunnels the closer they were to tunnel entrances when initially found. This suggests that roadside drift fences may have been acting as a barrier to long-toed salamander movement. In some situations, the barrier effect of fences might be even more harmful than the mortality due to vehicle collisions when there is no fence (Jaeger and Fahrig 2004). If the majority of salamanders are failing to reach Linnet Lake because they are being deterred by fencing, the resulting decrease in reproductive output and juvenile recruitment may be more harmful to the population than adult road mortalities.

In future studies, Park researchers could use visual observation or radio-telemetry to determine whether long-toed salamanders are turning around along fences or at tunnel entrances. If further research confirms that having to travel long distances along drift fences causes long-toed salamanders to turn back and forego breeding, Parks staff should consider either using shorter lengths of drift fences, or putting “holes” along the fence to allow some salamanders to pass. An alternative would be to only include drift fencing directing towards Tunnel 3 and Tunnel 4, where 84% of all long-toed salamanders crossed.

5.2.5 Protection from fish predation

My laboratory experiments discussed in Chapter 4 clearly show that a broad size-range of lake chub readily consume long-toed salamander larvae. My field observations over 2 years also indicated that recruitment of young salamanders to the Linnet Lake population is exceedingly low. In fact, despite extensive field surveys, I located no young-of-the-year and only 1 juvenile salamander, despite the presence of a reasonably large population of adult long-toed salamanders. Taken together, these data suggest that it is highly likely that predation by lake chub may currently limit population growth of long-toed salamanders in Linnet Lake, and that unless the current levels of predation are reduced, the persistence of the Linnet Lake population of long-toed salamanders is questionable.

Therefore, I recommend that Parks Canada consider substantially reducing the size of the lake chub population in Linnet Lake. One option would be to live-trap lake chub using minnow traps and transplant them back to Middle Waterton Lake, which is presumably where they originated. Live-trapping could be completed in 5-7 days. Additionally, Parks staff could enhance recruitment of long-toed salamanders by placing egg masses inside enclosures, in order to protect egg masses from fish predation. Enclosures could be removed once hatchlings become mobile and free-feeding, with the hope that older larvae would be able to evade predatory lake chub.

5.2.6 Salamander habitat protection

My results showed that a substantial cohort of the Linnet Lake population of long-toed salamanders overwinter close to Linnet Lake and did not migrate across the Entrance Road. While mechanical removal of invasive plant species has continued to the west of the Entrance Road, where long-toed salamanders have long been thought to overwinter (Pearson 2002), herbicide use has continued on other sides of Linnet Lake (K. Pagnucco, personal observation).

My results also showed that Stable Pond and Red Rock Roadside Pond contained large numbers of long-toed salamander eggs and larvae in 2008 and 2009. These other breeding sites may be important at contributing to overall juvenile recruitment at a metapopulation level. If fish periodically extirpate long-toed salamanders from Linnet Lake, the site may be re-colonized by dispersers from other ponds, such as Stable Pond (see Funk and Dunlop 1999). Stable Pond is <2 km away from Linnet Lake, well within the distance that long-toed salamanders can travel (Funk and Dunlop 1999). However, no measures are currently being taken to protect these potential source populations from habitat degradation and the use of herbicides.

Thus, I recommend that Parks staff review their removal of invasive species, including spraying, in key locations to ensure that these practices do not negatively affect long-toed salamanders and the habitats associated with Linnet Lake and other known breeding sites within the Park.

5.2.7 Installing future amphibian tunnels

My results showed that over 80% of all long-toed salamanders photographed using tunnels in 2009 used either Tunnel 3 or Tunnel 4. The majority of long-toed salamanders captured in 2008 were also found in areas adjacent to where Tunnel 3 and Tunnel 4 were later installed, indicating that these areas were “hotspots” of salamander movement during at least two successive breeding migrations. In 2009, only 5% of all salamanders using tunnels were recorded at Tunnel 1, which was at a location identified earlier as a movement corridor (Hewitt 2005).

Given the high costs of road tunnels, I recommend that in the future Parks Canada use mark-recapture techniques similar to those employed in my study to evaluate the effectiveness of tunnel locations prior to installation. Although preliminary surveys were conducted in 2001 and 2005 to assess priority sites for future installation of tunnels, either: i) these studies were insufficient to accurately reveal locations of actual “hotspots” of salamander movement, or ii) main corridors of salamander movement change dramatically, even over a few years, due to habitat changes or selective survival of animals using different routes. Upfront, well-timed efforts to optimize the location of tunnels would likely result in substantial cost-savings and greater benefits to animals using tunnels. The fundamental decision to install tunnels and to maintain them at all rests on the perceived importance and uniqueness of the conserved population.

5.3 Literature cited

Allaback ML, Laabs DM (2003) Effectiveness of road tunnels for the Santa Cruz long-toed salamander. 2002-2003 Transactions of the Western Section of the Wildlife Society 38/39:5-8.

Fukumoto J (1995) Long-toed salamander (*Ambystoma macrodactylum*) ecology and management in Waterton Lakes National Park. MSc. thesis, University of Calgary, Calgary, AB.

Funk WC, Dunlap WW (1999) Colonization of high-elevation lakes by long-toed salamanders (*Ambystoma macrodactylum*) after the extinction of introduced trout populations. Canadian Journal of Zoology 77:1759-1767.

Hewitt T (2005) Proposal for amphibian underpass on the Entrance Road near Linnet Lake, WLNP. Unpublished technical report. Parks Canada, Waterton Lakes National Park, Alberta, Canada, 16pp.

Jackson SD, Tyning TF (1989) Effectiveness of drift fences and tunnels for moving spotted salamanders *Ambystoma maculatum* under roads. In: Langton TES (ed) Amphibians and Roads: Proceedings of the toad tunnel conference. ACO Polymer Products, Shefford, UK, pp 93-99.

Jaeger JAG, Fahrig L (2004) Effects of road fencing on population persistence. Conservation Biology 18:1651-1657.

Pearson K (2002) Linnet Lake long-toed salamander (*Ambystoma macrodactylum*) roadkill prevention and population estimation summary report. Unpublished technical report. Parks Canada, Waterton Lakes National Park, Alberta, Canada, 32pp.