THE EFFECTS OF INTRODUCED FISH ON THE LONG-TOED SALAMANDER (*AMBYSTOMA MACRODACTYLUM*) IN SOUTHWESTERN ALBERTA, CANADA

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Abstract

Species that are introduced outside of their native ranges are an important threat to biodiversity. In southwestern Alberta, Canada, sport and bait fish have been introduced into most waterbodies. I examined the effects of introduced trout and minnows on the distribution, demography and behaviour of larval long-toed salamanders through a combination of field surveys, laboratory experiments and an outdoor mesocosm experiment. Results from field surveys at 30 high-elevation ($\geq$ 1500 m) lakes confirmed previous studies showing an allopatric distribution of trout and long-toed salamanders. The same pattern was also documented at 27 low-elevation (< 1500 m) ponds. In a mesocosm experiment, salamander survival was significantly reduced in ponds containing trout or minnows. Surprisingly, larvae exposed to minnows were 28-65% smaller than larvae in control ponds, suggesting strong interspecific competition for zooplankton prey. In a series of laboratory studies, trout preyed directly on salamander hatchlings and larvae, whereas minnows injured hatchlings but did not consume them. In laboratory aquaria, salamander larvae spent significantly more time within a refuge when exposed to minnow cues, but showed no behavioural response to trout. This confirmed my expectation that long-toed salamanders lack specific behavioural responses to trout, but respond generally to disturbances within the water column. Thus, direct predation and a lack of specific antipredator behaviour are among the likely mechanisms responsible for the observed allopatric distribution of trout and long-toed salamanders. My data also show that gape-limited fish reduce growth and survival of salamanders, perhaps more so than trout, through mechanisms such as competition and behavioural alteration.

Preface

In the end we will conserve only what we love; we will love only what we understand; and we will understand only what we have been taught.

Baba Dioum

When one tugs at a single thing in nature, he finds it attached to the rest of the world.

John Muir

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CHAPTER 1: GENERAL INTRODUCTION

Species that have been introduced outside of their native ranges are an important threat to biodiversity (Coblentz 1990; Richter 1997). Understanding the magnitude and extent of this threat is a major focus in conservation biology. Particularly well-documented is the role of introduced fish in amphibian population declines (Blaustein and Kiesecker 2002; Kats and Ferrer 2003). For example, allopatric distributions of non-native trout (*Oncorhynchus* spp.) and amphibians have been described in field surveys (Funk and Dunlap 1999; Knapp 2004) and hatchery-reared trout are known to transfer pathogens to amphibians (Kiesecker et al. 2001). Mesocosm experiments indicate strong and persistent effects of predatory fish on the survival and biomass of amphibian larvae (Semlitsch 1987; Semlitsch 1993). Introduced green sunfish (*Lepomis cyanellus*) and brown trout (*Salmo trutta*) reduced amphibian survival in field manipulations (Sih et al. 1992; Gillespie 2001) and oviposition preference studies show that adult amphibians avoid waterbodies that contain fish (Kats and Sih 1992; Hopey and Petranka 1994). Moreover, laboratory studies have indicated that in addition to fish-induced predation on amphibian eggs and larvae (Gamradt and Kats 1996; Monello and Wright 2001), indirect effects of fish on larval amphibian behaviour, especially on activity and refuge use (Stangel and Semlitsch 1987; Semlitsch and Reyer 1992) and food resource competition (Semlitsch 1987; Werner and Anholt 1996) can also lead to reductions in amphibian growth and reproduction. Thus, the evidence is overwhelming that both direct and indirect effects of introduced fish play important roles in determining amphibian distribution and abundance.

However, there are a number of shortcomings in our knowledge of the effects of fish on amphibians. One shortcoming is that most studies have evaluated the effects of single fish species and few comparisons have thus been made of the consequences that various fish species present to amphibians. Moreover, most research has focused on fish that are well known predators of amphibians (e.g., northern pike, *Esox lucius*, trout, *Onchorhynchus* spp. and bass, *Micropterus* spp.), despite the estimation that 93% of Canadian freshwater fishes are capable of consuming amphibian larvae (based on Scott and Crossman 1973) and evidence that shows the importance of indirect effects. Common introduced predators of amphibian larvae, mosquitofish (*Gambusia affinis*) have been shown to reduce the mass of frog tadpoles by causing injuries or reduced feeding behaviour (Gamradt and Kats 1996; Lawler et al. 1999). Previous studies have also demonstrated the indirect, negative effects of mosquito larvae (Blaustein and Marglait 1994; Mokany and Shine 2003) and crayfish (Gamradt et al. 1997) on amphibians. Throughout North America, various species of small-bodied cyprinid minnow have been introduced into lentic habitats outside of their native ranges (Crossman 1991). Although a small proportion of these introductions are likely a result of flooding, most occur as approved translocations or unauthourized introductions of baitfish or hobby fish (Crossman 1991). Though the small gape of most minnows limits them to feeding mainly on algae, zooplankton and small insects (Scott and Crossman 1973), considerable potential may exist for introduced minnows to impair amphibian populations through indirect mechanisms such as interspecific competition. Yet the effects of minnows on amphibians remain to be examined.

A second shortcoming is that many studies of the effects of introduced fish on amphibians, particularly in western North America, have involved high-elevation (> 1500 m above sea level) predator/prey populations. High-elevation lakes tend to have simpler habitat structure than those at lower elevations (Nelson et al. 1995), which may intensify the effects of fish predation on amphibians (Crowder and Cooper 1982; Diehl 1992). Further, amphibian larvae may require up to 3 years to metamorphose in high-elevation waterbodies, whereas metamorphosis typically takes place within one season at lower elevations (Licht 1975; Howard and Wallace 1985). Larvae are therefore exposed to potential fish predators for shorter periods within low-elevation habitats, while larvae at high-elevation sites containing fish must avoid predation over several years. Given these differences, the effects of fish on amphibians at low-elevation habitats may differ greatly from the reported impacts of introduced trout on high-elevation amphibian populations. Yet low-elevation habitats have been overlooked by previous studies on the effects of introduced fish on amphibians in areas where large elevation gradients exist. This is an especially significant shortcoming if low-elevation habitats support highly productive amphibian source populations and thereby play important roles in maintaining regional populations (Pulliam 1988).

A final shortcoming is that most previous studies have taken place within the core of amphibian species' ranges. It therefore remains uncertain whether introduced fish affect amphibians similarly at the species' range periphery, where the effects of such stressors often result in range contractions (Towns and Daugherty 1994; Channell and Lomolino 2000).

Given these shortcomings, the ideal system in which to further explore how introduced fish affect amphibians would be a combination of field studies at low- and high-elevations along the periphery of an amphibian species' range, and lab studies that elucidate the mechanisms involved. Long-toed salamanders (*Ambystoma macrodactylum*) are an excellent species on which to focus such a study. They are widely distributed throughout the northwestern United States, British Columbia and the Canadian Rocky

Mountains. Alberta's Rocky Mountains represent the easternmost limit of the species' range. The general biology of the long-toed salamander, including development, foraging strategies and behaviour, has been well-studied, especially at high-elevations (Petranka 2001). Adult long-toed salamanders are terrestrial and commence migration from upland areas to aquatic breeding habitats as soon as spring melt occurs (Graham and Powell 1999). The fundamental breeding requirement of the long-toed salamander is lentic habitat that contains water long enough for larvae to metamorphose. At high elevations (> 1700 m), larvae require up to 3 years prior to metamorphosis (Russell and Bauer 1993; Pilliod and Peterson 2001). Permanent lakes and ponds that are not prone to freezing solid are thus necessary for larvae to overwinter (Fukumoto 1995; Pilliod and Peterson 2001). Long-toed salamander larvae typically function as top vertebrate predators in habitats lacking fish (Petranka 2001). Larvae feed on crustacean zooplankton and benthic invertebrates, selecting prey in proportion with their own body size (Fukumoto 1995; Tyler et al. 1998). The larvae are known to be cannibalistic under conditions of high larval density and low food availability (Wildy 2001).

The majority of waterbodies within the long-toed salamander's range in Alberta, Canada, have been modified extensively over the past century by introduced fish (Donald 1987). Although long-toed salamander distribution surveys have taken place in Alberta (Nelson et al. 1995; Paton 2001; Huynh et al. 2002; Snyder et al. 2003), information on their distribution relative to fish remains anecdotal (Holroyd and Van Tighem 1982; Fukumoto 1995). Predation by trout is purported to be the primary mechanism responsible for long-toed salamander population declines in several protected areas described by Tyler et al. (1998), Funk and Dunlap (1999), Pilliod and Peterson (2001) and Bull and Marx (2002), but this has not been confirmed experimentally. It is also unknown whether the long-toed salamander has developed strategies to avoid predation by fish, such as unpalatability or antipredator behaviours.

Thesis Objectives

This thesis has two main objectives. The first is to determine the effects of introduced fish on the distribution of long-toed salamanders at the periphery of their range in southwestern Alberta. In Chapter 2, I evaluate the distribution of larval long-toed salamanders relative to introduced trout at 27 low- and 30 high-elevation habitats in Waterton Lakes National Park and the Castle Special Management Area. Based on previous studies (Funk and Dunlap 1999; Pilliod and Peterson 2001) and my own observations, I predicted that salamanders would be excluded from the majority of habitats containing introduced trout.

My second objective is to evaluate the effects of trout and minnows on long-toed salamander growth, survival and behaviour under controlled conditions. My main aim is to understand potential mechanisms responsible for the observed distribution of salamanders and trout in southern Alberta (Chapter 2). In Chapter 3, I evaluate the survival and growth of salamander larvae that were exposed to rainbow trout (*Oncorhynchus mykiss*), fathead minnows (*Pimephales promelas*) or dragonfly larvae (*Aeshna sp.*) for 30 days in an outdoor mesocosm experiment. I predicted that trout would strongly reduce salamander survival relative to minnows. I also expected the effects of minnows to be more chronic in nature, leading potentially to reduced salamander growth relative to the controls. Further, I verify whether rainbow trout and fathead minnows prey on long-toed salamander eggs, hatchlings or larvae in a series of predation trials in laboratory aquaria. I expected that neither fish would consume salamander eggs, that trout would consume salamander hatchlings and larvae and that minnows would be unable to consume either. Lastly, I evaluate the behavioural responses of salamander larvae to trout and minnows in a laboratory experiment. Given the recent nature of fish introductions in southwestern Alberta and throughout the long-toed salamander's range, and results from field surveys that indicate allopatric distributions of fish and salamanders (Funk and Dunlap 1999; Pilliod and Peterson 2001; Bull and Marx 2002), I anticipated that salamanders would lack detectable behavioural responses to fish.

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CHAPTER 2: THE DISTRIBUTION OF THE LONG-TOED SALAMANDER RELATIVE TO INTRODUCED TROUT IN SOUTHWESTERN ALBERTA

2.1. Abstract

The negative effects of introduced fish on amphibian populations are widely recognized. In particular, previous studies have demonstrated that long-toed salamanders and introduced trout are distributed allopatrically in high-elevation waterbodies in the northwest continuous United States. However, the effects of introduced trout on the distribution of long-toed salamander populations at low-elevation habitats have not been examined. Neither has the influence of introduced trout on long-toed salamanders been examined at the periphery of the salamander's range. In southwest Alberta, Canada, trout have been introduced into a large proportion of the lentic waterbodies for recreational angling and through flooding. Using a combination of visual encounter surveys, funnel trapping and snorkel surveys, I examined the distribution of long-toed salamanders relative to introduced trout at 27 low-elevation (< 1500 m) ponds and 30 high-elevation (≥ 1500 m) lakes in Waterton Lakes National Park and the Castle Special Management Area. The presence of salamanders was strongly associated with the absence of trout at both low- and high-elevation sites. Salamanders co-existed with trout at 4 (15%) of the 27 low-elevation sites, were absent from 6 (22%) other sites with trout, and inhabited all 17 (63%) sites where trout were absent. Similarly, salamanders were absent from all 14 (47%) of the 30 high-elevation sites containing trout and occupied 9 (30%) sites where trout were absent. These results suggest that introduced trout limit the distribution of long-toed salamanders at low- and high-elevation habitats at the eastern periphery of the salamander's range.

2.2. Introduction

Predation by fish is one of the main factors known to influence the current distribution and abundance of amphibians. In the context of global amphibian population declines and range contractions, predation by introduced fish is often described as a key issue (reviews by Blaustein and Kiesecker 2002; Collins and Storfer 2003). Strong evidence comes from field surveys that document almost complete allopatry of amphibians and fish within groups of wetlands. For example, Grinnell and Storer (1924) documented that the mountain yellow-legged frog (*Rana muscosa*) and Pacific treefrog (*Hyla regilla*) were once common inhabitants of naturally fishless, high-elevation waterbodies throughout the Sierra Nevada, California. Following the introduction of trout (*Oncorhynchus* spp., *Salvelinus fontinalis*) into most of those waterbodies, Zardus et al. (1977, in Bradford 1989) showed that in 133 lakes, 116 (87%) contained trout and two (2%) contained frog tadpoles, but none contained both. Similar results come from other field studies on frogs (Brönmark and Edenhamn 1994), newts (Aronsson and Stenson 1995; Gamradt and Kats 1996) and salamanders (Thompson et al. 1980; Sih et al. 1992).

The negative effects of introduced trout on high-elevation long-toed salamander populations have been particularly well documented (Tyler et al. 1998a; Funk and Dunlap 1999; Pilliod and Peterson 2001; Bull and Marx 2002). As an example, Funk and Dunlap (1999) showed that in 42 high-elevation Montana lakes, long-toed salamander larvae co-existed with trout at two (5%) lakes, and were present within 27 (64%) trout-free lakes. In the above-mentioned field studies and a mesocosm study by Tyler et al. (1998b), trout predation was implicated as the main mechanism involved in trout and long-toed salamander allopatry. Monello and Wright (1999) also documented the exclusion of all amphibians, including long-toed salamanders, from low-elevation habitats containing introduced goldfish (*Carassius auratus*) in Idaho, which they subsequently attributed to egg and larvae predation by goldfish (Monello and Wright 2001). These studies provide compelling evidence that introduced trout and goldfish are capable of limiting the distribution of long-toed salamanders.

However, our understanding of the magnitude and extent of trout effects on long-toed salamanders is limited by two factors. The first is that all previous studies have been completed on salamander populations located within the core of the salamander's range in the northwestern United States. Other than anecdotal reports such as those by Holroyd and Van Tighem (1982) and Fukumoto (1995), the negative effects of introduced trout on long-toed salamanders have thus far not been evaluated at the periphery of their range. Hamer et al. (2004) showed that an influx of fertilizers into the aquatic habitats of three frog species in southeastern Australia resulted in significant contractions of the frogs' ranges. Other stressors such as predation by introduced fish have the potential to cause similar contractions in the range of the long-toed salamander (Channell and Lomolino 2000).

A second factor is that previous studies of the relative distributions of trout and long-toed salamanders have focused exclusively on high-elevation sites (1500 to 2800 m) (Tyler et al. 1998a; Funk and Dunlap 1999; Pilliod and Peterson 2001; Bull and Marx 2002). Despite the fundamental differences between low-elevation (< 1500 m) habitats and high-elevation habitats, and therefore their potential to have markedly different salamander-fish interactions, similar studies have thus far not been completed at low elevations. Unlike naturally fishless high-elevation lakes, some low-elevation habitats are prone to natural fish introductions through flooding (Donald 1987), which may result in salamander populations in those low-elevation habitats being better adapted to avoid predation by fish. Further, low-elevation lentic habitats are typically more complex in structure than high-elevation sites (Nelson et al. 1995) which may lessen the susceptibility of salamanders to fish predation (Crowder and Cooper 1982; Diehl 1992). Low-elevation sites also have longer productive periods than high-elevation waterbodies, which allow salamanders to metamorphose within one season (Howard and Wallace 1985), thus reducing their exposure to potential fish predators. In contrast, salamanders at high-elevations may spend up to 3 years exposed to trout in the larval stage (Howard and Wallace 1985). Finally, low-elevation wetlands are typically more productive than oligotrophic high-elevation lakes (Wilhelm and Schindler 2000). Low-elevation wetlands thus have the potential to host larger salamander populations than high-elevation wetlands, and may consequently play a key role in maintaining regional salamander populations. Like other amphibians (Sjögren 1991; Trenham 1998), long-toed salamanders are thought to have metapopulation structure (Pilliod and Peterson 2001). Conservation of large source populations is thus imperative to the long-term persistence of regional salamander populations (Pulliam and Danielson 1991), especially in areas where a large proportion of high-elevation salamander source populations have been eliminated by introduced trout. Yet the extent to which introduced fish affect long-toed salamander populations at low-elevation habitats is poorly understood.

The objective of this field study was to determine whether the distribution of long-toed salamander larvae was influenced by introduced trout in low- and high-elevation waterbodies in southwestern Alberta, Canada. Based on previous studies, I predicted that long-toed salamanders would be excluded from the majority of trout habitats at both low- and high-elevations.

2.3. Methods

2.3.1. Study Area

All study sites were located within Waterton Lakes National Park (WLNP) and the Castle Special Management Area (CSMA) in the Rocky Mountains of southwestern Alberta, Canada (Figure 2.1). The areas have similar climate, topography and vegetation communities (Alberta Energy and Natural Resources 1978; Achuff et al. 2002). Disturbances in WLNP (525 km^2) are generally limited to low-impact recreation and roadways, whereas the CSMA (1700 km^2) is managed as a multi-use area where motorized recreation, forestry, grazing and gas extraction take place.

Most lakes and ponds in the study area were historically fishless (Rawson 1938; Donald 1987). However, nearly all have been stocked at some time since 1921, with various combinations of rainbow trout (*Oncorhynchus mykiss*), cutthroat trout (*O. clarki*), golden trout (*O. aguabonita*) and brook trout (*Salvelinus fontinalis*) to provide recreational angling opportunities (Anderson and Donald 1976a, b; Donald 1987). Fish stocking ceased in WLNP in 1979. Several introduced trout populations have been extirpated since, presumably by winterkill. Seventeen (50%) of the 34 high-elevation WLNP lakes currently contain self-maintaining trout populations (WLNP data from R. Watt, pers. comm. 2003). Five (27%) of the 18 high-elevation CSMA lakes contain self-maintaining trout populations, and three (17%) continue to be stocked annually (Alberta Fish and Wildlife data from D. Wig, pers. comm. 2004). Minnows have also been introduced to several lentic habitats in the region through flooding and their use as bait fish (Rawson 1938). In this study, I examined approximately 42% (41 of 98) and 11% (14 of 127) of the total number of lentic waterbodies in WLNP and the CSMA, respectively.

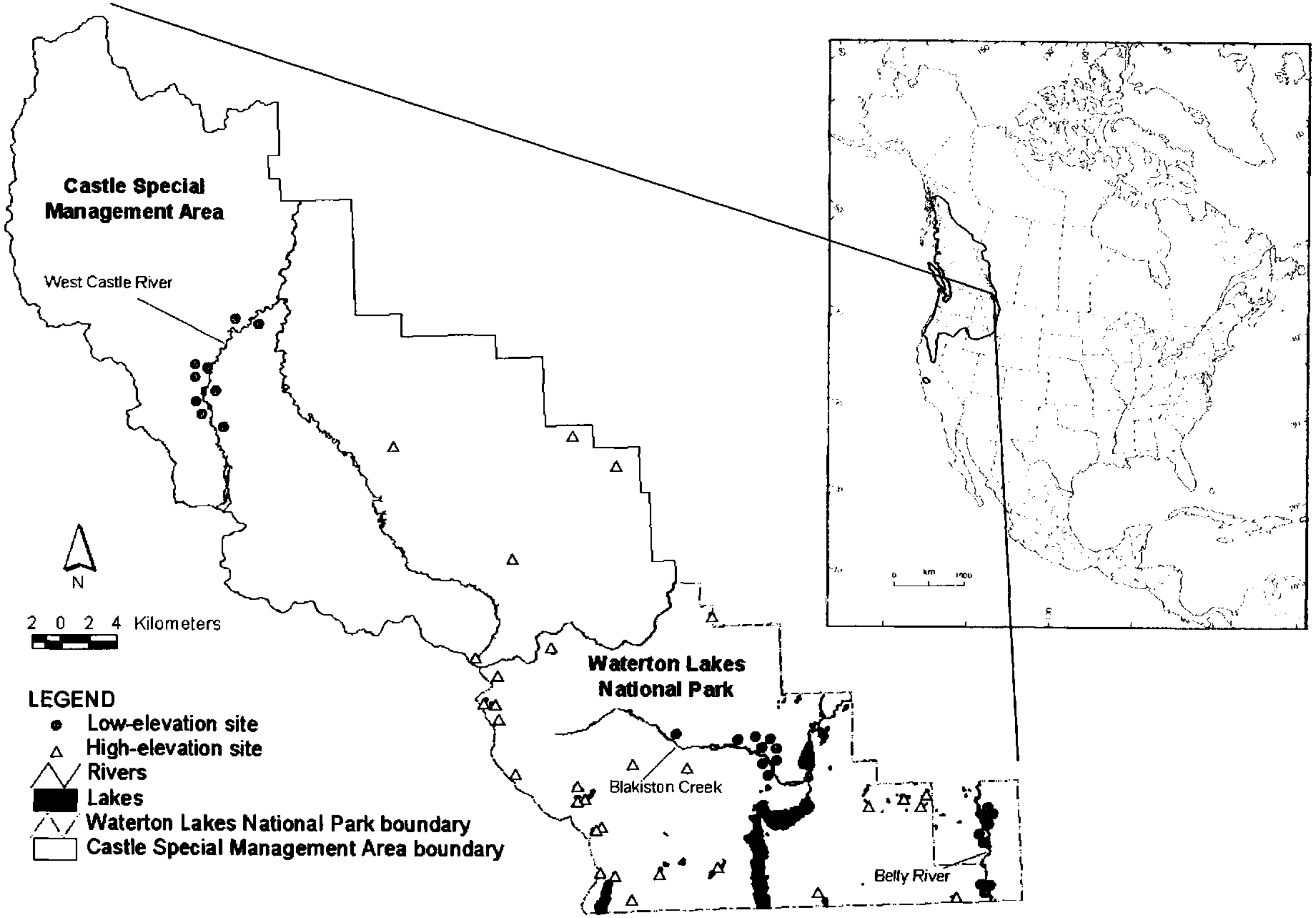

Figure 2.1. Location of the study area in North America and Alberta, Canada, and a GIS map of approximate low- and high-elevation study site locations in Waterton Lakes National Park and the Castle Special Management Area. The shaded area on the North American map (after COSEWIC 2004) depicts the long-toed salamander's range.

2.3.2. Low-elevation Surveys

Twenty-seven low-elevation (< 1500 m) ponds were surveyed June - August 2003 for the presence/absence of long-toed salamanders and fish. Three groups of nine ponds were selected along three major rivers in the area: the West Castle River (CSMA), Blakiston Creek (WLNP) and the Belly River (WLNP) (Figure 2.1). Low-elevation pond locations, elevations, maximum depths and areas are listed in Appendix A (Table I). Ponds were located 2 - 200 m from the rivers and the majority were within 100 m. Each river contained several species of native and introduced fish. Eight of the 27 ponds were prone to flooding from adjacent rivers, and thus to natural fish introductions. Eleven ponds were located far enough away from rivers that there was no risk of flooding. Six ponds contained brook trout and/or cutthroat trout that were stocked in the mid 1900s and winterkill had occurred in another pond that was formerly stocked. Finally, one pond that was prone to flooding also contained stocked trout. Low-elevation ponds were selected for their accessibility and proximity to the river systems. Ponds ranged in size from approximately 0.003 ha to 0.8 ha and in elevation from 1317 m to 1418 m above sea level. Low-elevation ponds were located away from human disturbances other than fishing, light cattle activity (CSMA only) and roadways.

Visual Encounter Surveys

Each low-elevation pond was surveyed at least twice during the 2003 field season, following the visual encounter survey methods described by Thoms et al. (1997) and implemented during several other long-toed salamander surveys in southwestern Alberta (Nelson et al. 1995; Paton 2001; Snyder et al. 2003). Visual encounter surveys took place during daylight hours and involved two or more trained observers independently surveying the entire periphery of each pond. All ponds contained clear water and had shallow shorelines. Surveyors also used long-handled, 30 x 28 cm, 1 mm mesh dipnets, making two, half-metre net sweeps per 5 m of shoreline in areas obscured by emergent and/or submerged vegetation. Surveyors walked either on the pond bank or within approximately 1 m of the shore so that any fish or amphibians present were flushed. The blunt ends of surveyor's dipnets were used to disturb substrates in which larvae or fish might hide.

At least one long-toed salamander larva was captured per site for positive identification using characteristics described by Corkran and Thoms (1996). Fish were identified by using a key (Joynt and Sullivan 2003) and through communication with local fish biologists. Observations of salamander larvae and/or fish were typically made within the first 10 minutes of a survey's commencement. Data for sites where salamanders and/or fish were not observed were confirmed by comparing my results with data from other amphibian surveys (e.g., Paton 2001; Taylor and Smith 2003) and through communication with local fish biologists. In all of those cases, the results were identical except for one site where mountain whitefish (*Prosopium williamsoni*) were present in 2003 but not in previous surveys.

Funnel Trapping

Aquatic funnel traps are an effective method of capturing ambystomatid larvae and small fish, and are especially useful in making observations at low-elevation waterbodies where vegetation or woody debris may inhibit visual encounter surveys (Richter 1995; Adams et al. 1997). Funnel traps also offer an advantage in that they can be left overnight when visual encounter and netting surveys are not feasible and when amphibian larvae and fish activity patterns vary from diurnal movements (Neish 1971; Alanara and Brannas 1997).

At sites where salamanders and/or fish were not detected during two or more visual encounter surveys, funnel trapping was used to make supplementary observations or confirm absence. Unbaited traps were placed according to the shoreline-based protocol described in Adams et al. (1997), with slight modification. One galvanized hardware cloth minnow trap was placed within each of a series of contiguous, 10 m or 20 m intervals around the entire shoreline of each pond. Interval size varied with the size of the pond; larger ponds were assigned 20 m intervals while smaller ponds were allotted 10 m intervals to ensure that they contained a sufficient number of traps to make effective observations. Five to 12 traps were typically set per site. Traps were placed in water 12 - 21 cm deep so they permitted captures but were not completely submersed, to prevent drowning of captured animals. Traps were set for at least one 12 - 24 hr overnight period per site where fish or larvae presence or absence needed confirmation. All animals captured in funnel traps were released alive.

Funnel trapping took place at sites along the Belly River and Blakiston Creek between 8 July and 6 August 2003, when larvae observed at nearby sites were large enough that they could not escape through the trap mesh, but prior to metamorphosis. Funnel trapping was not possible at the West Castle sites during that period, as the area was closed due to the Lost Creek forest fire. Fortunately, those sites had been thoroughly surveyed and salamander larvae had been identified at all sites except one (where their absence was confirmed) prior to the area's closure. Funnel trapping was completed at some West Castle sites between 30 September and 9 October 2003, to confirm fish absence. In total, 130 traps were set at 15 low-elevation sites for 20 overnight periods. On average, 22.1 ± 3.1 hours of trapping took place at each site.

Prior to data collection, I assessed the effectiveness of funnel traps in capturing long-toed salamander larvae during four overnight periods at two low-elevation ponds where larvae had recently been observed. At least one larva was captured per site during each of those test periods, in which only two or three traps were set per site. At two other sites where no salamander larvae were observed during visual surveys, their absence was confirmed by setting funnel traps for three overnight periods.

Snorkel Surveys

Snorkel surveys are another effective method of detecting fish and salamander larvae at field sites (Thoms et al. 1997; Tyler et al. 1998a; Hoffman et al. 2003). To further confirm presence or absence of fish, snorkel surveys were completed at three low-elevation ponds that were of great enough depth that the pond bottom could not be seen from the shoreline. Those surveys involved systematically snorkeling the entire pond in search of fish for approximately 15 – 20 minutes.

2.3.3. High-elevation Surveys

Thirty high-elevation (≥ 1500 m) lakes were surveyed July – September 2002 and July - October 2003 (Figure 2.1). Twenty-five lakes were located within WLNP and five were within the CSMA. High-elevation lake locations, elevations, maximum depths and areas are listed in Appendix A (Table II). All of the high-elevation lakes were fishless prior to most being stocked with trout in the early to mid 1900s (Donald 1987). High-elevation sites were selected for their accessibility and the availability of accurate fish stocking records. They ranged in size from 0.2 ha to 30.2 ha and in elevation from 1525 m to 2200 m. All high-elevation lakes were located away from human disturbances other than hiking and fishing. High-elevation sites were surveyed for presence or absence of long-toed salamanders and fish using the visual encounter survey methods described above. Similar methods were employed in high-elevation long-toed salamander and trout surveys by Funk and Dunlap (1999) and Pilliod and Peterson (2001). Brokes (1999) and Funk and Dunlap (1999) found that shoreline visual surveys were as effective as snorkel surveys in determining presence or absence of larval long-toed salamanders and trout at similar high-elevation lakes in Washington and Montana.

The remote locations of most high-elevation lakes imposed several constraints on my sampling protocol. Thus, only visual encounter surveys were completed at high-elevation sites. Six high-elevation sites were surveyed only in 2002, 10 sites were surveyed only in 2003 and 14 sites were surveyed in both years. Results were identical in 2002 and 2003 at sites surveyed in both years. For further confirmation, funnel trapping or snorkel surveys were completed at three high-elevation sites.

2.3.4. Data Analysis

The analysis was restricted to trout and salamander presence/absence data. Abundance data were not collected because they would not be comparable across the extended sample periods. Variation in the time of day and daily weather conditions would further confound abundance estimates. Analysis of presence/absence data was therefore the most feasible and conservative way to examine the effects of introduced trout on the distribution of long-toed salamanders within the study area.

Data were analyzed using Fisher's exact test, which produces an exact probability that two variables are statistically independent when one or more expected frequencies are less than 5 (Sokal and Rohlf 1981). Separate tests were conducted for the low-elevation and high-elevation data. 'Salamander presence/absence' and 'trout presence/absence' were the two variables and each study site was assigned to either 'salamanders present' or 'salamanders absent' and 'trout present' or 'trout absent'. The null hypothesis was that there was no association between 'salamander presence/absence' and 'trout presence/absence'. The alternate hypothesis was that there was a negative association between those two variables. Funk and Dunlap (1999) utilized the same test to determine whether introduced trout and long-toed salamanders were independently distributed in high-elevation lakes in Montana.

An additional chi-square analysis was carried out to determine whether there was an association between elevation and the number of study sites that contained trout or salamander larvae. Elevation ('low-elevation' or 'high-elevation') and trout/salamanders present ('trout present' or 'salamanders present') were the two variables. The null hypothesis was that there was no association between elevation and the number of study sites that contained trout or salamanders. The alternate hypothesis was that there was an association between the two variables.

2.4. Results

The proportion of low-elevation ponds that contained trout and salamander larvae was significantly different from the proportion of ponds without trout that contained larvae (Figure 2.2; $p = 0.001$). Of the 27 low-elevation ponds surveyed, 10 (37%) contained trout and 21 (78%) contained salamander larvae; four (15%) of the sites contained both. One site along the West Castle River contained mountain whitefish and no salamanders. Based on their diets (Scott and Crossman 1973), I considered mountain

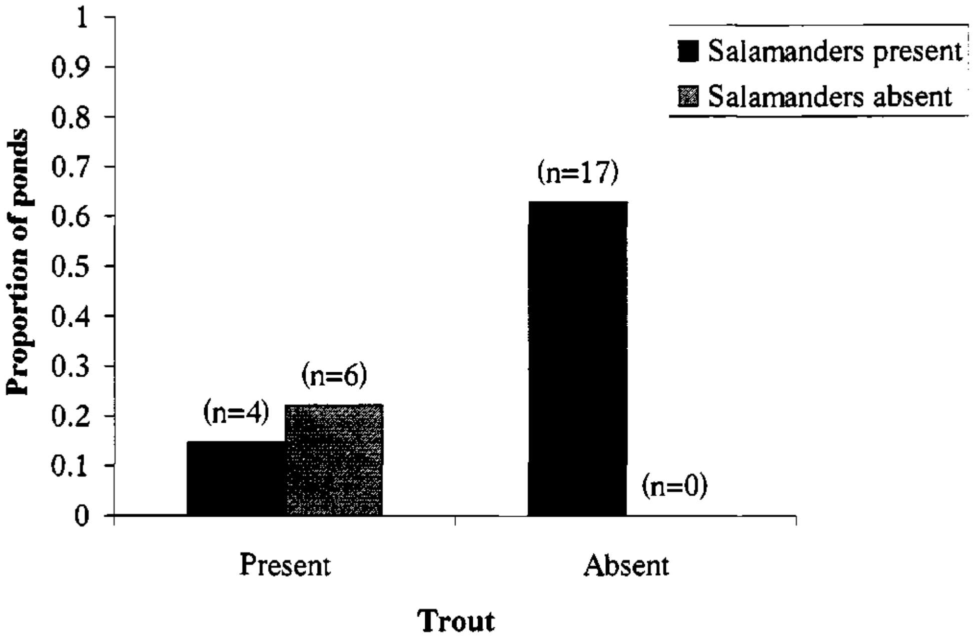

Figure 2.2. Frequency histogram showing the proportion of the 27 low-elevation ponds with and without long-toed salamander larvae and trout.

whitefish similar to trout in their ability to consume salamander larvae. Because mountain whitefish were the only predatory fish species documented at a site where trout were absent, I treated that site as one that contained trout in my analysis. Four (40%) of the 10 low-elevation ponds with trout also contained larvae, while all of the 17 ponds without trout contained larvae. Brook trout were present at all low-elevation trout ponds except one, which contained bull trout (*S. confluentus*). In addition to brook trout, burbot (*Lota lota*), longnose sucker (*Catostomus catostomus*) and mountain sucker (*C. platyrhynchus*) were observed at one Belly River site, and an unknown sculpin species (*Cottus* sp.) and mountain sucker were documented at another. At low-elevation sites where salamander larvae were observed during visual encounter surveys, between 1 and approximately 500 individuals were observed per site. At sites where salamanders were captured in funnel traps, 1 - 8 individuals were captured at each site, and 0.1 - 1.6 individuals were captured per trap. The presence/absence of trout, long-toed salamander larvae, other fish and other amphibians at each low-elevation study site are shown in Appendix A (Table I).

The proportion of high-elevation lakes that contained trout and salamander larvae was also significantly different from the proportion of lakes without trout that contained larvae (Figure 2.3; $p = 0.001$). Of the 30 high-elevation sites surveyed, 14 (47%) contained trout and 9 (30%) contained salamander larvae, while none contained both. None of the lakes with trout contained larvae, whereas nine (56%) of the 16 lakes without trout contained larvae. Trout species present at high-elevation lakes were brook trout, cutthroat trout and rainbow trout. At high-elevation sites where salamander larvae were present, between 1 and approximately 30 larvae were observed. The presence/absence of trout, long-toed salamander larvae and other amphibians at each high-elevation study site are shown in Appendix A (Table II).

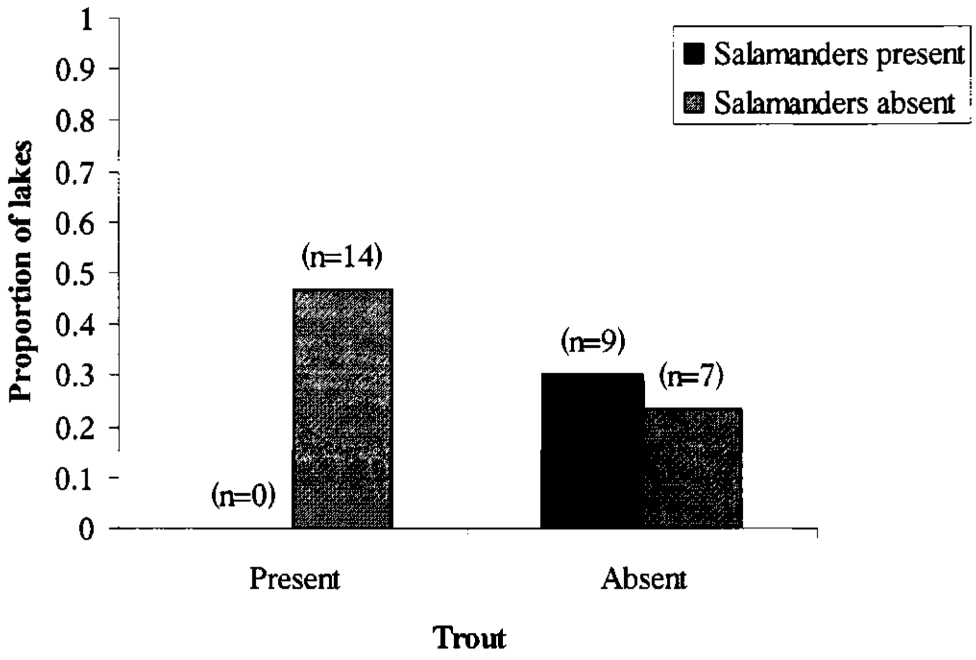

Figure 2.3. Frequency histogram showing the proportion of the 30 high-elevation lakes with and without long-toed salamander larvae and trout.

There was an association between elevation and the number of sites that contained trout or long-toed salamander larvae (Pearson Chi-square; F = 4.38, p = 0.036). Fourteen (58%) of the 24 sites where trout were present were high-elevation sites, while 10 (42%) were low-elevation sites. Conversely, 9 (30%) of the sites where salamander larvae were present were high-elevation sites and 21 (70%) were low-elevation sites.

Although I had initially aimed to examine the distribution of long-toed salamanders relative to minnows as well as trout, minnows were present at too few sites. Minnows were present at a low proportion (16%) of the 57 study sites and only three of those sites did not also contain trout. Long-toed salamander larvae were observed at all three of those minnow sites.

2.5. Discussion

Results of my surveys at high-elevation sites corroborate previous studies indicating that trout eliminated or reduced the density of long-toed salamander larvae (Tyler et al. 1998a; Funk and Dunlap 1999; Pilliod and Peterson 2001; Bull and Marx 2002). Further, my data provide the first evidence that the distribution of long-toed salamanders at low elevation habitats is also restricted by introduced trout. That salamander larvae were present in all low-elevation sites that did not contain trout strongly suggests that trout are a significant limiting factor in the distribution of low-elevation salamander populations within the study area. Reports of very large long-toed salamander populations at low-elevation habitats in Alberta (Fukumoto 1995; Powell et al. 1997) indicate that those habitats have the potential to host highly productive source populations, from which individuals may disperse to less productive habitats (Pulliam 1988; Trenham 1998). Alteration of the availability of such source habitats by introduced trout may therefore have significant, previously-unrecognized impacts on regional long-toed salamander population dynamics. Although amphibian metapopulation dynamics have been well-studied (review by Marsh and Trenham 2001), I have been unable to locate information describing the dynamics involved in areas where amphibian species exist across large elevation ranges, such as in my study area. An assessment of how individual salamander populations at various elevations contribute to the maintenance of regional populations would provide a much-improved context in which to consider the consequences of salamander declines caused by introduced fish.

Unlike other amphibian species that evolved in close proximity to predatory fish (Resetarits and Wilbur 1991; Kats and Sih 1992), adult long-toed salamanders do not appear to avoid ovipositing in fish-inhabited ponds (Tyler et al. 1998a). Habitats containing trout are therefore likely salamander population sinks, where juvenile recruitment is insufficient to compensate for adult mortality (Pulliam 1988). Lakes and ponds in which introduced trout populations are able to thrive are typically deeper and more permanent in nature than ones that do not support trout. Pilliod and Peterson (2001) noted that many high-elevation habitats that are unsuitable for maintaining fish populations may also act as sinks to long-toed salamander populations that require waterbodies of sufficient depth to permit successful breeding and overwintering. Thus, they predicted that the maintenance of introduced trout populations may result in the eventual extirpation of long-toed salamanders from entire regions, including fishless sites. Though the high-elevation lakes I examined that were occupied by introduced trout but not by long-toed salamanders (mean depth = 13.4 ± 14.3 m, n = 14) were not significantly deeper than sites containing only salamanders (mean depth = 3.5 ± 3.3 m, n = 9) (one-way ANOVA; $F_{1, 21} = 4.03$, $p = 0.0579$), this prediction may also be relevant in southwestern Alberta.

The majority (71%) of the seven high-elevation sites where neither long-toed salamanders nor trout were present during my study were stocked historically, each containing trout for 8 to 38 years (WLNP data from R. Watt, pers. comm. 2003). Given the 6 to 10 year life-expectancy of Alberta long-toed salamanders (Russell et al. 1996), and if trout predation severely reduced or eliminated recruitment of juvenile salamanders, salamander populations would have been extirpated from those sites within a decade of trout introductions. There being no known records of amphibian distribution prior to fish stocking for any of my study lakes and ponds, it is unknown whether long-toed salamanders inhabited those sites historically. However, having accounted for habitat differences between sites, Pilliod and Peterson (2001) found significantly larger long-toed salamander and Columbia spotted frog (*Rana luteiventris*) populations in fishless lakes than in lakes containing introduced trout. This suggests that populations of those amphibian species historically existed at sites that now contain trout (Pilliod and Peterson 2001). I expect that the same holds true in my study area, where several stocked, high-elevation sites have habitat characteristics similar to fishless sites that support salamander populations.

It is uncertain how salamander larvae were able to co-exist with trout at four low-elevation ponds and not at others. Eight metamorphosing larvae were observed at one of those sites, which indicates that they had co-existed with trout throughout their larval period. One possibility is that habitat complexity, and thus the availability of predator-free refugia, was higher in those ponds than in ponds where trout excluded larvae. Crowder and Cooper (1982) and Gotceitas (1990) showed that foraging efficiency of fish was reduced in habitats of high structural complexity, permitting predator-prey coexistence. Indeed, Tyler (1996) found that structurally complex, high-elevation habitats containing fish supported long-toed salamanders in low densities. However, ambystomatid larvae exposed to predatory fish for prolonged periods may grow at slower rates than larvae in fishless environments (Figiel and Semlitsch 1990). In turn, reduced larval growth rates are known to reduce the fitness of adult salamanders (Semlitsch et al. 1988). Examination of the habitat characteristics that permit long-toed salamander larvae to co-exist with fish in low-elevation habitats and assessment of salamander fitness in fish versus fishless habitats would further our understanding of fish-salamander dynamics at low-elevation habitats.

In contrast to sites containing trout, all three of my study sites that contained minnows and no other fish contained long-toed salamander larvae. This suggests that minnows may not affect the distribution of salamanders as severely as trout. The minnow species observed at my study sites, fathead minnow (*Pimephales promelas*) and northern redbelly dace (*Phoxinus eos*), are gape-limited species (Scott and Crossman 1973) unlikely to prey extensively on salamander larvae (see Chapter 3). However, Duffy (1998) showed that zooplankton consumption by fathead minnows is extremely high. Potential therefore exists for minnows and larval salamanders to compete for invertebrate prey (see Chapter 3). Minnows were observed in low densities (approximately 1 to 100 individuals) at my study sites and likely did not deplete food resources sufficiently to affect salamanders. Nonetheless, I would expect any negative impacts of minnows on salamander populations to be chronic, making them less apparent than the more acute effects of trout. Qualitative studies of long-toed salamander distribution relative to minnows, and comparison of salamander growth rates in minnow and fishless habitats would improve our understanding of the potentially significant impacts that these non-predatory fish may have on salamanders.

In summary, my results show that introduced trout strongly limit the distribution of long-toed salamanders at low-elevation ponds and high-elevation lakes in southwestern Alberta. Given this evidence, data provided by previous studies, and the contemporary nature of fish introductions in the region, the long-toed salamander appears to lack effective defenses against fish predation. By limiting salamander populations to fish-free habitats, the maintenance of current trout populations might have the potential to result in the extirpation of regional long-toed salamander populations and perhaps significant reductions in their range.

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Appendix A: Low- and high-elevation study site location and data tables.

Table I. Low-elevation study site locations, physical characteristics and incidence of trout, long-toed salamander larvae (LTSA), other fish and other amphibians in 2003. UTM coordinates collected using NAD 83 datum.

Drainage	**Site Code**	**Elevation (m)**	**Easting**	**Northing**	**Maximum Depth (m)**	**Area (ha)**	**Trout***	**LTSA**	**Other fish****	**Other amphibians*****
Belly	BR1	1396	303735	5436775	5.0	0.4	-	+	-	CSFR, WETO
	BR2	1380	303750	5436420	1.3	0.4	-	+	-	CSFR, WETO
	BR3	1378	303710	5436306	1.0	0.2	-	+	-	CSFR
	BR4	1386	303577	5436150	2.2	0.5	$+_{K}$	-	BT, LS, MS, UM	-
	BR5	1368	303953	5435277	0.5	0.3	$+_{U}$	-	RD	-
	BR6	1389	303993	5435110	3.0	0.4	-	+	RD	CSFR
	BR7	1373	303297	5431853	0.7	0.7	-	+	-	CSFR
	BR8	1383	303463	5431641	1.0	0.8	-	+	-	WETO
	BR9	1385	303583	5431877	0.7	0.2	-	+	SC, MS, RD	-
Blakiston	BC1	1418	282424	5443631	0.8	0.1	-	+	-	-
	BC2	1350	288850	5442173	1.0	0.8	-	+	-	CSFR
	BC3	1335	289278	5441871	1.5	0.5	-	+	-	-
	BC4	1322	289283	5441858	0.5	0.1	$+_{K}$	-	-	-
	BC5	1341	289241	5441761	1.5	0.7	$+_{K}$	-	-	-
	BC6	1317	289257	5441726	0.5	0.2	-	+	-	-
	BC7	1360	289252	5441689	1.3	0.3	$+_{K}$	+	-	-
	BC8	1318	289080	5441110	2.0	0.3	$+_{K}$	+	-	CSFR
	BC9	1306	289034	5441115	1.5	0.8	$+_{K}$	-	-	-
West Castle	WC1	1397	691014	5472798	0.5	0.1	-	-	MW	-
	WC2	1391	688083	5469406	0.5	0.2	$+_{K}$	+	-	-
	WC3	1390	687628	5467614	0.6	0.3	-	+	-	CSFR, WETO
	WC4	1390	687652	5467806	1.3	0.3	-	+	-	CSFR, WETO
	WC5	1415	687677	5467968	0.9	0.2	-	+	-	-
	WC6	1404	687998	5466855	1.2	0.8	$+_{B}$	+	-	-

	Site Code	Elevation (m)	Easting	Northing	Maximum Depth (m)	Area (ha)	Trout*	LTSA	Other fish**	Other amphibians***
	WC7	1394	688066	5469414	0.6	0.2	-	+	-	-
	WC8	1409	690497	5472616	2.0	0.5	-	+	-	-
	WC9	1394	688208	5469454	0.4	0.1	-	+	-	-

* Trout species observed: K = brook trout, B = bull trout, U = unknown trout species

** Other fish species observed: BT = burbot, SC = unknown sculpin species, LS = longnose sucker, MS = mountain sucker, MW = mountain whitefish, RD = northern redbelly dace, UM = unknown minnow species

*** Other amphibian species for which breeding evidence was observed: CSFR = Columbia spotted frog, WETO = western toad (*Bufo boreas*)

Table II. High-elevation study site locations, physical characteristics and incidence of trout and long-toed salamander larvae (LTSA) in 2002-2003. UTM coordinates collected using NAD 83 datum.

Drainage	Site Code (Site Name*)	Elevation (m)	Easting	Northing	Maximum Depth (m)	Area (ha)	Trout**	LTSA	Other amphibians***
Waterton	W1 (Sofa Wetland A)	1525	798300	5438300	1.5	3.0	$+_{C, K}$	-	-
	W2 (Sofa Wetland B)	1525	799450	5437700	2.0	2.6	$+_{C, K}$	-	-
	W3 (Kesler Lake)	1525	786000	5451900	2.0	7.7	-	+	BCFR
	W4	1525	799900	5438500	2.0	0.2	-	+	-
	W5 (Crandell Lake)	1550	283287	5441528	15.5	4.5	$+_{K}$	-	-
	W6 (Sofa Mountain Pond 1)	1550	795800	5437950	4.0	2.5	-	+	CSFR
	W7 (Little Akamina Lake)	1695	716333	5434396	5.0	4.7	$+_{R, K}$	-	CSFR
	W8 (Deer Lake 2)	1725	301113	5431210	4.0	2.5	-	+	CSFR
	W9 (Akamina Pass Pond)	1775	715432	5434426	1.5	0.2	-	+	CSFR
	W10 (Crypt Pond)	1798	291776	5432408	2.0	2.7	-	-	-
	W11 (Bertha Lake)	1800	784800	5433900	50.3	30.2	$+_{R}$	-	-
	W12 (Lost Lake)	1877	708021	5447862	12.0	1.6	-	+	-
	W13 (Peck's Basin Pond)	1925	708700	5444750	1.5	0.5	-	-	-

Drainage	**Site Code (Site Name*)**	**Elevation (m)**	**Easting**	**Northing**	**Maximum Depth (m)**	**Area (ha)**	**Trout****	**LTSA**	**Other amphibians*****
	W14 (Summit Lake)	1931	717700	5432400	2.0	2.0	-	+	CSFR
	W15 (Lower Twin Lake)	1934	707712	5446150	8.0	2.7	$+_{R, K}$	-	-
	W16 (Lower Rowe Lake)	1971	708021	5447862	8.0	2.0	-	-	-
	W17 (Goat Lake)	2000	712150	5449500	9.3	2.4	$+_{C}$	-	-
	W18 (Upper Twin Lake)	2000	707300	5446100	13.0	6.4	$+_{R, K}$	-	-
	W19 (Lone Lake)	2027	709494	5441196	13.0	2.5	$+_{C}$	-	-
	W20 (Ruby Lake)	2050	717678	5442116	4.2	2.4	-	-	-
	W21	2150	714250	5439550	1.5	1.9	$+_{C}$	-	-
	W22 (South Lineham Lake)	2165	713972	5439670	17.0	5.8	-	-	-
	W23 (Middle Rowe Lake)	2162	715150	5437390	12.0	2.2	-	-	-
	W24 (North Lineham Lake)	2170	713548	5440055	29.0	19	$+_{C}$	-	-
	W25 (Upper Carthew Lake)	2200	781150	5434400	13.0	4.4	-	-	-
Castle	C1 (Bathing Lake)	1547	716409	5462762	2.1	0.4	$+_{K}$	-	WETO
	C2 (Butcher's Lake)	1585	713689	5465460	5.0	2.5	$+_{K}$	-	WETO

	Site Code (Site Name*)	Elevation (m)	Easting	Northing	Maximum Depth (m)	Area (ha)	Trout**	LTSA	Other amphibians***
	C3	1774	706723	5449322	3.0	1.0	-	+	-
	C4	2027	700944	5464009	1.2	0.2	-	+	CSFR, WETO
	C5 (Bovin Lake)	2050	709200	5456200	32.0	10.9	+$_K$	-	-

* Site name used by Anderson (1976a,b), Snyder et al. (2003), Taylor and Smith (2003) or Sports Scene Publications (2004)

** Trout species observed: R = rainbow trout, K = brook trout, C = cutthroat trout, U = unknown trout species

*** Other amphibian species for which breeding evidence was observed: CSFR = Columbia spotted frog, WETO = western toad, BCFR = boreal chorus frog (*Pseudacris maculata*)

CHAPTER 3: RELATIVE EFFECTS OF INTRODUCED TROUT AND MINNOWS ON THE GROWTH, SURVIVAL AND BEHAVIOUR OF LONG-TOED SALAMANDERS

3.1. Abstract

Most studies on the effects of introduced fish on the growth and survival of salamander larvae have focused on single, well-known predators. The indirect effects of small-bodied bait fish on salamander demography are poorly studied, despite their widespread introduction into wetlands containing salamanders. In this study, long-toed salamander larvae were reared in outdoor mesocosms that contained trout, minnows or odonates. Larval survival over 30 days was reduced by 39% and 42% in the trout and minnow tanks respectively, compared to controls. The mean mass of surviving larvae in the minnow and trout treatments was reduced by 37% and 14%, respectively, compared to controls. Palatability trials in laboratory aquaria confirmed that trout preyed directly on salamander hatchlings and larvae. In contrast, minnows caused injuries to hatchlings, but never consumed them. In a laboratory experiment that measured the difference in larval salamander behaviour prior to and during exposure to trout and minnows, salamanders spent significantly more time within a refuge when exposed to minnows, but no behavioural responses to trout were detected. Thus, direct predation by trout and a lack of larval antipredator behaviours likely best explain the low survival observed in trout mesocosms. Low survival and growth rates in the minnow mesocosms are best explained by exploitative competition for zooplankton prey. These results confirm predictions made in previous studies of the negative effects of trout on amphibian larvae. Further, these data are the first to show that minnows also have the potential to negatively affect salamander demography, perhaps even more so than trout.

3.2. Introduction

In recent decades, the widespread introduction of non-native species of fish has been identified as one of the foremost causes of amphibian population declines and range reductions (Collins and Storfer 2003). In particular, several studies have addressed the common role of introduced fish in the decline of salamander populations. Results from field surveys (Funk and Dunlap 1999; Bull and Marx 2002), field manipulations (Sih et al. 1992; Gillespie 2001) and oviposition preference studies (Kats and Sih 1992; Cunnington 1998) have demonstrated a strong link between reductions in salamander population size and introduced fish. Laboratory studies have further documented both the direct effects of fish predation on salamanders (Semlitsch 1988; Monello and Wright 2001), and the indirect effects such as competition and behavioural alteration (Semlitsch 1987; Werner and Anholt 1996). Thus, there is solid evidence indicating that introduced fish can negatively affect salamander populations through a variety of mechanisms.

Despite these advances in understanding the effects of introduced fish on salamanders and the means by which they affect them, a number of deficiencies exist. Specifically, previous studies tended to focus on the effects of single fish species (Semlitsch 1988; Aronsson and Stenson 1995; Sih et al. 2000). Comparisons involving other species of fish, many of which are also introduced, have thus far not been completed. Further, fish species examined in previous studies are mainly piscivorous predators that exert acute, easily-detectable effects on salamanders (Kats and Sih 1992; Tyler et al. 1998b). Given their high rates of zooplankton consumption (Duffy 1998), gape-limited species such as cyprinid minnows are likely to affect salamanders in a more chronic nature; possibly to the same extent as better-recognized predators. Previous studies have described the negative, indirect impacts of organisms such as mosquitoes

(Blaustein and Marglait 1994; Mokany and Shine 2003) and crayfish (Gamradt et al. 1997) on amphibians. However, the indirect effects of gape-limited fish species on salamanders and other amphibians appear to have been thus far overlooked in published literature.

Several studies have described the absence or very low density of long-toed salamander populations in historically fishless, high-elevation lakes that presently contain introduced trout (Tyler et al. 1998a; Funk and Dunlap 1999; Pilliod and Peterson 2001; Bull and Marx 2002). A mesocosm study by Tyler et al. (1998b) showed reduced survival of salamanders exposed to trout and clarified that trout were responsible for the observed salamander declines in the field. Predation by trout was alleged to be the primary mechanism involved. However, the precise means by which trout negatively affect long-toed salamanders remain to be examined.

In their assessment of amphibian antipredator defenses, Kats et al. (1988) found that species which regularly co-existed with fish were better able to avoid predation via unpalatability and antipredator behaviours. Anecdotal reports of long-toed salamander larvae being found in trout stomachs indicate that the larvae are palatable to trout. Yet, direct predation on the various aquatic stages of the salamander by trout has not been confirmed. The ability of minnows to consume long-toed salamander larvae at various stages of larval development has also not been addressed. Further, the role of behavioural defenses by long-toed salamanders against fish predation is unclear. Many studies have described antipredator defenses such as reduced activity and increased refuge use by amphibian larvae (Sih et al. 1988; Lawler 1989; Semlitsch and Reyer 1992). Non-cannibalistic long-toed salamander larvae are known to reduce their activity level in the presence of cannibalistic conspecifics (Chivers et al. 1997). However, the magnitude and extent of long-toed salamander behavioural defenses to fish in general, and to trout in particular, is unknown. I expect that long-toed salamanders have not developed effective chemical or behavioural defenses against the relatively recent threat of fish predation.

In this study, I compared the survival and growth of long-toed salamanders reared in outdoor mesocosms that contained rainbow trout (*Onchorhynchus mykiss*), odonates (*Aeshna* sp.) or fathead minnow (*Pimephales promelas*), species that I have observed within the same watersheds as long-toed salamanders in southwestern Alberta, Canada. I also examined the palatability of long-toed salamander eggs, hatchlings and larvae, and the propensity of trout and minnows to consume them, in a series of predation trials. Finally, long-toed salamander behaviour in response to minnows and trout was observed in a laboratory experiment.

3.3. Methods

3.3.1. Mesocosm Experiment

Long-toed salamander larvae that were stocked into the mesocosms originated from approximately 700 eggs collected from three adjacent, fishless ponds located 6 km southwest of Beaver Mines, Alberta (49°27'N, 114° 12'W) on 30 May 2002. Eggs were maintained at 10°C in the laboratory under a 14:10 hour photoperiod and hatched 10-12 days later. Larvae were maintained at 10°C for the subsequent 10 days, while they fed from their yolk reserves. On 21 June, 30 larvae (total length: mean = 9.87 mm, SD = 0.23, n = 11) were apportioned into each of 16 separate containers. Each container was then assigned at random to one of 16 screened, plastic, 1200 L livestock watering tanks (108 cm diameter x 120 cm high) located within a fenced enclosure on University of Lethbridge property. The procedure used to set up the tanks followed those described for other studies involving larval salamanders (e.g., Semlitsch 1987). In brief, the tanks were filled on 17 May 2002 with 1150 L of irrigation water, 800 g of air-dried reeds (*Typha* sp.) and 200 g of mixed, wet leaf material, both of which were collected from the edges of a local pond. One litre inoculations of concentrated zooplankton and amphipods collected from 3 - 6 local ponds commenced on 14 June and continued every 14 - 16 days until the end of the experiment. Inoculations were aimed at providing an ad-libitum food source for fish and salamander larvae throughout their development (Anderson 1968) and to minimize intraspecific cannibalism (Wildy et al. 2001).

Fish, odonate and control treatments were assigned to the 16 tanks at random. Larval odonates (mean length = 34 ± 3 mm, n = 8) had been collected from a pond 8 km southwest of Beaver Mines on 30 May. Minnows (mean standard length = 46 ± 5 mm, n = 8) were collected at Bathing Lake, AB (49°16'N, 114° 02'W) on 1 June. Trout (mean standard length = 69 ± 6 mm, n = 4) were obtained from the Allison Creek Brood Trout Station in the Crowsnest Pass, Alberta. Four tanks received two larval odonates, four received two young-of-the-year fathead minnows, four received one rainbow trout fingerling and four were predator-free controls. The treatments were added to the tanks on 28 June, allowing the larval salamanders a seven-day acclimation period in the absence of fish and odonates. Twice weekly observational surveys of the tanks were conducted throughout the experiment to ensure survival of fish and odonates and to monitor larval development status. The experiment was concluded after 30 days on 28 July when no salamanders were observed in some minnow and trout tanks. The survival of larvae was evaluated as each tank was drained and each surviving individual was weighed (± 0.001 g).

Larval survival was evaluated as the proportion of larvae within a tank that survived the 30-day experiment. Survival data were arcsin-square-root-transformed and analyzed using a one-way ANOVA. Analysis of fish and odonate effects on larval mass was based upon the average mass (g) of the survivors within an individual tank. No transformation of mass data was necessary to meet the assumptions of normality or equal variances. Differences between pairs of treatment means were evaluated with Tukey's HSD procedure.

3.3.2. Predation Trials

To evaluate palatability and rate of predation, long-toed salamander eggs, hatchlings and larvae were exposed to trout and minnows in the laboratory. An outline of the general experimental design, including predator treatments, size of fish and number of replicates is described in Table 3.1. Methods followed those used by Gamradt and Kats (1996) to assess palatability of and predation rates on California newt (*Taricha torosa*) larvae and egg masses by crayfish (*Procambarus clarkii*) and mosquitofish (*Gambusia affinis*).

Long-toed salamander eggs, hatchlings and larvae used in laboratory predation trials were collected as eggs on 12 May 2003 from a fishless pond located 15 km northwest of Twin Butte, Alberta (49°16'N, 114° 59'W). Rainbow trout fingerlings were obtained from the Allison Creek Brood Trout Station (Crowsnest Pass, Alberta). Adult female fathead minnows were collected from Bathing Lake on 20 May. All fish and salamanders were maintained in aerated, 20 L aquaria (40 x 20 x 25 cm) filled with dechlorinated tap water at 10°C and on a 16:8 hour photoperiod. Trout and minnows were fed standardized daily rations of Silver Cup Feed # 3 Crumbles (1 g) and Nutra-fin flakes

Table 3.1. Treatment types, mean standard fish lengths (± 1 SD) and replicates used in long-toed salamander egg mass, hatchling and larva predation trials.

Salamander stage	**Treatment**	**Mean fish length (mm)**	**Replicates**
Egg mass	One rainbow trout	60.1 ± 3.7	4
	Two juvenile minnows	29.4 ± 2.1	4
	Control		2
Hatchling	One rainbow trout	60.1 ± 3.7	4
	One adult minnow	54.2 ± 3.0	4
	Control		4
Larva	One rainbow trout	71.0 ± 3.0	4
	One adult minnow	51.5 ± 2.5	4
	Control		2

(0.25 g), respectively, at approximately 1000 hr. Salamander hatchlings were allowed to feed off of their yolks. Thereafter, larvae were fed zooplankton collected from various local ponds on an ad-libitum basis.

For each trial, treatments were replicated simultaneously in a random spatial arrangement of 20 L aquaria on a laboratory bench and maintained under the same conditions described above. One refuge, consisting of a plastic aquarium plant weighted by a rock, was placed at the centre of each aquarium. Trout and minnows were fed at approximately 1000 hours the day prior to each trial and then deprived of food.

The egg mass palatability trial commenced on 26 May. One egg mass containing 22.8 ± 5.7 embryos was added to each aquarium. Upon conclusion of the egg mass palatability trial, eggs were returned to their original aquaria and allowed to hatch. The hatchling predation trial began on 2 June, when salamanders were of mean length 11.5 ± 0.3 mm, had not yet developed legs, but had stabilizing balancers. Hatchlings did not require supplemental feeding during the trial as they were absorbing their yolks. The larva (30.2 ± 1.6 mm) predation trial started on 25 June. Larvae were at development stage 8 (Watson and Russell 2000) with visible gills, hind limb buds and forelegs bearing three toes. Approximately 175 ml of concentrated zooplankton were added to each tank at the start of the larva trial and again at 48 hr. Eight hatchlings or larvae were added to each aquarium.

Individual trout and minnows were used in only one of the hatchling or larva predation trials. Aquaria were monitored periodically for predation and to ensure that no fish mortality had occurred. At 96 hours, fish were removed and the proportion survival of eggs, hatchlings or larvae was evaluated. The locations of surviving hatchlings or larvae (water column or refuge) were evaluated upon termination of the trials.

Proportion survival data were arcsin-square-root-transformed prior to analysis. A two-way analysis of variance was used to compare the effects of treatment (trout, minnow and control treatments) and salamander development stage (hatchling and larva). Tukey's HSD test was used for comparisons between pairs of means.

3.3.3. Behaviour Experiment

To examine behavioural responses of long-toed salamanders to trout or minnows, individual salamander larvae were exposed to rainbow trout fingerlings or adult fathead minnows in laboratory aquaria. Methods were modified from Semlitsch and Reyer (1992) and Stauffer and Semlitsch (1993).

Salamander larvae used in the experiment were collected from a fishless pond located 17 km southwest of Beaver Mines on 24 July, 2003. Larvae were maintained in pairs in a series of 2 L (29 x 18 x 11cm), clear containers at 21°C and on a 16:8 hour light-dark cycle throughout the experimental period. Each pair was fed 50 ml of zooplankton and amphipods collected daily from a local pond. Trout and minnows were obtained from the same sources as those described for the predation study and were maintained under similar conditions.

The behaviour experiment took place 26 - 28 July, 2003, between 0900 and 1800 hours each day. Two 52 L aquaria (60 x 31 x 28 cm) were arranged adjacent to one another on a laboratory bench. Opaque, white paper was placed at the ends, bottom and back of each aquarium. Each aquarium was divided into two water-tight halves (test chambers) by a piece of opaque (painted white) Plexiglas secured with silicon (Figure 3.1). Each test chamber was further divided by a piece of 2 mm plastic netting secured lengthwise to divide the rear third of the chamber from the front section. The mesh

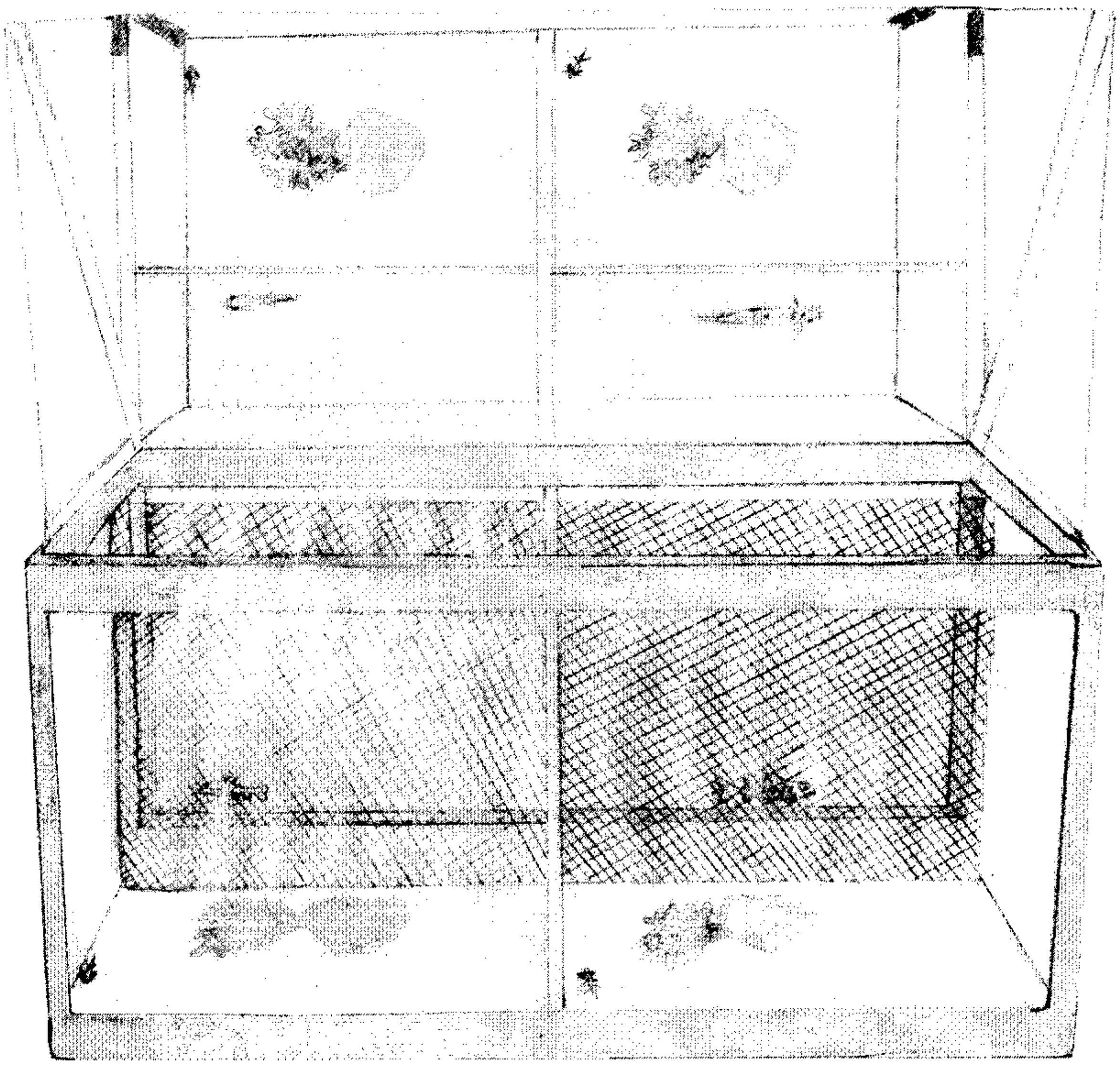

Figure 3.1. Aquarium (60 x 31 x 28 cm) design used to test behavioural responses of long-toed salamander larvae to rainbow trout and fathead minnows. Refer to text for description.

separated larvae and fish, but permitted transmission of chemical, tactile and visual cues. In each test chamber, an artificial plant and small rock refuge was placed centrally in the front section. Each test chamber was filled with 10 L of aged water. An angled mirror was placed on the top of each aquarium to permit simultaneous front and top view observations. A video camera was positioned on a tripod 1.2 m from each aquarium so that the entire aquarium and the image in its mirror were visible, and larvae were easily observed. Responses of larvae in all four test chambers were video-recorded simultaneously.

Salamander larvae (47.2 ± 5.0 mm) were approximately eight weeks of age, when I assumed that the development of locomotory and sensory abilities required for behavioural responses would be well advanced. One randomly selected larva was released within the front portion of each test chamber and allowed a 30 minute pre-exposure acclimatization period. In the last ten minutes of the pre-exposure period, video recording was commenced. At the end of the pre-exposure period, a randomly-selected minnow (mean standard length = 55.5 ± 5.7 mm) or trout (107.2 ± 3.7 mm) was added to the rear enclosure of each test chamber. The fish and control treatments assigned to each test chamber and each trial were chosen randomly. Video recording continued for a second, five-minute acclimatization period, followed by the 25-minute exposure period. At the end of the exposure period, video recording was stopped and larvae and fish were removed from the tanks. Individual larvae were not reused in the experiment; however trout and minnows were returned to their tanks and used repeatedly on a random basis. Twelve replicates of each treatment were completed. Upon conclusion of each trial, aquaria, plants and rocks were rinsed with tap water. The relative locations of the aquaria were alternated between each trial to eliminate positional bias.

I recorded the proportion of time that each larva spent swimming or resting (activity level), at the aquarium edge, in open-water or within the refuge (microhabitat use) and at the front or back half of test chamber (proximity to fish), for the pre-exposure and exposure periods. Data were recorded from the videotapes at 30-second intervals. Response variables analyzed were those in which larvae would be most (swimming, open-water or back of chamber) or least (within refuge) vulnerable to predation. Outliers were removed from the dataset prior to analysis following methods described by Sokal and Rohlf (1981). All proportional data were arcsin-square-root-transformed to improve normality and homogeneity of variances prior to analysis. Behavioural responses of the larvae were analyzed by repeated measures analysis of covariance for the effects of treatment and exposure period. Repeated measures analysis of variance was used because the same salamanders were evaluated before and during exposure to fish. Thus, the variable 'exposure period' was a repeated measure, assessing the effects of fish addition on the response variables. A preliminary analysis indicated high variation in activity levels of individual larvae during the pre-exposure period. Thus, the proportion of time that larvae spent swimming in the pre-exposure period was used as a covariate in analyses of the other response variables. Tukey's HSD procedure was used to analyze pairwise differences.

3.4. Results

3.4.1. Mesocosm Experiment

At the end of the mesocosm experiment, each tank contained two minnows, one trout or two odonate larvae. The mean survival of salamander larvae was reduced significantly in the minnow and trout tanks compared to the odonate and control tanks

(Figure 3.2; $F_{3,12} = 9.76$, $p = 0.002$). An average of 5% and 3% of the 30 salamanders that were added to the trout and minnow tanks, respectively, survived the 30-day experiment, whereas 24% and 44% of larvae survived in odonate and control tanks.

Mean mass of surviving larvae in the minnow tanks was significantly lower than in the other three treatments (Figure 3.3; $F_{3,12} = 5.45$, $p = 0.013$). Mean larval mass in the minnow treatment was reduced from the control by 37%, while mass of larvae in the trout treatment was reduced by 14%.

In mesocosm experiments, treatment effects have the potential to be confounded by density effects. Thus, the mass of survivors in the mesocosms may be due to size-selective predation, but can also be due to a reduction in larval density within ponds. If salamander mass was density-dependent, then the heaviest salamanders would be present in low density tanks. Rather, there was a significant positive correlation between proportion survival and mean mass of larvae ($r = 0.551$, $p = 0.0271$, $n = 16$), indicating a positive association between survival and body size.

3.4.2. Predation Trials

Egg survival was 100% in each treatment; all embryos and surrounding layers remained intact, indicating that neither trout nor minnows attempted to prey on eggs. For the hatchling and larva trials, there was a significant interaction between treatment and salamander development stage, indicating that survival of salamanders at the two stages was affected differently by trout and minnows (Figure 3.4; two-way ANOVA, treatment x development stage; $F_{2,15} = 15.39$, $p < 0.0001$). Salamander survival was significantly different in each of the control, minnow and trout treatments. Salamander hatchlings and larvae suffered no mortality in the control treatments. Hatchling survival

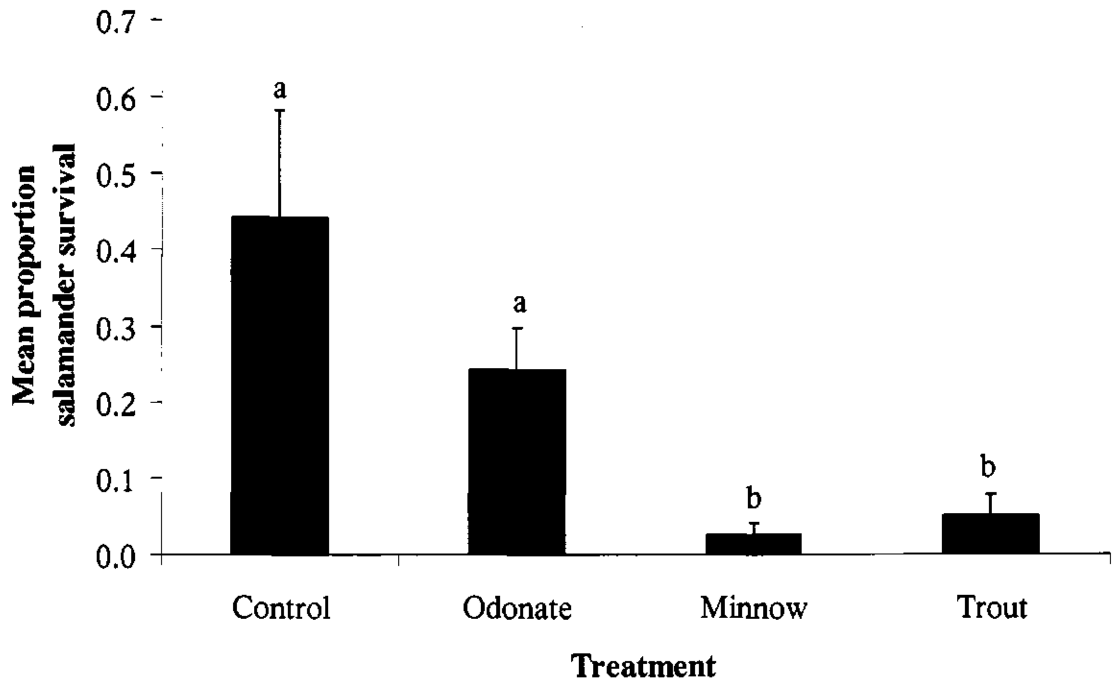

Figure 3.2. Mean proportion survival (± 1 SE) of larval long-toed salamanders reared in experimental mesocosms containing odonate larvae, fathead minnows and rainbow trout. Different alphabetic subscripts indicate significantly different means.

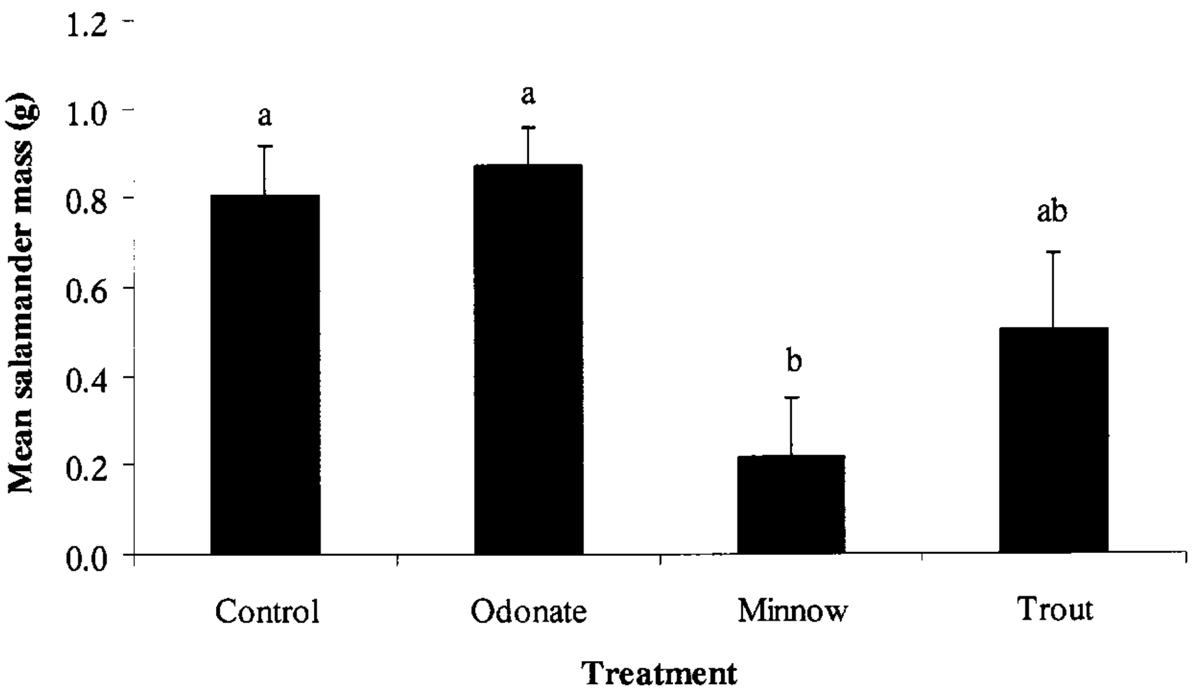

Figure 3.3. Mean mass (± 1 SE) of larval long-toed salamanders reared in experimental mesocosms containing odonate larvae, fathead minnows and rainbow trout. Different alphabetic subscripts indicate significantly different means.

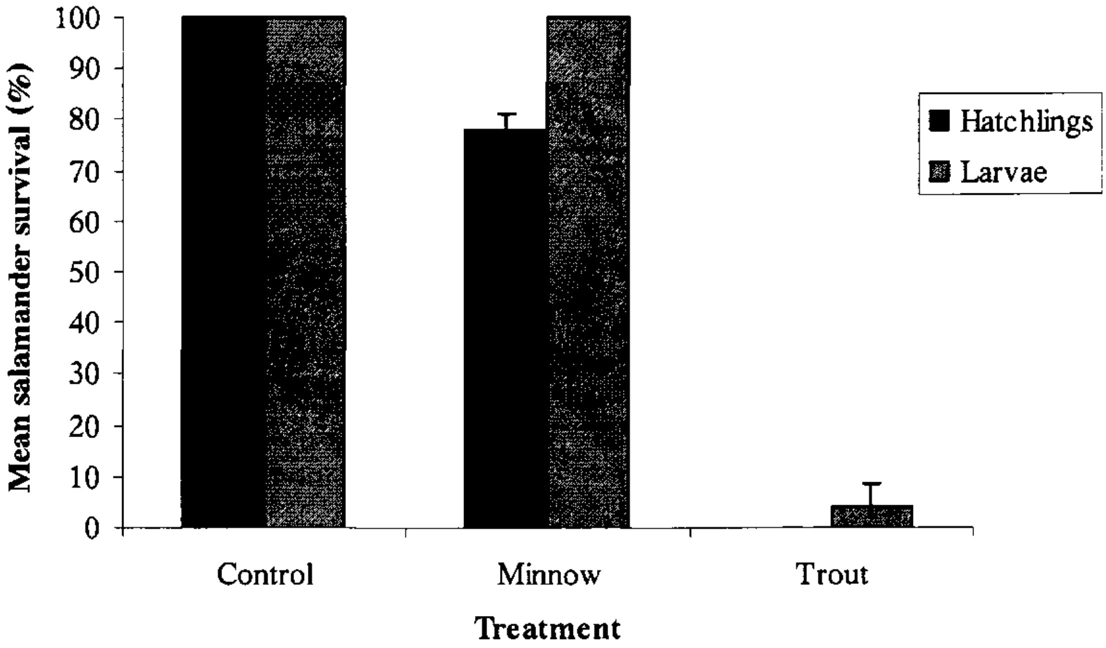

Figure 3.4. Mean percentage survival (± 1 SE) of long-toed salamander hatchlings and larvae exposed to fathead minnows and rainbow trout in laboratory predation trials.

was reduced by 22% in the minnow treatment, whereas all larvae survived when exposed to minnows. No hatchlings survived exposure to trout, while survival of larvae in trout treatments was reduced by 96%.

My anecdotal observations indicated that trout consumed all hatchlings within approximately 30 minutes of the hatchling trial's commencement. In contrast, minnows were observed causing fatal injuries to hatchlings but avoided consuming them. One of the trout replicates in the larva predation trial was not included in the analysis because the trout escaped from the aquarium after consuming 25% of the larvae and was not replaced. The sole larva that survived in the other trout replicates was within the refuge at the end of the trial. Trout were observed preying directly upon larvae; most had been consumed within a half hour of that trial's commencement.

3.4.3. Behaviour Experiment

Throughout the behaviour experiment, responses of individual salamander larvae were highly variable across treatments and exposure periods. This may have restricted my ability to detect differences in larval behaviour in response to the fish treatments. Table 3.2 shows the mean proportions of time that larvae spent in each activity, microhabitat and portion of the test chamber during the pre-exposure and exposure periods and across treatments. In all treatments and exposure periods, larvae spent the majority of their time resting, within the edge microhabitat and at the back of the test chamber.

Salamander use of the open-water microhabitat or back of the test chamber was not significantly different across treatments or exposure periods (Table 3.3). However, the presence of fish had a significant effect on the proportion of time that larvae spent within the refuge (Table 3.3). The pairwise comparison showed that larvae in the

Table 3.2. Mean proportion of time long-toed salamander larvae spent swimming or resting, within the refuge, open-water or edge microhabitats, and at the front or back portion of the test chamber. Means for the pre-exposure period are in grey font and exposure period means appear in bold.

		Control		Minnow		Trout	
Activity level	Swimming	0.21	**0.37**	0.34	**0.28**	0.43	**0.27**
	Resting	0.79	**0.63**	0.66	**0.73**	0.58	**0.74**
Microhabitat use	Refuge	0.00	**0.02**	0.01	**0.17**	0.00	**0.03**
	Open-water	0.15	**0.12**	0.18	**0.15**	0.06	**0.08**
	Edge	0.79	**0.82**	0.79	**0.68**	0.93	**0.89**
Proximity to fish	Front	0.25	**0.41**	0.35	**0.42**	0.47	**0.41**
	Back	0.75	**0.59**	0.65	**0.58**	0.53	**0.60**

Table 3.3. Summary of two-way repeated measures analysis of covariance for proportion of time larvae spent within the refuge, in open-water and at the back of the test chamber. Significant results are shown in bold.

Response variable	Source of variation	df	Mean square	F-value	P-value
Refuge use	Swimming (pre-exposure)	1	0.008	0.286	0.5967
	Treatment	2	0.118	4.404	**0.0207**
	Error	31	0.027		
	Exposure period	1	0.020	0.589	0.4485
	Exposure period * Swimming	1	0.050	1.438	0.2395
	Exposure period * Treatment	2	0.103	2.990	0.0649
	Error	31	0.034		
Open-water use	Swimming (pre-exposure)	1	0.044	0.514	0.4784
	Treatment	2	0.082	0.960	0.3936
	Error	32	0.085		
	Exposure period	1	0.025	0.458	0.5036
	Exposure period * Swimming	1	0.073	1.329	0.2575
	Exposure period * Treatment	2	0.001	0.027	0.9735
	Error	32	0.055		
Back of chamber	Swimming (pre-exposure)	1	4.306	23.210	**0.0001**
	Treatment	2	0.016	0.085	0.9191
	Error	32	0.186		
	Exposure period	1	0.173	1.062	0.3105
	Exposure period * Swimming	1	0.062	0.381	0.5414
	Exposure period * Treatment	2	0.091	0.559	0.5773
	Error	32	0.163		

minnow treatment spent significantly more time within the refuge than larvae in trout and control treatments (Table 3.2). The nearly significant interaction between exposure period and treatment (Table 3.3) indicated that long-toed salamanders exposed to minnows tended to spend more time within the refuge during the exposure period. These results and my informal observations indicated that variations in the activity level of the fish may have been responsible for the significant difference in the proportion of time that larvae spent within the refuge during the exposure period. I tested this notion by collecting additional data on the proportion of time that each trout and minnow spent swimming, and then evaluating the correlation betweenfish activity and the larval use of the refuge. Minnows spent a significantly higher mean proportion of time swimming (71%) than did trout (11%) (one-way ANOVA; $F_{1,22} = 40.77$, $p < 0.0001$). However, this difference was not correlated with any of the behavioural responses of long-toed salamanders.

3.5. Discussion

The direct effects of rainbow trout on long-toed salamander populations have been well-documented. Tyler et al. (1998b) used a mesocosm approach to show that trout predation was likely the primary factor leading to observed allopatric distributions of trout and salamanders in subalpine lakes in Washington State, USA (Tyler et al. 1998a). Similarly, trout predation is likely responsible for the patchy distribution of long-toed salamanders within subalpine watersheds in Montana (Funk and Dunlap 1999), Idaho (Pilliod and Peterson 2001) and Oregon (Bull and Marx 2002). My results further highlight the significance of rainbow trout as predators of long-toed salamanders. My results also demonstrate that trout are capable of rapidly consuming all larvae within their sight, and that long-term (30 d) exposure of larvae to trout leads to severe mortality.

Predation by trout is therefore the most likely fish-related mechanism leading to long-toed salamander declines in habitats containing introduced trout.

Results from my mesocosm study are the first I am aware of to show that fathead minnows, gape-limited fish not typically known to include amphibians as prey, are capable of reducing survival of salamander larvae to the same extent as trout. Moreover, larvae raised in minnow mesocosms weighed significantly less than larvae from trout, odonate or control treatments. The main implication of the mesocosm experiment is thus that indirect effects of minnows, such as competition for limited invertebrate prey or behavioural alteration, may play a more important role, over the longer term, in causing fish-induced reductions in salamander growth and survival than direct effects induced by predation.

Results of the predation trials indicate that a portion of the observed salamander mortality in minnow mesocosms was likely a result of attempted predation by minnows on hatchlings. However, the lower mass of surviving salamanders in minnow tanks suggests the importance of indirect minnow impacts. Fathead minnows are known to depress the abundance, biomass and species richness of zooplankton and other invertebrates (Hanson and Riggs 1995; Duffy 1998; Zimmer et al. 2001). The implication of these studies is that fathead minnows, and likely other small-bodied cyprinids, have the potential to act as exploitative competitors with larval long-toed salamanders. Although competitive interactions were not evaluated in my experiments, they can be inferred as a key factor in minnow mesocosms. Crustacean zooplankton are also a main prey item of rainbow trout and *Aeshna* larvae (Van Buskirk 1993; Donald et al. 2001). Interspecific competition may thus be expected to have taken place in trout and odonate treatments as well. However, my mesocosm results and those of Tyler et al. (1998b) show that surviving salamanders in trout treatments did not differ in size with those in control tanks. This suggests that little interspecific competition occurred in trout mesocosms, perhaps because trout fed mainly on salamanders, while the few surviving larvae accessed higher concentrations of zooplankton. Behavioural indirect effects, defined as the altered intensity or nature of competitive interactions among prey caused by the presence of a potential predator (Werner and Anholt 1996), may have been another unmeasured factor in trout, minnow and odonate mesocosms.

In the behaviour experiment, the proportion of time that salamanders spent within the refuge when exposed to minnows was significantly higher than when they were exposed to trout. This was a surprising result, as I had not expected for either fish to elicit such a strong response. It is difficult to distinguish the cues that led to such a response to minnows but not to trout. One possibility is that the salamanders have a generalized response to high rates of activity, but lack a specific behavioural response to the presence of fish. Larvae in the minnow treatment may have been hiding in response to the physical or visual stimuli from the active fish. Alternatively, as Stauffer and Semlitsch (1993) suggested, the abundance of abrupt movements by minnows may have caused concentrated waves of chemical cues, rather than otherwise gradually diffused chemical signals, to reach larvae and stimulate a response. Whatever the motivation, prolonged hiding by amphibian larvae may result in diminished foraging opportunities (Semlitsch 1987) and thus to decreased larval size, as was observed in the minnow mesocosms. Decreased salamander growth rate and body size have many potentially severe implications such as diminished reproductive potential, mating success and survival, heightened susceptibility to disease or starvation, reduced overwintering success and delayed sexual maturity (Semlitsch 1987; Semlitsch et al. 1988). Reduced growth rate may also prolong the larval period, thus extending exposure to aquatic predators (Werner and Anholt 1996). If long-toed salamander microhabitat use in natural habitats is altered by introduced minnows as my data suggest, such behavioural alteration, in combination with interspecific competition, has the potential to severely reduce salamander fitness.

My results also provide evidence of several indirect mechanisms involved in trout-salamander allopatry. Larval unpalatability and antipredator behaviours are positively associated with amphibian co-existence with fish in natural habitats (Pearson, unpublished data). My observations in the laboratory verify that long-toed salamander hatchlings and larvae are palatable to trout. Further, larvae did not exhibit behavioural responses to trout. Long-toed salamanders usually inhabit temporary lowland ponds and high-elevation, historically fishless lakes (Petranka 2001) where defense mechanisms against fish predators have not been necessary until recently (Donald 1987). Moreover, larvae inhabiting ephemeral ponds or cold mountain lakes must develop quickly to avoid pond drying or overwintering as larvae. High activity and feeding rates are thus favoured (Sih et al. 2000). Sih et al. (1988) demonstrated high rates of emergence from a refuge by *Ambystoma* larvae in the presence of fish. Similarly, Kats et al. (1988) verified that ambystomatids occupying temporary habitats similar to those of the long-toed salamander were palatable and showed ineffective antipredator behaviour. My predation and behaviour experiments confirm that long-toed salamanders also lack such antipredator strategies. In further support of this, Tyler et al. (1998a) found no significant differences in the proportion of long-toed salamander larvae hiding in bottom substrate in fishless lakes and lakes with fish. This apparent lack of long-toed salamander adaptations to predation by fish supports previous suggestions that predation is the primary mechanism involved in the exclusion of salamanders from introduced trout habitats.

Although conditions were controlled in the mesocosms, reduced survival of larvae in the control tanks was similar to survival rates observed in fishless field enclosures in similar studies (Travis 1983; Semlitsch 1988; Petranka 1989; Scott 1990). This suggests that, rather than the perhaps unrealistically high survival attained in many mesocosm studies (Semlitsch 1987; Tyler et al. 1998b), my mesocosms more closely approximated the conditions found in natural pond communities. Moderate rates of salamander mortality in the control mesocosms indicate that factors in addition to behavioural alteration, interspecific competition and predation played a role in determining survivorship. High rates of aggression and intraspecific cannibalism have been demonstrated for long-toed salamanders, particularly under conditions of restricted resources and high density (Wildy et al. 2001). Food limitation induced by minnows may be expected to have resulted in higher cannibalism among salamanders. However, my observation that larvae in the minnow mesocosms were significantly smaller than in other treatments is not consistent with the general rule that larger, cannibalistic larvae consume smaller conspecifics (Walls et al. 1993; Wildy et al. 1998). Cannibalistic morphologies described by Walls et al. (1993) were not observed in any treatments upon termination of the mesocosm experiment, yet we were not able to rule out the possibility that some cannibalism took place within the mesocosms. Intraspecific competition may have also had an effect on larval survival in control mesocosms; however the high mass of those larvae at the experiment's termination indicates that it was not a major factor.

Unfortunately, it is not possible to separate the effects of predation, competition, behavioural alteration and cannibalism on the growth and survival of salamanders in the mesocosm experiment. Yet in combination with my laboratory studies, these results demonstrate how the former three factors may play a role in salamander populations where predatory or gape-limited fish have been introduced. Factorial experiments designed to evaluate interactions between these factors could usefully address the complexity that is undoubtedly a feature of natural salamander populations, particularly those within watersheds that contain introduced sport and bait fish.

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CHAPTER 4: KEY ADVANCES, FUTURE DIRECTIONS AND CONSERVATION IMPLICATIONS

4.1 Key Advances

This thesis had three primary goals. The first was to describe the distribution of long-toed salamanders relative to introduced trout at low- and high-elevation habitats along the periphery of the salamander's range. Second, I investigated the relative effects of trout and minnows on long-toed salamander growth and survival in outdoor mesocosms. My third goal was to examine whether fish-induced behavioural alteration was an important determinant of salamander growth and survival. Significant advances have been made on each of these goals.

One key advance was the demonstration that long-toed salamanders and trout were distributed allopatrically at both low- and high-elevation habitats in southwestern Alberta (Chapter 2). Similar patterns have been observed at high-elevations within the core of the salamander's range (Tyler et al. 1998a; Funk and Dunlap 1999; Pilliod and Peterson 2001; Bull and Marx 2002). Yet, my data are the first to show that trout also negatively affected the distribution of salamanders at low-elevation habitats. The relatively high productivity of low-elevation ponds may make them especially important for the persistence of regional salamander populations (Pulliam and Danielson 1991). One implication of these results is that the presence of introduced trout in low-elevation habitats may therefore have greater potential to reduce salamander population sizes than those in high-elevation habitats. The fact that the effects of introduced trout at the periphery of the long-toed salamander's distribution are similar to those within its range core suggests that these effects can potentially result in contractions in the salamander's overall range.

A second advance comes from the controlled mesocosm experiment. Results from this component of my study indicate that trout have strong negative effects on the survival of long-toed salamanders, corroborating the results of Tyler et al. (1998b). Further, they are the first results to identify the potential of relatively small-bodied, gape-limited minnows to reduce salamander survival and growth (Chapter 3). Previous studies have shown that mosquitofish (*Gambusia affinis*) (Gamradt and Kats 1996), and mosquito larvae (Mokany and Shine 2003) can similarly reduce the size of individual amphibian larvae. My results are the first I am aware of to indicate that the widespread introduction of minnows as baitfish and hobby fish may also pose an important threat to amphibian populations.

A final advance was an improved understanding of the mechanisms by which trout and minnows affect long-toed salamanders (Chapter 3). Previous studies have suggested, but not tested, that predation by trout on long-toed salamanders is the main mechanism responsible for trout-salamander allopatry (Tyler et al. 1998b; Pilliod and Peterson 2001; Murphy 2002). However, this is the first study to confirm, in an experimental setting, that trout readily consumed long-toed salamander hatchlings and larvae. Conversely, gape-limited minnows were observed to present a much lower predation risk, which indicates that decreased salamander survival in minnow mesocosms was more likely a result of interspecific competition. Further, my behaviour experiment expanded on work by Kats et al. (1988) to show that long-toed salamander larvae appear to lack specific behavioural responses to fish, but show a more general response, possibly to high rates of fish activity (Chapter 3). Thus, long-toed salamander larvae do not appear to have developed unpalatability or antipredator behaviours as strategies to minimize the relatively recent risk of predation by introduced fish. This evidence supports the results of my field surveys and mesocosm study, where salamanders were absent from or had reduced survival in habitats containing fish.

4.2 Future Directions

The combined results from the field surveys and laboratory experiments provide strong evidence that the observed patterns of trout and salamander allopatry are due to predation by trout. However, this evidence must still be considered indirect. Ideally, a direct test of the effects of introduced fish on salamanders would involve adding fish to extant salamander sites or eliminating fish from sites where salamanders have not been observed, and monitoring long-term recovery. This approach has been attempted previously, but the outcomes of those studies were restricted by low replication, a lack of density data and the short-term nature of the measured responses (Macan 1966; Sexton and Phillips 1986; Aronsson and Stenson 1995). Parker and Schindler (2000) made significant progress in removing introduced trout from high-elevation alpine lakes using gillnets. More structurally complex, low-elevation ponds may be more challenging to restore to their naturally fishless state. Additional studies that address the feasibility of restoring low- and high-elevation lakes and ponds to fishless states are needed.

Results from the mesocosm experiment, while verifying the importance of trout predation, also indicated the importance of indirect and interactive effects in determining long-toed salamander growth and survival. There is a need for additional mesocosm experiments that confirm whether interspecific competition between minnows and salamanders was the mechanism that caused the reduction in salamander growth. For example, minnows could be enclosed within a mesh structure to separate direct predation effects from competition effects. While long-toed salamanders and fish are an appropriate model system to evaluate the linkages between competition and predation, they are complicated by the fact that long-toed salamanders are cannibalistic (e.g., Wildy et al. 2001). Follow-up studies using the mesocosm approach are probably the most effective method of examining interactions between competition, predation and cannibalism. Conditions outlined by Wildy et al. (2001) that tend to induce cannibalism between long-toed salamander larvae should be avoided in future mesocosm studies. Periodic surveys for cannibalistic salamander morphs should also take place throughout future mesocosm studies.

Future studies might also consider the implications of my results at a broader scale. Results from Chapter 2 show that long-toed salamanders are widely distributed in lakes and ponds within southwestern Alberta watersheds. My anecdotal observations indicate that at some of those sites, particularly those at low-elevations, they can occur in high densities. Long-toed salamanders thus have the potential to play an important role within an ecosystem context; as important prey to terrestrial organisms such as mountain garter snakes (*Thamnophis elegans*) (Matthews et al. 2002) and as top vertebrate predators within fishless lakes (Petranka 2001). It is noteworthy that the extensive series of studies that have examined the impact of introduced trout in Rocky Mountain aquatic ecosystems have not considered the functional role of salamanders as top vertebrate predators (Donald et al. 2001; Parker et al. 2001; Schindler and Parker 2002). Lamontagne and Schindler (1994) described a method of determining the historical status of fish populations in Rocky Mountain lakes from fossilized *Chaoborus* mandibles. A similar technique should be developed to identify lakes that historically hosted salamander populations, and thus to clarify the roles of salamanders in those ecosystems. This could potentially be accomplished by examining historic zooplankton assemblages.

4.3 Conservation Implications

A growing body of research on the effects of introduced fish on amphibians now provides strong evidence that amphibian populations have declined or are at risk of declining as a result of the creation and maintenance of introduced fish populations. This has been especially well-documented in the case of the long-toed salamander. The results of this study have several implications for the management of long-toed salamanders, fish and other organisms within my study area and beyond.

In order to ensure the long-term persistence of long-toed salamanders at the landscape scale, I strongly recommend that management agencies restore to a fishless state all lentic habitats that have the potential to host salamander source populations. This is especially important at high-elevations, where long-toed salamanders require the same deep lakes that are most often occupied by stocked trout. Rates of long-toed salamander dispersal between low- and high-elevation breeding habitats, or between habitats at any elevation, are unknown. Such information, though challenging to gather, would be of tremendous value in identifying habitats that require restoration. Further, a series of biotic and abiotic habitat characteristics could be measured and correlated with salamander distributions to determine those habitats that are best-suited to support long-toed salamander populations (Tyler et al. 1998a; Knapp 2004). If such additional information does not soon become available, all lentic habitats containing stocked trout should be restored to a fishless state. The presence of long-toed salamander populations at all of my low-elevation study sites except those that contained trout strongly suggests that if all trout were removed, each of the sites would contain salamanders. However, most of the low-elevation ponds where trout occurred were vulnerable to trout introductions via flooding rivers. From a conservation perspective, those are largely unavoidable, and thus acceptable, salamander sink habitats. On the other hand, I expect that the removal of stocked trout from all other low-elevation ponds would result in the reestablishment of sizeable long-toed salamander populations.

Once introduced trout are removed from selected habitats, the restoration of salamander populations should be closely monitored. I expect that, due to their close proximity to existing salamander populations and the presence of fewer geographic barriers, low-elevation habitats would likely be colonized by salamanders within a few years of their restoration. In contrast, I expect that the higher degree of isolation and sizeable geographic barriers would slow recolonization of high-elevation salamander habitats considerably. Funk and Dunlap (1999) documented recolonization by long-toed salamanders at high-elevation lakes in Montana that had been restored to their fishless states. Despite genetic evidence that suggests low levels of dispersal between long-toed salamander populations in that area (Tallmon et al. 1999), salamanders recolonized the lakes within 20 years of the confirmed absence of trout and likely travelled overland to those sites from lakes located within a 1 km radius. Similar potential exists for long-toed salamanders to recolonize restored high-elevation habitats within my study area. For example, long-toed salamanders have been observed since 1994 (Fukumoto 1995; Snyder et al. 2003) at Lost Lake, a high-elevation site in Waterton Lakes National Park that contained stocked trout from 1926 – 1972 (Rawson 1938; Goble 1973). The nearest known long-toed salamander habitat to Lost Lake has never been stocked with trout and is located approximately 2 km away, on the other side of a steep, treed ridge. Powell et al. (1993) documented a long-toed salamander at least 900 m from the nearest potential breeding habitat, which further confirms that individuals are indeed capable of travelling between neighbouring breeding habitats. If natural recolonization is not feasible due to isolation or small salamander populations, translocation of salamander eggs or larvae from the nearest known source could be an alternative method of restoring long-toed salamander populations.

Introduced species have been identified as the most challenging human-caused threat to amphibian conservation to reverse or remove (Kiesecker 2003). Additional research, effective communication between scientists and managers and sensible management actions are imperative to ensuring the long-term conservation of long-toed salamander populations throughout their range.

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