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Effects of habitat features on long toed salamander (*Ambystoma macrodactylum*) populations

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Effects of habitat features on long toed salamander (*Ambystoma macrodactylum*) populations

by

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Abstract

Amphibian populations are experiencing worldwide declines, occurring at higher rates than for any other taxon. Long toed salamanders *Ambystoma macrodactylum*, Baird, 1850, have been listed as a species of special concern in Alberta since 1999 when they were considered not at risk. I investigated the effects of potential habitat threats on long toed salamander populations by comparing historical with present-day populations in 13 breeding ponds in Southwestern Alberta and assessing indicators of habitat quality as possible population predictors. My goals were to: (1) to update presence/absence status of previously confirmed populations (Graham & Powell 1999), (2) measure variation between population and habitat characteristics to study relationships between the two, and (3) collect preliminary evidence of population turnover (which could be a preliminary indicator for metapopulation structure). First, I found that six of the 13 ponds (46%) occupied by salamanders two decades ago had since lost their populations. I also found that the limited measures of environmental variables I considered do not seem to accurately predict variation in population abundance. Finally, I observed preliminary evidence of population turnover, with population status changes in four of the nine ponds that could be measured in both years. This study helps to fill a gap in provincial population data for this species, and points to some variables that may be vital habitat characteristics, providing information that can be used to direct conservation policy and future research.

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Chapter 1: Introduction

1.1.1 Amphibian population declines

Amphibian populations have experienced worldwide declines in recent years, occurring at higher rates than for any other taxon (Kenison et al. 2016). Of over 5,700 known amphibian species, more than 40% are facing losses (Davidson & Knapp 2007). This unfortunate trend illustrates what has been referred to as the largest and most widespread bout of extinction events since the loss of the dinosaurs (Hopkins 2007). Amphibians are more sensitive to disturbance in their environment than many other animals for several reasons and are often good indicators of the health of ecosystems they inhabit (Marsh et al. 2005). One reason for their pronounced sensitivity is their use of both aquatic and terrestrial habitats for their life histories, so that the alteration of even one of these habitat types can result in loss at the ecosystem level and possibly lead to population loss. Their physiology as ectotherms allows them to make use of resource poor environments, which can expose them to greater risk of bioaccumulation of pollutants and exposure to disease through their permeable skins (Hopkins 2007). The health of amphibian populations can often be used as an indication of environmental health because as a group they span a wide range of trophic niches, facilitating resource transfer through the food web (Hopkins 2007). Although it is generally agreed that decreases in habitat quality, fragmentation, and overall loss are the main drivers of the widespread population loss trend (Funk & Dunlap 1999, Government of Alberta 2017), our knowledge of its causes on the finer scale is lacking. Amphibians' need for both terrestrial and aquatic environments and migration/dispersal facilitation throughout them to complete their life histories (Pagnucco et al. 2012, Lee-Yaw et al. 2015, Cosentino & Brubaker 2018) highlights the importance of both the "pondscapes" and landscapes adjacent to and among them being viewed with equal importance when accurately assessing habitat quality and the needs of at-risk species.

Declines in amphibian species are seen even more severely in Canada than in other parts of the world. By 2016, COSEWIC had categorized 23 of the 52 species and subspecies known in Canada as endangered, threatened, or of special concern (Canadian Herpetological Society 2021). In addition to the habitat fragmentation, loss, and degradation, known to contribute to declines globally, struggling Canadian populations are also affected by vehicle collisions, illegal harvesting, climate change, chemical pollution, targeted eradication efforts, disease, and non-native species introductions (Canadian Herpetological Society 2021). Making matters worse is the fact that many Canadian amphibians are at the northern end of their range, often limited by shorter breeding seasons, resulting in smaller populations to start. These populations are often located exclusively in the Southern areas of the country, where anthropogenic development is greatest. Canada's economic structure also means natural resource extraction and agriculture are often responsible for large scale habitat loss (Canadian Herpetological Society 2021). There is also the problem of data deficiency for many amphibian species, and lack of points of reference for populations resulting in difficulty with monitoring and understanding environmental impacts (Lesbarreres et al. 2013). There

are many landscape features and anthropogenic influences that can harm amphibian populations, both directly and indirectly at a broader landscape level. Here, I will discuss three factors that may negatively affect amphibian populations: roads, fish stocking, and fragmentation.

1.1.2 Effects of roads on amphibian populations

Roadways cause vehicle/animal collisions in many species, but slow-moving migratory ones such as amphibians are at higher risk. Collisions occur mainly during their spring and fall migrations to and from breeding ponds, where in some areas hundreds of unfortunate individuals can be observed flattened on highway surfaces (Powell 2019 personal communication). Injury mitigation measures include installation of under- or over- passes to facilitate amphibian crossings, which have been shown to decrease mortality of at-risk populations by up to 80% in Waterton Lakes National Park (Pagnucco et al. 2012). Roadways also cause less direct impacts to amphibians; for example, anthropogenic infrastructure that degrades local habitat and fragments what remains (Marsh et al. 2005, Pagnucco et al. 2012). Many salamander species have well-developed homing abilities, preferentially returning to the same pond where they were born for breeding each year (Marsh et al. 2005). Observations of long-toed salamanders entering breeding ponds and exiting in the same direction indicates that they follow similar annual migration patterns (Pagnucco 2010). This is a practice that can be interrupted by roads. Travel success was found to decrease by 51% in red-backed salamanders (*Plethodon cinereus*) when roads fragmented their migration routes (Marsh et al. 2005). Based on these observations, the orientation of local roads within the breeding pond/upland habitat complex should affect the level of impact on travel and mortality. The physical characteristics of local roads also appear to play a role in the level of danger presented to migrating amphibians. Roads with steep edges, especially where these slopes lead downward to a road, for example, were found to further decrease road permeability for migrating salamanders (Marsh et al. 2005). Because of both direct mortality and behavioral avoidance by migrating amphibians, roads should be considered partial barriers to travel, which is required for successful migration, reproduction, and dispersal.

1.1.3 Effects of fish stocking on amphibian populations

Another major threat to amphibian habitat is the stocking of breeding ponds and lakes with non-native fish species, which occurs widely over amphibian ranges in Alberta. Fish stocking is commonly chosen for the benefit of recreational anglers but renders many otherwise suitable amphibian breeding areas uninhabitable. Amphibian eggs and larvae are palatable to the vast majority of fish species, meaning that cohabitation between the two is naturally rare (Pearson & Goater 2008, 2009). Although cohabitation is possible, it is likely due to sufficient within pond heterogeneity to facilitate safe oviposition sites and hiding from predators. Females usually avoid laying eggs where fish are detected, and where laying does occur survival rates are extremely low due to consumptive effects (Pilliod & Peterson 2001). The effects of fish stocking on long toed salamander populations are fairly well documented (Funk & Dunlap 1999, Pearson & Goater 2008, Pearson & Goater 2009, Pagnucco et al. 2011, Pilliod et al. 2013, Kenison et al. 2016). This species is especially vulnerable to population declines caused by fish presence and is more likely to be extirpated by trout introductions than many other amphibian species (Welsh et al. 2006). Stocked fish present a novel challenge because fish are often restocked annually. This introduces fish to habitats that do not naturally support permanent populations, such as shallow water bodies that

freeze through in winter. These would normally remain available to amphibians if not stocked, representing a case of resource partitioning under normal conditions.

Introduced fish present a threat to amphibian populations by means of both predation and competition. Salmonids, one commonly introduced fish type, are top predators in most aquatic environments they inhabit, and prey directly on amphibians mainly in the egg and larval stages (Pearson & Goater 2009, Pagnucco et al. 2012, Kenison et al. 2016). Large fish also have less direct impacts such as affecting growth and development, likely through behaviour alteration with increased focus on predator avoidance rather than foraging. A decrease in growth rate of up to 85% and a 56% lower chance of metamorphosis was observed in long toed salamander larvae raised in the presence of trout cues (Kenison et al. 2016). Minnows are also commonly introduced as bait fish for anglers, but their limiting effects on amphibian populations are poorly understood (Pearson 2004, Pearson & Goater 2009). The smaller size of these minnows renders them gape limited with dietary restrictions based on prey size, including zooplankton, algae, and small insects (Pearson 2004). Their small body size and similar diet to long toed salamander larvae means they pose little predatory threat but are more likely to provide direct competition for food resources (Pearson & Goater 2009). A decrease in body size of 30-40% was observed in long toed salamander larvae reared in the presence of fat-head minnows (*Pimephales promelas Rafinesque*) (Pearson & Goater 2009).

The location of breeding ponds and lakes can be an important factor in long toed salamander survival in the presence of fish because of differences in characteristics caused by elevation. Individuals in lower elevation ponds tend to show higher rates of dispersal and are more likely to cohabitate with fish due to the presence of ponds' greater vegetative cover and resulting higher structural complexity (Kenison et al. 2016). Lower elevation ponds also tend to facilitate more rapid development, with larvae commonly overwintering for up to several years in higher elevation ponds (Pearson 2004), thereby increasing the amount of time spent in the vulnerable larval stage. This evidence strongly indicates that higher elevation ponds are disproportionately affected by fish introductions. Loss of amphibian habitat to nonnative fish is emphasized by the fact that amphibians have been found to recolonize lakes where fish stocking has ceased for long enough for these introduced species to become extirpated (Funk & Dunlap 1999).

The effects of competition and predation by fish are also evident at the landscape level. Long toed salamanders are significantly less likely to inhabit even fishless ponds within a landscape of greater overall pond occupancy by trout (Pilliod & Peterson 2001). Additionally, fish can invade ponds that are not intentionally stocked during acute events such as spring floods (Pilliod & Peterson 2001). This was supported by my own observations of one pond, fishless in 2019, producing two fish in traps during the 2020 season. Further investigation revealed that this particular pond had connectivity with another through an under-road culvert. Although above water level during most of my observations, it could have provided connectivity during seasonal flooding events. These landscape level effects and indications of metapopulations in fish suggest strongly plausible influences on habitat connectivity and population webs for long toed salamanders.

1.1.4 Metapopulations in amphibians

A metapopulation is a network of populations that have some form of connectivity with each other, usually on a spatial scale (Griffiths et al. 2010). While each population is independent with individuals breeding with other population members, they are connected with others to varying extents, often through the dispersal of juveniles moving from natal ponds to new breeding habitats (Muths et al. 2018). Metapopulations can be important for amphibian species persistence by providing compensation and in some cases, rescue, for populations facing decline or extirpation (Lee-Yaw et al. 2015). While multiple factors are to blame for declines in amphibian populations, habitat fragmentation is among the most important. Increased isolation of populations with decreases in both habitat patch size and occupancy have been observed in multiple amphibian species (Vos & Chardon 1998). Higher genetic variation between amphibian populations than for other taxa also suggest that amphibians face unique dispersal challenges (Funk & Dunlap 1999). Amphibians' relatively small range size, tendency toward site fidelity, and small body size resulting in limited travel capacity makes them particularly sensitive to population isolation amplified by fragmentation and loss of dispersal corridors. While many species require habitats that are more continuous (e.g., the general use of forest habitat), the discrete nature of breeding ponds for amphibians makes traversing the landscape a necessary challenge if some level of connectivity between salamander populations is to be maintained. Salamanders and newts have a relatively limited dispersal range of 300-1260 meters as recorded among several different species (Marsh & Trenham 2001). Dispersal facilitates the maintenance of genetic variation, population persistence or recovery, and ability to select preferred habitats in changing environments (Funk & Dunlap 1999, Marsh et al 2005, Lee-Yaw et al. 2015). Patterns of dispersal can be intrinsically linked to the existence of metapopulations. These can be vital to the survival of a species in the face of population declines or even extirpation by providing potential for recolonization.

In amphibians, metapopulations in a given area depend heavily on landscape features, the proximity of these features to one another, and habitat types (Lee-Yaw et al. 2015). Therefore, areas containing multiple ponds connected by moist habitat over relatively small distances should be conducive to metapopulations. The suitability of movement corridors is farther dependent on microhabitat features, as the ability of small-bodied individuals to travel with minimal physical obstruction while avoiding desiccation are, at extremes, mutually exclusive. In this case, dense ground cover would prevent desiccation, while also impeding travel, and a balance between the two appears to be ideal (Lee-Yaw et al. 2015). While metapopulations have been observed in some amphibian species (Marsh & Trenham 2001, Corser & Dodd 2004, Smith & Green 2005), microhabitat use in relation to dispersal and metapopulations in amphibians remains poorly understood (Smith & Green 2005, Lee-Yaw et al. 2015). This represents a gap in our knowledge of population dynamics and species persistence, from the microhabitat to the metapopulation level.

1.2.1 Study species

The long toed salamander (*Ambystoma macrodactylum* Baird, 1850) belongs to the order Caudata, which includes other salamanders and newts, and the family Ambystomatidae which includes the mole salamanders that are found exclusively in North America (Fukamoto 1995). Although long toed salamanders range from Alaska to California, the eastern subspecies (*A.m. krausii*) is found in Alberta mainly in the front ranges of the Rocky

Mountains (Atkinson-Adams 2015). This is one of five subspecies, which can be identified by distinct colouration, with classification confirmed by genetic sampling (Lee-Yaw & Irwin 2012). Because this subspecies is by far dominant in Alberta, I assumed that all specimens collected in the areas of this study belonged to it. The long toed salamander is listed as a species of special concern in Alberta (Government of Alberta 2017), having been “up listed” since 1999 when they were considered not at risk (Graham & Powell 1999). This turn for the worse in less than two decades has been attributed mainly to habitat loss, alteration, and fragmentation (Funk & Dunlap 1999, Government of Alberta 2017). They can breed in a variety of pond types, ranging from small ephemeral ponds to larger bodies such as lakes at a wide range of elevations from subalpine to near sea level (Pearson & Goater 2008). Breeding occurs in spring as soon as ice has melted enough for access to water, and eggs may even be laid under the ice (Oseen et al. 1995). Eggs are deposited in clumps and anchored to aquatic vegetation such as stems or small sticks, and hatch five to seven weeks after being laid (Fukumoto 1995). The larvae remain fully aquatic and are predatory from the start, feeding on zooplankton in early larval stages and preying on small aquatic invertebrates as they grow larger (Fukumoto 1995). Larvae are cryptic and tend to stay near the pond bottom among aquatic vegetation for hiding. Although I spotted a couple of larvae in the water during trapping, this was a rare occurrence. Time from hatching to metamorphosis can vary greatly and is heavily dependent on elevation. In lower elevation ponds, development from aquatic larvae to terrestrial juvenile normally takes a single summer, but at higher elevations can take up to three years (Pearson 2004). After reaching the juvenile stage (usually in late summer or sometime in the fall), young of the year emerge into the terrestrial landscape with the adults, where they forage for invertebrates. They usually reach sexual maturity when two to three years old (Atkinson-Adams 2015). The overwintering habits and terrestrial habitat use by long toed salamanders remains poorly understood (Alberta Government 2003, Atkinson-Adams 2015). It appears that they stay underground over winter for insulation, often using the abandoned burrows of other small animals (Pagnucco et al. 2012).

While long toed salamanders are themselves predatory from hatching through adulthood, they are also prey to a variety of predators. Predation in the egg stage is frequent, when individuals are sessile and unable to swim to safety and occurs mainly through consumption by fish (Fukumoto 1995). Fish continue to be the main predator in the larval stage, but they are also preyed upon by large aquatic insects and some birds (Fukumoto 1995). Dragonfly larvae were common catches in my minnow traps, especially in the Bow Valley. These are likely to be predators of long toed salamander larvae. Several of the larvae captured in my survey ponds had damaged tails, indicating attempted predation episodes. Cannibalism has been observed in a laboratory setting (Fukumoto 1995) and was more likely to occur when there was a pronounced size difference between individuals. The perils of the adult and juvenile stages are more often associated with migration and dispersal (e.g. crossing roads (Pagnucco 2012), while predation is a more significant threat in the egg and larval stages (Pearson et al. 2008). This is because mature individuals develop chemical defense in the form of a sticky white excretion from the skin when stimulated, which acts as an effective deterrent against most predators (Powell 2019, personal communication).

1.2.2 Research significance

Much of the current literature has focused on single variables causing amphibian declines (Pearson 2004, Pagnucco 2010, Kenison et al. 2016), while a closer look suggests deeper complexity at play. For example, while amphibians have proven capable of recolonizing previously stocked ponds after fish stocking has ceased (Funk & Dunlap 1999), some areas show continued declines or failed recolonization even after the extirpation of fish populations (Davidson & Knapp 2007). This indicates the involvement of at least one other variable and the need for multifaceted investigation. Another gap occurs in our understanding of amphibian dispersal and the potential for metapopulations. Similar to the tendency toward focusing on a relatively narrow set of parameters in research is that more of a landscape approach is needed to truly understand habitat influences on amphibian species success. Much research focuses on the pond as an independent entity or isolated habitat patch, with the assumption that amphibians do not venture far from them. This may be an inaccurate representation, as amphibians can move up to 10 km in extreme circumstances (Smith & Green 2005). It has been confirmed that amphibians may move considerably farther than anticipated by common models via unexpected events such as flooding (Oseen et al. 1995). Even though recent studies have been conducted on long toed salamanders in similar ecological regions, the majority of interest seems to be focused on Waterton Lakes National Park (Pearson 2004, Pagnucco 2012, Atkinson-Adams 2015). Few extensive surveys, if any, have been recently conducted in Southern Alberta to my knowledge, leaving current distribution in this area largely unknown. To my knowledge, my study is the first to assess the status of previous populations since the species up-listing in 2003. Data for the most recent long toed salamander management plan points out a lack of data especially with reference to population continuity and refers to information on habitat disturbance collected in 1999 (Alberta Environment & Parks 2016). Long toed salamanders have been identified as data deficient (Government of Alberta 2003, Atkinson-Adams 2015, Lee-Yaw et al. 2015, Alberta Environment & Parks 2016), which my research attempts to rectify.

1.2.3 Thesis outline and goals

Based on the former evidence, the central goals of my research are to (1) to update presence/absence status of previously confirmed populations (Graham & Powell 1999), (2) measure variation between population and habitat variables to study relationships between the two, (3) collect preliminary evidence of population turnover (which could be a preliminary indicator for metapopulation structure). I hypothesized that if long toed salamanders are affected by anthropogenic development, then population abundances should be lower in ponds with indication of habitat disturbance such as roads within one kilometer and fish stocking within breeding ponds. I predicted that there would be fewer present populations than two decades ago, and that human disturbance, particularly adjacent roads and fish stocking, would negatively impact relative population abundance. I also expected to observe some evidence of population turnover marked by inter-year inconsistencies within the observed populations. I studied the long toed salamander populations of 13 sample ponds in the Bow and Kananaskis Valleys in recent years and compared observations with surveys conducted before large scale population declines as a model for better understanding the species' up-listing during the past couple of decades. I went about this by comparing presence/absence status of populations in 2019 and 2020 with historical data, while measuring habitat features for comparison against current population abundances to determine which features best

accounted for population success or decline. Finally, I present findings from a review of the current literature on amphibian metapopulations and observations of population changes in my survey ponds across the two-year study period.

Chapter 2: Methods for quantifying long toed salamander populations and habitat quality

2.1 Study area

The Bow and Kananaskis River Valleys are part of Kananaskis Country and the front ranges of the Rocky Mountains in southwestern Alberta, Canada. This area, which lies between the Rocky Mountains and the Interior Plains, provides the temperate, moist climatic conditions that historically support viable populations of long toed salamanders (Graham & Powell 1999). I sampled 13 ponds in total (seven of these were in the Kananaskis Valley, $n=7$; and six in the Bow Valley, $n=6$ (Table 3.1); previously surveyed in the late 1990s when populations were considered stable (Graham & Powell 1999). Surveying both valleys provided an expanded sampling range while maintaining ecological similarity as both valleys were at similar elevations and in close geographical proximity to each other. The ponds surveyed in the two valleys during my study were representative of a variety of typical Rocky Mountain foothill ponds, with differing sizes, depths, substrates, and surrounding vegetation. Most ponds had snail shells along the shoreline, indicating the remnants of at least some moisture year-round. During the later season surveys of 2020, while all Bow Valley ponds remained intact with sufficient water levels for sampling, one Kananaskis pond had disappeared completely and three others had water levels too low for sampling. This indicated that some of the ponds in the Kananaskis were of an ephemeral nature. Most were too small to support fish populations and were historically fishless (all ponds in the Kananaskis Valley were fishless, as were four of the six Bow Valley ponds (Table 3.1)). All appeared to be suitable breeding habitat for long toed salamanders. They ranged in size from a few to a few hundred square meters, and in depth from approximately 30 cm to an estimated 2 meters (Table 3.1). The surrounding vegetation of most of the ponds was characteristic of the area and featured white spruce and trembling aspen stands, with willow species growing near the water. Some of the ponds had substrates with fine mucky silt, and some with large rocks. I observed that those with finer silts tended to show more aquatic vegetation growth mainly in the form of grasses and sedges. Most of the survey ponds in the Kananaskis Valley were considerably larger in surface area and depth than those of the Bow Valley

2.2 Methods

2.2.1 Characterizing populations

In 2019, I visited survey ponds weekly beginning in mid-April for the start of egg laying and spotted the first clutch on May 8th. I conducted observational egg searches within 2 m of the shoreline. This minimized disruption of nesting areas and is the depth at which egg clusters can be expected (Pretzlaw et al. 2002). The entire shoreline of most ponds was fully accessible, but in circumstances where the entire shoreline could not be reached, I used a transect system of repeated observations every 10-30 m, recording my visual estimate of total number of eggs observed. I returned for three replicate visits at each pond during spring 2019 (Table 2.1). At these times, I observed phenological differences between the Bow and Kananaskis Valleys. In the Bow Valley, pond ice had mostly lifted while some snow remained on the ground in patches. More ice and snow were observed in the Kananaskis Valley, with delayed budding of vegetation and temperatures at an average of 5 °C lower during the day.

Because eggs take several weeks to hatch and climatic variation was expected (Atkinson-Adams, 2015) and observed, I commenced minnow trapping several weeks after egg laying was confirmed. Trapping allowed me to identify, measure body length, and count toes to assign developmental stage of the individuals I captured, in addition

to identifying other amphibians and/or fish presence. Because of more rapid seasonal advancement, I surveyed the Bow Valley first (beginning on June 21st), followed by the Kananaskis (ending on July 13th) (Table 2.1). Collapsible funnel net traps (American Maple Minnow/Craw Trap Model #TR-501) were set at approximately 30–50 m intervals to scale for pond size, depending on accessibility and anchor points. This resulted in placement of two to eight traps per pond at marked locations. Each trap was made of cloth mesh with wire frame, 25 x 25 x 46 cm, with funnel entrance holes 6 cm in diameter. Traps were 50% submerged to ensure that any captive amphibians had access to both water and air and left in situ for 24 hours (Pretzlaw et al. 2002). The next day, traps were retrieved in the same order and at the same times of day in which they were set. The contents of each trap were poured into a sorting pan containing several cm of pond water. Upon identification, long-toed salamander larvae were removed from the pan and handheld for examination using nitrile gloves. Larval appendages develop in the sequential fashion of anterior to posterior limb buds followed by toes. For each individual captured, I recorded the emergence of limb buds and counted number of toes where applicable to assign a developmental stage using a stage identification table created for this species (Russell & Watson 2000). I also measured the body length in cm from snout to tail tip of each individual using a flexible plastic measuring tape and rounding values to the nearest mm. After observations, individuals were immediately returned to the same area of the pond in which they were captured. All animals were handled within Canadian Council of Animal Care standards (University of Calgary Animal Care Protocol # AC19-0043).

Due to COVID-19 restrictions, approval for 2020 field work and mobilization took until late summer, thus replication of my 2019 sampling was limited by time constraints. Because of the late season, water levels in four of the 13 ponds were too low to support minnow trapping. On August 11, I set the first traps in both the Bow and Kananaskis survey ponds (Table 2.1). Because it seemed reasonable to expect that hatching had already occurred, both valleys were surveyed simultaneously. This had the added benefit of allowing me to directly compare body length and developmental stage of individuals between all ponds. I carried out three trapping replication periods in available ponds until August 14th. Protocols for trapping and handling were the same as in 2019.

2.2.2 Habitat features

Across 2019 and 2020, I made observations during each pond visit of abiotic and biotic pond features. In addition to the more obvious variables needed to test some of my predictions, selected variables to be included in the scope of this study were based on our current understanding of threats to long toed salamander habitat, as well as referencing research protocols (Pretzlaw et al. 2002). Turbidity can affect individual survival of amphibians and be an excellent indicator of aquatic pollutants (Suh & Choe 2016, Calderon et al. 2019), which I measured using a LaMotte 2020i Turbidity Meter. Because water temperature can positively or negatively affect species abundance (directionality dependent on species observed) (Dubos et al. 2020) and low dissolved oxygen concentration (hypoxia) can affect population abundance in amphibians (Calderon et al. 2019), I measured both using a Myron L Ultapen PT5 Dissolved Oxygen and Temperature Pen. Extreme values of water pH have been shown to decrease population size and rate of development in amphibians, so I measured pH using an EcoSense pH10A pH & Temperature Pen. A single reading was made on each visit, but at a different area of the pond each time to gain

accurate representation of the entirety of each waterbody, during three replicate visits in each year. Fish presence or absence was established by querying government databases (Alberta Fish Stocking Reports 2019, 2020) and interviewing local anglers. A small number of fishes (white suckers and brook trout) were also captured in minnow traps (and reported to ACC as per animal care protocol requirements). Other amphibian species (western toads and Columbia spotted frogs) were captured primarily in minnow traps as tadpoles and adults. I identified them to the species level. As with fish, these non-target species captures were reported to ACC. All roads and other pond access points located within one km of a pond were recorded, as this is the expected dispersal range of long toed salamanders in most cases (Atkinson Adams 2015). Roads outside of this radius were expected to have much less impact and were not recorded. I recorded features of all roads and trails within this radius, including number of lanes, surface type (i.e., paved or not), and whether an edge barrier was present. Any foot paths within the area were also recorded for size and therefore expected volume of traffic and classified using a numerical coding system. Single track footpaths were coded as 4, double track unpaved footpaths as 3, double tracked paved footpaths or single lane roads as 2, and multi lane paved roads as 1. None of the roads had edge barriers and point of access observations were made on site.

I classified ponds for estimated aquatic vegetation cover within one meter on each side of the waterline: none (0% cover), low (1-30% cover), medium (31-60% cover), and high (61-100% cover). Pond surface area was measured using walking paces between widest points along the shore, with each pace counting as one meter, and maximum depth was measured in small ponds where the deepest area was accessible but could only be visually estimated in larger ponds. Visual estimation was aided where possible by observation of rocks or sticks on the pond floor, or the point at which dogs using the area for recreation began to swim when entering a pond. I used Google Maps to establish coordinates and elevation on site.

In 2020, I replicated the environmental sampling variables of water temperature, turbidity, and dissolved oxygen. These were taken using the same equipment and methods as in the previous year. I also recorded the presence of fish and other amphibians for both years. Because of repeated equipment failure, pH measurements could not be replicated in 2020. Observations of the more fixed habitat characteristics of aquatic vegetation, pond surface area and depth, and road/trail proximity and type were made but not recorded when no change was observed since the previous year, and data collected in 2019 was used representative of both years where the variables were perceived to be equal.

2.2.3 Population turnover

Because of the COVID-19 pandemic and associated restrictions, my plans for the 2020 field season were considerably downsized. I had planned to resample all ponds in the same way as in 2019 and in the meantime, I had also developed an increasing interest in the question of metapopulations in the species. Although studying metapopulations would need to be considerably more time intensive than was allowed in a M.Sc. project, I planned to assess population turnover as a possible, albeit very preliminary, indication of metapopulations. To do this, I had planned to expand my sample ponds by scouting the landscape for possible breeding ponds to add to the original 13 and increase sample size. I also planned to make observations of the landscape which supported all breeding ponds for features such as pond density, directional orientation, and possible barriers to travel in

between. Unfortunately, a second field season that would not only replicate sampling but also expand the parameters of the first in hopes of probing the base conditions for population turnover in the survey areas could no longer proceed. One simple smaller scale approach I took was to assess how many ponds stayed in the same state (e.g., salamanders detected in 2019 and 2020) or changed state (e.g., salamanders not detected in 2019 but present in 2020 or vice versa) and observe population fluctuation in ponds I surveyed both years.

I also conducted a literature review to characterize metapopulations in amphibians in general, and long toed salamanders specifically. I queried the University of Calgary library search engine with the keywords “amphibians”, “long toed salamanders”, “metapopulations”. Because the topic of climate change effects as a potential future research path came up during my reading, I needed specific evidence on whether plasticity in response to climatic variables has been observed in amphibians, so I conducted another search using the key words “climate”, “life history”, “plasticity”, “amphibians”. For all keyword searches, I applied filters for peer reviewed research articles, in the 2000-2020 publication year range. I then uploaded all files into Covidence for abstract screening. Articles were selected based on topic relevance and uploaded to Zotero for organization, data extraction, and reference management.

2.2.4 Data Analysis

All data analysis was completed using Microsoft Excel 365 MSO (16.0.13127.21210) 32-bit, R version 3.6.3 (2020-02-29), and Spearman’s Rank Calculator (geographyfieldwork.com).

2.2.4.1 Population estimates

Because of the lack of accurate population detection in egg counts evidenced by no observations of eggs in ponds where I later trapped multiple individuals (indicating the potential for false negatives when relying on egg counts), egg values were not incorporated in formal analysis. I only analyzed population data collected from minnow trapping as it appeared to be the more reliable method. Because trapping took place over a span of only three days during 2020, I selected the highest number of captures over a 24-hour period from the three replicates per site and used this single value for analyses (Table 3.1). While it was unlikely that individuals captured at each visit were novel, using the highest number of captures allowed for representation of the nearest-to-accurate population estimates as possible.

The shortened 2020 season allowed me to observe a single life stage in both valleys on the same days, and I was able to use this small but temporally consistent dataset to make more specific comparisons of population status between ponds and valleys. I did this by applying pairwise comparisons (two-sample t-test assuming unequal variance) between individuals captured, body length (cm), and developmental stage (number of toes emerged) to determine statistical significance of differences in these variables between both valleys and both years. This could be tested only among ponds with sufficiently large populations that had been sampled across both years (n=6). Because of differing methodology in data collection and processing between Graham and Powell (1999)’s project and my own, only presence/absence could be compared with historical data, with quantitative population analysis confined to the present study.

2.2.4.2 Pond characteristics

Because of the range in variation, pond surface area measurements were natural log transformed prior to analyses. For all habitat features, I calculated the means of the three replicates taken from 13 ponds in 2019 and nine ponds in 2020 (pH for 2019 only), respectively, and used those means for two separate analyses, one for each year. I used pairwise comparisons (two-sample t-test assuming unequal variance) to identify differences in pond features, comparing both valleys to test my expectation of ecological similarity between the two areas.

I used principal components analysis (PCA) in R to determine which variables most strongly correlated with variation in long toed salamander abundance. I selected only independent variables with quantitative values, removing categorical variables (aquatic vegetation, roads, and fish and amphibian abundances were excluded), which left: water temperature, pH, turbidity, dissolved oxygen, surface area, depth, and elevation. Many of the independent variables (pond surface area and pond depth, for instance) were likely to be strongly correlated with one another. A correlation matrix confirmed that about one in four pairwise comparisons produced strong correlations (>0.60) for 2020, indicating the need to account for multicollinearity. PCA is a statistical method that explicitly accounts for the lack of independent predictor variables. I retained components from this analysis that accounted for acceptable standards of variance within minimal components (Wildi 2013), selecting the first and second principal components (PC1 and PC2, respectively), which collectively captured 63% of variance in 2019 and 71% in 2020. I identified habitat variables as significant contributors to their respective PCs when their loading values were ≥ 0.5 , a threshold selected based on its ability to account for most contribution weight with fewest variables (Kienzle 2018). Eigenvalues related to the PCA were found using the R factoextra package (Kassambara & Mundt 2020).

I followed this with a generalized linear mixed model (GLMM) with zero inflated negative binomial distribution using github NBZIMM package (Yi, 2018) to determine whether PC1 and PC2 were significant predictors of population abundance. I chose negative binomial distribution based on its ability to account for higher-than-expected variation in my data, thus producing residual deviance values closer to the degrees of freedom. Using the GLMM allowed me to incorporate the random effects of Valley in these analyses, with the general formula for these analyses being: $\text{Abundance} = \text{PC1} + \text{PC2} + (\text{random factor} = \text{Valley})$, conducted separately for 2019 and 2020. The zero inflated model accounted for the fact that six of 13 ponds in 2019 and three of nine ponds in 2020 had zero individuals. I conducted two separate GLMs with the R MASS package (Ripley 2020) to determine relationship significance between PC1 and PC2 and the means of body size and developmental stage, respectively, in 2020. I was not able to use mixed models for these analyses because my sample size was small (only two ponds in one of the valleys), leaving insufficient degrees of freedom to estimate the random effects of “Valley”. Finally, because I predicted that roads would have specific effects on populations and these important field measurements could not be included in the former models, I conducted a GLM for each year to determine the effects of road type and distance between nearby roads and breeding ponds on population abundance. For this analysis, I used R MASS package (Ripley 2020) with a Poisson error family (I tried various other families including quasipoisson to reduce residual deviance but Poisson produced the lowest deviation possible) with the general formula $\text{glm}(\text{individuals} \sim \text{road type} * \text{distance to nearest road})$. I also used SRC for vegetation cover (one analysis for each year), which could not be included in the PCA. I had intended to analyze the impact of the presence of fish on

salamander presence and relative abundance; however, there was insufficient power for such analysis. I found fish in only one pond in 2019 (Quarry) and two in 2020 (Quarry and Sheppard's). With regard to other amphibians, I encountered two species (western toad [*Bufo boreas*] and Columbia spotted frog [*Rana luteiventris*]) but I did not conduct formal analysis for two reasons. First, it is not clear that these species should impact long toed salamander abundances and may be present simply as a response to similar habitat requirements. Second, the distribution of the different presence statuses for these species did not lend itself to formal analysis: western toad only: N=2, Columbia spotted frog only N=0, both present: N=6, both absent N=5.

In all analyses, I used $p < 0.05$ as the threshold for statistical significance.

Chapter 3: Results

3.1 Populations

The detection of eggs in only three of 13 ponds while minnow trapping later confirmed populations mainly consisting of larvae in seven of the 13 indicates that egg counts are an unreliable method for population detection when used on their own. Because I detected eggs only in smaller ponds, this suggests a possible bias toward ponds where the observable shoreline included a greater percentage of the entire pond (Table 3.1). Minnow trapping, which had the notable advantage of also confirming fish populations and allowing me to observe long toed salamanders in various life stages, proved to be a more reliable method. There were no significant differences in abundance ($t_7=0.64$, $p=0.56$), body size ($t_7=-0.92$, $p=0.41$), or developmental stage ($t_7=-0.90$, $p=0.42$) of populations between the two valleys in 2020 (Table 3.1). Despite the lack of statistically significant differences overall, I did observe considerable variation among populations. Ponds represented a span in capture rates, ranging from no individuals detected to what may indicate healthy populations in others (Table 3.1). This range was especially evident in the Bow Valley, with less variation and generally lower captures observed in the Kananaskis Valley (Table 3.1). In 2019, the only year when all 13 ponds could be sampled, 55% of all individuals were captured in the Bow Valley (16 individuals out of 29 total). In the same year, I detected populations in 50% of Bow Valley ponds and 57% of ponds in the Kananaskis (Table 3.1). When presence/absence was compared with historical values (Graham and Powell 1999), I observed an overall 46% (six of 13 ponds) decrease in population presence over the past two decades. Bow Valley ponds saw a 50% decrease (three of six ponds) while intact populations in the Kananaskis saw a 43% drop (three of seven ponds).

I caught almost entirely larvae in the minnow traps, with the exception of a couple of juveniles to whom I also assigned a developmental stage. Although the difference was not statistically significant ($t_4=-0.92$, $p=0.41$), larvae in the Kananaskis Valley were on average just over 10% longer (mean length=60.72 mm, SD=35.10, n=40) than in the Bow Valley (mean length =53.86 mm, SD=28.83, n=162) (Table 3.1). Not surprisingly, Kananaskis larvae were also about 5% more advanced in developmental stage, at a mean stage of 17.30 (SD=9.99, n=40) as opposed to a mean stage of 16.55 (SD=8.59, n=162). in Bow Valley individuals (Table 3.1, Figure A.1). This difference was not significant ($t_4=-0.90$, $p=0.42$).

3.2 Within and among valley pond characteristics

Few of the pond habitat variables (2019 = 7 variables, 2020 = 6 variables) were significantly different between the two valleys (Table 3.2). Turbidity did differ, being significantly higher in the Bow Valley than at Kananaskis in both years ($t_{11}=2.77$, $p=0.05$). Although mean pH and dissolved oxygen in the survey ponds were nearly identical in both valleys (Table 3.2), Kananaskis ponds tended to have less aquatic vegetation (Table 3.1). Ponds were larger in the Kananaskis Valley, with 7.1% greater depth and 40% greater surface area on average (Table 3.2). Not surprisingly, they also tended to be colder (average difference = 1.68°C).

3.2.2 Principal components analyses

PCA on seven habitat variables from 2019 resulted in the first two components explaining 63% of variation in the dataset (Table 3.3, Figures 3.5, 3.6). The primary contributors to the first component accounting for 38% of variation was surface area ($r = -0.53$) (Figure 3.5). PC2 accounted for 25% of variation in the dataset (Table 3.3, Figure 3.6). The main drivers behind PC2 were water temperature ($r = 0.60$) and elevation ($r = -0.54$). GLMM revealed nonsignificant associations between PC1 and number of individuals ($p = 0.68$) and also between PC2 and number of individuals ($p = 0.58$). PCA for six habitat variables in 2020 showed PC1 and PC2 accounting for 71% of variation collectively. Variables with significant contributions to PC1 (51% of variation) were pond depth ($r = 0.53$) and a borderline contribution from water temperature ($r = 0.50$). A significant contribution to PC2 (20% of variation) was made by dissolved oxygen ($r = -0.68$). The following GLMM resulted in a non-significant association between PC1 and number of individuals ($p = 0.74$) and a significant association between PC2 and number of individuals ($p = 0.01$).

GLMs including larvae measured in 2020 found no significant relationship between PC1 and body size ($p = 0.15$), a weakly significant association between PC2 and body size ($p = 0.04$), and no significant relationships with developmental stage (PC1: $p = 0.91$, PC2: $p = 0.76$).

3.2.3 Fish, roads, and aquatic vegetation

Fish were confirmed in two of the Bow Valley sample ponds (Table 3.1). I captured fish in some of my minnow traps in Quarry Pond (2019) and Sheppard's Pond (2020), while all Kananaskis ponds were fish free (Table 3.1). Because fishes were found in only 2 ponds, formal analysis for this variable was not suitable.

The types of roads and trails that occurred within a one-km radius of survey ponds were very similar in both valleys, with only a very slightly greater size and perceived traffic volume in the Kananaskis Valley than the Bow Valley (Table 3.1). Roads and trails were closer to survey ponds located in the Bow Valley, however, where the average buffer was 14 m less than in the Kananaskis. GLMs revealed nonsignificant associations between road type and distance to nearest road with population abundance in both years (2019 [$n = 13$]: road type: $z = -1.47$, $p = 0.14$; distance to nearest road: $z = 1.62$, $p = 0.10$; road type x distance to nearest road interaction: $z = -0.72$, $p = 0.47$; 2020 [$n = 9$]: road type: $z = -1.22$, $p = 0.22$, distance to nearest road: $z = -0.31$, $p = 0.76$, road type x distance to nearest road interaction: $z = 0.45$, $p = 0.65$) (Figure 3.1).

Aquatic vegetation amounts had a negative but statistically nonsignificant correlation with population abundance in both years (2019: $R_{13} = -0.12$, $p = 0.50$; 2020: $R_9 = -0.06$, $p = 0.05$). Overall, amounts of aquatic vegetation were higher on average in the Bow Valley (mean = 2.17, SD = 0.98, $n = 6$) than in the Kananaskis (mean = 1.71, SD = 0.76, $n = 7$).

3.3 Population turnover

Comparison of survey pond populations during the two years of observation indicate the potential for considerable turnover. Of the nine ponds that were sampled during both years, I detected populations in five. Of the four that changed from year to year, three transitioned from absence to presence, and one from presence to absence (Table 3.1). Three ponds with substantial captures in both years demonstrate the potential for population fluxes (Figure 3.3).

Chapter 4: Metapopulation in amphibians: a literature review

4.1 Introduction

Amphibians are the most threatened group of vertebrates in the world, with one in every three species at risk of extinction (Ribeiro 2011, Hale et al. 2012). Because habitat fragmentation is believed to be one of the leading causes of these population declines (Ribeiro 2011, Cox et al. 2017), the structure and function of metapopulations is a worthwhile focal point for study and better understanding particularly in amphibians. Because metapopulations are defined by at least some level of connectivity among populations, knowledge of how they work could provide a linkage between the positively correlated variables of habitat fragmentation and population declines. Population connectivity is valuable because it can have a rescue effect by facilitating recolonization of struggling or extirpated populations, in addition to providing gene flow that promotes genetic diversity and reduces inbreeding depression (Lee-Yaw et al. 2015).

While populations are affected by birth/death rates, competition, and immigration/emigration, metapopulations are affected by spatial proximity of participating populations and migration patterns, which ultimately leads to connection through the individuals that move among them (Hanski 1996). This connectivity makes the network stronger by facilitating flow between populations of varying sustainability.

Although each participating population breeds distinctly within its own individuals, each group is reliant upon one another for long term survival, with no one population able to persist without support from the others (Billerman et al. 2019). This support is provided by sufficient connectivity to provide immigrants in cases of declining local populations. Additionally, populations are usually varied enough from one another in both distance and characteristics that environmental perturbation is not likely to affect the habitats of all in the same way, but rather affect some and leave others as havens (Billerman et al. 2019).

The central goals of this review were to compile existing evidence to discover whether metapopulations are likely to play a significant role in the population dynamics and species persistence in my study ponds, and to recommend future research directions based on identified knowledge gaps. Because there is only limited evidence of metapopulations in long toed salamanders, I present the topic here in terms of pond breeding amphibians in general. The intention is to extrapolate this onto the study species, using whatever species-specific evidence does exist to support this.

4.2 Source/sink dynamics

Metapopulations are characterized by episodes of extirpation and recolonization (Billerman et al. 2019). In terms of loss/growth dynamics, they function much like the populations of which they are composed but within different parameters. While population dynamics are dictated by birth/immigration versus death/emigration rates, metapopulations are a function of the dynamics of local population extinction/recolonization rates. If local recolonizations are more than extinctions the metapopulation will grow, but when the opposite is true it will shrink and eventually become imperiled (Carlson & Edenhamn 2000).

Metapopulations can be analogized to other structures using similar source sink dynamics such as the island mainland theory. The main difference in this case, however, is that unlike many established island populations, no single population within the metapopulation is stable enough to persist long term on its own (Billerman et al. 2019). Sink populations depend on larger, more stable populations with higher birth than death rates to provide a source of immigrants for support during declines due to unfavourable conditions. Because source populations are usually stronger in the relationship (more robust) while sink populations are more fragile (often declining), it may seem intuitive to place higher conservation priority on sink populations. This may at times be a backward approach, however, as maintaining strength in the source population can in fact be preserving the survival of multiple frail sink populations that without source immigrants would face long term extirpation. Just as populations blink in and out of existence through extirpation and recolonization, they can also alternate roles of source or sink, usually as a result of environmental perturbation.

One example of a way that source/sink roles can be switched is by fish introductions. Fish are an important limiting factor in amphibian breeding ponds when present, and while the introduction of fish to historically fishless ponds usually results in local decimation of amphibians, metapopulations can in some cases facilitate coexistence. Safe ponds that are part of the same network as invaded ponds can provide refugia for amphibians, mitigating the effects of breeding habitat loss by fish introductions (Tiberti 2017). The risk of extirpation in invaded tiger salamander (*Ambystoma tigrinum*) populations is lower in ponds that are associated with others, while the chance of recolonization is significantly higher, meaning greater survivability for the metapopulation as a whole, even where predatory fish are present in some of the ponds (Cosentino et al. 2011). Because predatory fish do not occur in all suitable amphibian breeding ponds, their distribution is patchy in comparison. Although the presence of fishes greatly increases the risk of tiger salamander extirpation, patches where they do exist become sinks, dependent upon nearby source populations where fish do not exist (Cosentino et al. 2011). Just as fabric woven together is stronger than individual fibers on their own, metapopulations provide strength to populations through coalition, but this is not without limitations. Fish/amphibian coexistence is only likely when fish invasions occur in populations that already function as sinks, as invasion into preexisting source habitats could produce the opposite effect with a broader extirpation risk inclusive of multiple dependent sink populations. For source/sink dynamics to function, adult survival in the sink population must also be high enough or the rate of immigration will not be able to overcome the rate of population decrease due to deaths and lower reproductive rates. Adult survival in the survey ponds of great crested newts (*Triturus cristatus*) is normally between 25-80%, but when this amount falls below 30% for a period of time, the rescue ability of source populations lowers considerably (Griffiths et al. 2010).

Safe habitat patches can, to an extent, provide haven for individuals fleeing unsuitable conditions. Italian crested newt (*Triturus carnifex*) populations in ponds where fish were introduced plummeted to or near zero as a result of environmental stress, while populations at nearby ponds were found to increase subsequently, implying that the newts were able to sense the presence of the novel predator and evacuate to safer connected ponds (Tiberti 2017). This may, however, only serve as a short-term solution, as intraspecific competition for limiting resources would likely already have capped populations at the new location near sustainable levels. Additionally, the migration from invaded to haven ponds was observed to take several seasons, reflecting the time lag required for

new habitat exploration and limited travel rate, and is therefore not indicative of a simple relocation as many costs such as casualties and missed breeding events are incurred along the way (Tiberti 2017).

4.3 Dispersal in amphibians

Translocation of individuals in the landscape is a well researched yet poorly understood aspect of ecology (Muths et al. 2018). Because dispersal is the primary process by which metapopulations exist, understanding its function should be a focal point. Migration and dispersal, both of which are terms prone to misuse, are in fact entirely different processes. Migration is commonly undertaken by adults as a recurring event in search of resources or breeding habitat and usually involves a round trip. In pond breeding amphibians, this is commonly seen as annual travel from upland overwintering grounds to breeding ponds and back. Dispersal on the other hand, is usually a single, unidirectional event and more likely undertaken by juveniles. In amphibians this is likely to occur when young individuals leave the natal pond for a new breeding habitat and is an important mechanism in the function of metapopulations. Because of these differences, dispersal usually occurs among individuals less frequently than migration but requires more time and availability of travel corridors as farther distances are usually covered (Ficetola et al. 2009). Dispersal can be prompted by various factors, but variation in habitat quality is expected to be the main cause (Cayuela et al. 2018). Our knowledge of exactly how decreasing habitat quality affects dispersal decisions is quite limited with some conflicting results, and is likely circumstantial. Poor local habitat quality may increase dispersal as individuals seek resources elsewhere. Individual yellow-bellied toads (*Bombina variegata*) are over 10 times more likely to disperse from highly unpredictable environments compared with stable environments (Cayuela et al. 2016). In other cases, dispersal may decrease if poor environmental conditions lead to less suitable travel conditions (Ficetola et al. 2009).

Because individuals even in stable habitats are known to disperse (Cayuela et al. 2018), habitat variation is not the only factor at play. In environments with less variation and more stability, dispersal is likely to be caused by social factors such as the need for less competition, which can be the case both inter- and intraspecifically (Cayuela et al. 2016).

High dispersal among species tends to be positively correlated with traits typical of R strategists, such as higher reproductive rates, shorter life spans, and rapid maturation, which can be observed even within species (Cayuela et al. 2016). Species persistence is rarely random and is closely connected with ability for dispersal and therefore likelihood of colonization (Ficetola & De Bernardi 2004). Persistence is likely to favour those less sensitive to changing environments, more likely to disperse, and with a lifestyle that uses the landscape more continuously rather than in discrete patches (Ficetola & De Bernardi 2004). Species with lower dispersal, slower travel, or higher site fidelity more frequently exist in genetically isolated populations and are more likely to be endangered or even extinct (Ficetola & De Bernardi 2004, Zamudia & Wieczorek 2007). These factors are often linked to body morphology or behavioural patterns. Many Ambystomid salamanders are slow travelers and exhibit high breeding site fidelity, and the fact that frogs and toads have been shown to have greater travel capacity than salamanders highlights the differences we observe in dispersal and eventually population turnover among amphibian species (Ribeiro et al. 2011).

A high degree of intraspecific variation in dispersal rates also occurs at the individual level. It is unclear whether these differences are due to phenotypic plasticity in changing environments or genetically based personality traits, but individual amphibians that engage in dispersal have a higher chance of doing so again (Cayuela et al. 2018). There are some conflicting results on amphibian dispersal distances (Zamudia & Wieczorek 2007) with some showing farther than expected dispersal and others relatively small within areas of greater isolation, but it is generally believed that dispersal distances in amphibians are not very large. Although outliers, long range dispersers do exist and should be taken into account as equally if not more importantly than the norm (Ficetola & De Bernardi 2004). These individuals highlight the differences in dispersal tendencies and decisions in individuals, but more importantly they represent increased opportunity for recolonization, gene flow, and diversity in metapopulations.

4.4 Landscape features and metapopulations

Just as species persistence is rarely random, neither is the suitability of habitats for amphibians, with some areas being more supportive of populations than others. Because of this, one or a small number of participating ponds in a metapopulation may be nearly entirely responsible for the persistence of all other populations within the network (Heard et al. 2015). In most cases, there are differences between the features of the ponds and certainly between the ponds and the landscapes between them. It is heterogeneity in the landscape that creates metapopulations in the first place, causing a single population to become fragmented and, apart from mate selection, removing stochasticity from breeding (Hale et al. 2012). Metapopulations can be more supportive of local species persistence than a single population by providing a source of immigrants for struggling populations, and by providing genetic diversification potential through mixing of populations (Hale et al. 2012). The heterogeneity that creates metapopulations in the first place is also crucial to their survival. This is largely because if all were the same, the episodic change events that are so characteristic of most breeding ponds, especially ephemeral ponds, used by amphibians would affect all ponds in the region to the same extent. Heterogeneity increases the likelihood that some ponds will be less affected due to differing susceptibility of their features and be able to provide a source for patches that are more affected and whose populations may suffer as a result. In the growling grass frog (*Litoria raniformis*), extirpation rates increase during years of low precipitation (Heard et al. 2012), highlighting the benefit of having ponds of differing hydroperiods so that not all patches can be expected to dry up before breeding activities are complete. Chytrid fungal disease is capable of wiping out entire amphibian populations and the primary suspect in the uplisting of the growling grass frog to endangered status (Heard et al. 2015). Environmental heterogeneity provides refuge for potential source populations not yet infected, as the fungus is more successful in some environments than others (Heard et al. 2015). Dispersal versus isolation from host facilitated disease therefore appears to be an important tradeoff between population rescue with healthy individuals and the risk of increasing spread in the other direction. The benefits to growling grass frogs from dispersal are still advantageous to a metapopulation overall, and these benefits increase where environmental heterogeneity allows for some habitat patches to act as refugia and therefore healthy sources to provide rescue for nearby infected populations (Heard et al. 2015).

Just because ponds are near enough to each other for travel should not lead to the assumption that this will happen, as many obstacles to movement present themselves in the landscape matrix (Willson & Hopkins 2020). On

a macro level, mountainous areas can impact connectivity in metapopulations of amphibians associated with this habitat type. Genetic diversity is significantly lower in long toed salamanders at higher elevation ponds than lower ones (Giordano et al. 2007), likely because of more challenging landscape features and climatic conditions affecting travel. Although Columbia spotted frogs (*Rana luteiventris*) are able to travel relatively long distances and use large range areas with single populations inhabiting more than one pond, gene flow still decreases significantly as a function of elevation and when mountain ridges present barriers (Funk et al. 2005). In addition to the structural impediment that ridges cause, they also present greater desiccation risk to travelers due to harsh climates and less vegetative cover than areas of lower elevation (Funk et al. 2005). Travel barriers in the landscape are frequently created by anthropogenic disturbance such as roads. Collisions with vehicles are a major cause of casualties in amphibians who are difficult to see, slow moving, and must migrate through areas that sometimes contain roads (Pagnucco et al. 2012). Roads also affect these species indirectly by fragmenting habitats and reducing gene flow (Ficetola et al. 2009, Pagnucco et al. 2012). Because metapopulations rely on dispersal ability, obstacles such as roads can have severe impacts on these population networks.

On the microhabitat level, one of the greatest risks to a travelling amphibian is desiccation (Cosentino et al. 2011), and this coupled with their small body size, slow travel rate, and relatively small range of optimal moisture and temperature means they require specialized conditions to travel. This means that the features of micro habitats they must travel through to disperse have a great deal of impact on their success. Microhabitat factors that affect travel involve tradeoffs between substrates that provide few obstacles for efficient travel but higher risk of desiccation such as sand, and those that provide better moisture conservation but are more difficult to move through such as high amounts of litter (Lee-Yaw et al. 2014). A controlled study on travel substrate selection in long toed salamanders found they tend to choose substrates with a balance of both, sacrificing some of one quality for some of the other (Lee-Yaw et al. 2014). Of all the experimental substrates (moss, sand, deciduous litter, coniferous litter, grass), moss was selected by most individuals as travel media (Lee-Yaw et al. 2014). This illustrates not only the importance of landscape features in travel capacity, but also the behavioural aspect of travel route decisions, and how this can be heavily dependent on individual preference and experience.

4.5 The Landscape Perspective

Amphibians are unique among vertebrates in their use of multiple habitat types for their life cycles, from aquatic to terrestrial. Because their life cycles cannot be completed and populations could not persist or grow without breeding ponds, these are often the focal point of amphibian conservation efforts. Although breeding ponds are critical, many adult amphibians spend the majority of their lives in upland habitat. Ambystomid salamanders spend 90-95% of their lives in forested areas (Compton et al. 2007). This suggests that conservation of upland areas should be given at least equal priority to that of breeding ponds (Bauer et al. 2010).

Amphibian breeding habitat exists on a number of ecological scales from the breeding pond itself to the breeding pond inclusive of upland habitat to surrounding pools with their upland habitats to groups of these pond assemblages on a regional scale (Compton et al. 2007). This indicates that conservation of breeding ponds is good and conservation of ponds with upland habitat is better, while still likely to be only partially effective. For example, in frog and toad species, two ponds with dispersal of individuals occurring between them is more effective for

conservation than four ponds without inter-pond dispersal (Gilioli et al. 2008). Therefore, conservation of breeding ponds, even where some surrounding upland habitat is maintained, may well be insufficient without the ability for dispersal, which should be in higher priority than simply conserving either discrete patches or travel corridors (Bauer et al. 2010, Muths et al. 2018). This requires a landscape approach to conservation, and an understanding that with metapopulations, the whole is truly worth more than the sum of its parts.

We must then expand our perspective farther yet to include the matrix which extends into the areas between these habitat patches and realize that conservation of one pond may also require the conservation of another pond. While ponds and their surrounding upland habitats tend to be used somewhat discretely by pond breeding amphibians, these populations are usually dependent on others in the region for long term success. As discussed earlier, the microhabitat features of these areas are very important for travel, but so is the distance between ponds. The landscape variables that mainly determine the functionality of metapopulations not only include habitat quality at individual ponds, but also distance between ponds and ability to traverse the matrix between them (Murphy et al. 2010). Change happens, and even in an ideal breeding pond, the local population may be highly prone to extirpation if that pond does not have connectivity with a metapopulation in case of environmental upheaval and subsequent population declines. Although pond size is not correlated with population size, isolation is, with isolated ponds showing lower populations overall (Corser & Dodd 2004). In the growling grass frog, connectivity with other ponds has only a slightly lowering effect on extirpation rates, but heavily impacts the probability that these areas will be recolonized when needed (Heard et al. 2012). Put another way, metapopulations will not likely save a population from decline, but are likely to provide invaluable rescue when necessary. This is why when it comes to amphibian habitat conservation, movement corridors should be viewed with equal importance as breeding habitats if populations are to persist (Bauer et al. 2010). Distance to the nearest stream that can be used for travel without desiccation is the most important habitat factor for connectivity in the red listed mountain tree frog (*Dryophytes wrightorum*) populations (Parsley et al. 2019). The more isolated a population is, the greater its long-term extirpation risk (Billerman et al. 2019), and connectivity is positively correlated with the density of pond arrangement in the landscape (Compton et al. 2007). Therefore, it is both suitable travel corridors and distance between ponds that are responsible for successful connectivity between populations. Loss of even very small ponds in the landscape can decrease the function of metapopulations by increasing inter pond distances (Compton et al. 2007, Willson & Hopkins 2020). Not surprisingly, rescue effects by which source American toad (*Bufo americanus*) populations were able to contribute to sink populations decreased with increasing distance between ponds (Willson & Hopkins 2020).

Habitat degradation at a single pond can have consequences for the entire metapopulation, often having cumulative effects. This causes something of a double negative, where risk of local extirpation increases, while the chance of population rescue decreases, as not only habitat of individual populations is affected but often the travel corridors that allow for recolonization as well (Hale et al. 2012). While this cumulative damage can be due to loss of ponds in the landscape increasing travel distance, it can also be through influences on source/sink dynamics, for example the provision of less migrants for sink population support (Murphy et al. 2010). Larger source populations provide most migrants, and because habitat quality is positively correlated with population size, habitat degradation

in a local population can harm the metapopulation by provision of less rescuing dispersers (Hale et al. 2012). In a source population of American toads, mercury contamination of one breeding pond affected the entire metapopulation negatively as this vital contributing population was suddenly able to provide less migrants due to population declines and could therefore support less associated sink populations (Willson & Hopkins 2020).

4.6 Recommendations for future research

From the evidence presented here, it seems reasonable to conclude that amphibians can exist in metapopulations. What we do not understand well is the structure and function of these population networks. Largely responsible for this is the fact that we do not know much about the moving parts that metapopulations are the function of such as dispersal, gene flow, or the distances at which these may occur in amphibians (Purrenhage et al. 2009, Ribeiro et al. 2011, Zamudia & Weiczorek 2017). Species-specific traits have a significant role in dictating the isolation or connectivity of populations. Species with higher site fidelity and lower travel potential will tend to be isolated within a smaller geographical range (Zamudia & Weiczorek 2017). Because dispersal is the main contributor to the function of metapopulations, it is often studied as a proxy. Dispersal is mostly unpredictable however, and tends to occur in discrete events revolving largely around age of individuals and environmental perturbation, making it difficult to directly observe these activities. Therefore, much of our study of dispersal in amphibians is indirect, using methods such as genetic tracing and mark recapture techniques (Lee-Yaw et al. 2014). Still, dispersal is very challenging to quantify. Although genetic qualifications of populations are often used, there is no generally agreed upon protocol for spanning what remains as the gap between presumed rates of dispersal and metapopulation structure (Purrenhage et al. 2009). To identify whether a metapopulation exists in an area, a considerable time investment is required to accurately assess if longer term events of extirpation and recolonization are occurring (Billerman et al. 2019). This would take several years and may not be feasible depending on resource constraints of the project. There should also be a considerably larger sample size in terms of survey ponds, and I would recommend at least twice the number that I was able to use here. Finally, the study of a metapopulation would be largely ineffective unless individuals could be identified to ensure against recapture or misidentification of native individuals as immigrants. This can be done using mark recapture techniques, individual photographs, or genetic sequencing to identify movement and in turn gene flow and/or mixing. Most of these methods were outside of the scope of this study.

Another challenge is the differentiation between spatial and functional connectivity. While metapopulations are often assumed to exist based on indirect indicators such as apparent structural connectivity, there is a distinct difference between the two (Billerman et al. 2019). Structural connectivity is largely theoretically based and largely defined by landscape indicators such as inter-pond distance, density, and ease of travel (Ribeiro et al. 2011), making it much easier to assess and the only fully conceivable perspective for the researcher. Functional connectivity is based on observations of how individuals actually move between populations rather than how they could move between them. This is usually more accurate to reality but is also more convoluted and requires accounting for factors such as behavioural avoidance of environmental features in the landscape (Ribeiro et al. 2011). While this means that functional connectivity is more directly indicative of metapopulations, it is also much more challenging to quantify. Ideally, we would use structural connectivity as an indicator of functional

connectivity, which could finally indicate and confirm metapopulation existence. A multi-amphibian study found a clear correlation between structural and functional connectivity (Ribeiro et al. 2011), indicating that although less direct, this method can show us a lot about how connectivity may be functioning to create metapopulations.

Despite constraints and challenges, there remains much work to be done in this field. As our understanding of metapopulations and the factors impacting them remains limited, there are several directions for future research to take. There is potential for the use of metapopulation theory to guide artificial recolonization of severely fragmented urban landscapes and areas where populations have declined to critical levels or below. Introductions of European toads (*Bufo bufo*) and European common frogs (*Rana temporaria*) into artificially created ponds has been proven successful and could be a viable way to start new populations, rather than relying on random dispersal events (Gilioli et al. 2008). This approach is not without risk from complications such as the spread of Chytrid fungus (Hale et al. 2012) or other diseases. More research needs to be done before this can be confirmed as a viable and biologically safe option.

Climate change is another important consideration for future research, as temperature and precipitation changes can strongly affect the hydroperiod of ephemeral ponds while not only rendering some unsuitable for breeding, but also increasing travel distance between ponds in the network (Murphy et al. 2010). We know that amphibians exhibit phenological plasticity in response to climatic conditions. Rose's mountain toadlets (*Capensibufo rosei*) put increased effort into reproduction during high rainfall years when there is more access to aquatic breeding patches (Becker et al. 2018). Mediterranean parsley frogs (*Pelodytes punctatus*) are able to breed in spring, fall, or both and alternate depending on environmental conditions (Jourdan-Pineau et al. 2012). We also observe developmental plasticity in amphibians responding to varied climates. Long toed salamanders hatch later and may delay metamorphosis for a year or more in higher elevation breeding ponds (Atkinson-Adams 2015). Great Basin spadefoot (*Spea intermontane*), Pacific chorus frog (*Pseudacris regilla*), and northern red-legged frog (*Rana aurora*) tadpoles reach metamorphosis earlier in warmer water temperatures, which likely offsets the associated earlier drying of vernal ponds in warmer drier conditions (O'Regan et al. 2014). What we do not know is to what limit this plasticity is able to reach, and at what point genetic adaptation may or may not be able to influence survivability at rates rapid enough for sufficient response to changing climates.

4.7 Conclusions

Although our understanding of metapopulation structure and function remains limited, we can form some very reasonable conclusions from the evidence reviewed here. The first and perhaps most basic from a conservation perspective is that metapopulations have a critical role in population maintenance and species persistence in amphibians. To conserve amphibians, we must conserve metapopulations, and pivotal to this is the fact that dispersal is the main driver of metapopulation existence. This is where it becomes more complex, because dispersal as a process is difficult to quantify, variable by species and individual, and strongly influenced by features of the landscape on multiple levels. Dispersal, convoluted yet critical as it is, may be viewed as a limiting factor for metapopulations, but in turn it is also limited by fragmentation of the landscape, untravellable distances between habitat patches, and source populations affected by local habitat degradation that decreases available dispersers. This inevitably leads back to the landscape perspective, where we know that a disperser is only as good as the terrain they

travel, that behind many an intact population is a generous source population, and that no habitat patch, however ideal, is much good without a network.

Chapter 5: Discussion

With goals to update population statuses, search for correlations between habitat variables and number of individuals and explore the possibility for population turnover, I successfully tested my hypothesis that if long toed salamanders are affected by anthropogenic development, then population abundances should be lower in ponds with indication of habitat disturbance. Based on this hypothesis, I had expected more of the habitat variables I measured to show significant correlation with populations. However, many of the relationships I examined and trends I observed (dissolved oxygen, turbidity, water temperature) may have biological relevance. The fact remains that while concerns have been raised about recent long toed salamander declines in the Rocky Mountain foothills, we know little about the potential causes and drivers of this trend or about current populations in the area (Government of Alberta 2003, Atkinson-Adams 2015, Lee-Yaw et al. 2015, Alberta Environment & Parks 2016).

5.1 Habitat variables

5.1.1 Dissolved oxygen

Although there was no significant difference in dissolved oxygen between Bow and Kananaskis Valley ponds across both years, concentrations were higher on average in the Kananaskis (Table 3.2). Our knowledge of the effects of dissolved oxygen on the distribution and abundance of amphibians, including long toed salamanders, is generally limited. Anuran amphibian abundance in both species and individuals was found to be positively correlated with dissolved oxygen (Calderon et al. 2019). Larval natterjack toads (*Epidalea calamita*) showed signs of slower development after only 12 days in conditions leading to hypoxia (Ortiz-Santaliestra & Adolfo 2015). Although there is little evidence on long toed salamanders and we know very little about how this could affect them specifically, we can only extrapolate from other species. Tiger salamanders, although generally less tolerant of environmental stress than many other amphibian species, are on the other hand better able to withstand anoxic conditions (Wassersung & Seibert 1975). In addition to increased tolerance, Ambystomids have well developed lungs the closer they get to metamorphosis and are able to approach the surface for air. This tolerance for lower oxygen environments along with advanced lung development may have been developed in response to a greater need for oxygen and may even be helpful in ponds where predators are more plentiful. Amphibians must consider tradeoffs between their need for oxygen which is often found more abundantly near the water's surface with the need for refuge from predators, which is often more readily available at lower depths (Wassersung & Seibert 1975). This points toward the idea that predator prey dynamics could play a key role in the ways that salamanders are able to make use of aquatic habitats even if oxygen levels are lower. If the risk of predation is scarce, then individuals should be able to approach the surface more freely and utilize richer sources of oxygen there without being eliminated by predators. When more predators are present, they may have to rely on increased tolerance levels that allow them to remain nearer to the pond bottom.

5.1.2 Turbidity and population abundance

Higher turbidity levels in the Bow Valley than the Kananaskis Valley was not surprising for several reasons. Kananaskis ponds tended to be deeper without obvious inflows, unlike many Bow Valley ponds, which often had clearly defined tributaries in the form of small streams. Turbulence from running water would likely carry contributions of upland materials. I also observed more fine silt and clay substrates in the Bow Valley (likely linked

to the increased aquatic vegetation there) as opposed to more rocks in the Kananaskis. Although increased turbidity can occur naturally and often fluctuates based on rain events and spring run-off, it can also result from anthropogenic sources. Higher levels are indicative of the addition of foreign materials into aquatic systems, which can lead to sediment pollution (Snodgrass et al. 2007). When these originate from agricultural or industrial sources, they can be composed at least partially of substances toxic to aquatic life, such as hydrocarbons, metals, and salts (Snodgrass et al. 2007). Increased traffic in the Bow Valley in recent years (Yellowstone to Yukon Conservation Initiative 2021), as well as the closer proximity of roads and access trails, point to physical disturbance and industrial and road runoff as possible causes of higher turbidity. The town of Canmore saw a population increase of about 60% between 1996 and 2016, while visits to Banff increased by 21% in the three years between 2014 and 2017 alone (Alberta Government 2018). More directly related to habitat loss is the fact that Canmore's ecological footprint is estimated to have increased by more than five-fold since the 1970s (Yellowstone to Yukon Conservation Initiative 2021). This can be expected to have negative consequences on breeding habitat and biodiversity. The number of amphibian species present in breeding ponds has been found to be lower in more turbid water (Calderon et al. 2019). Turbidity can also lead to behavioral modifications, which may alter predator/prey relationships. For example, Pacific tree frog (*Pseudacris regilla*) tadpoles were less likely to survive in turbid waters due to a decrease in hiding behaviour, which led to more successful attacks by their predators (Suh & Choe 2016).

5.1.3 Water temperature and population abundance

Ponds tended to be colder on average in Kananaskis Valley ponds. My observations of later snow melt and delayed budding of trees and greening of vegetation in the Kananaskis Valley point to cooler temperatures and possible delayed onset of spring and summer. This climatic difference could be caused by elevation differences, with Kananaskis ponds at an average of 284 m higher than Bow Valley ponds. Slope aspect could also be a contributing factor, as the Bow Valley tends to run west/east, while the Kananaskis Valley has more of a north/south orientation (Figure C.1). Temperature appeared to affect population distributions, with a general trend toward higher individual abundance in warmer ponds (Figure 3.2). Although little is published about the effects of water temperature on long toed salamanders, temperature has been found to have significant effects on population abundances in 12 amphibian species (Dubos et al. 2020). The directionality of the relationship was different for some species than for others, suggesting that ideal temperatures are not equal among species.

5.1.4 Rate of development and water temperature

The preference for warmer water temperatures should coincide with higher rates of development in long toed salamander larvae, as supported by the literature which focuses mainly on larval development across elevation gradients. Higher elevation ponds can be expected to be colder, and long toed salamander larvae there can take a few years to reach metamorphosis, as opposed to those at lower elevations that normally reach the juvenile stage by the end of their first summer (Pearson 2004). Ezo salamanders (*Hynobius retardatus*) delayed metamorphosis and remained in the larval stage for extended periods in colder water temperatures (Hirofumi 2011). However, the situation observed in my survey ponds was to the contrary, where larvae captured in the colder, higher elevation survey ponds of the Kananaskis tended to be larger and more advanced in developmental stage.

One driver of increased growth rate could be differences in seasonal longevity of ponds in the survey areas. In the later season of 2020, four of the Kananaskis survey ponds had dried up, which could also be linked to my observations of colder water temperatures, later spring, and therefore assumed shorter summer there. Contrary to previously presented research but in support of my observations, plasticity may allow for more rapid development in ephemeral ponds with brief growing seasons in some amphibians. Pool frogs (*Pelophylax lessonae*) reared in colder more northern climates showed greater plasticity and briefer developmental stages than those from more southern areas (Orizaola & Laurila 2016).

Bow Valley Pond presents a particularly interesting case study by which to observe the possibility of temperature cued developmental rate plasticity in ephemeral breeding ponds. Although it yielded the highest captures of all ponds in 2019, no population was detected there in the later season of 2020. Although it was the smallest of all survey ponds, it did not dry up by the later dates of 2020 as even some of the much larger ponds did. In July 2019 it was the second warmest pond, but by the much later seasonal stage of August 2020, it had become the coldest. As discussed previously, water temperature can be an important factor in developmental rate. This pond's small size may have caused increased temperature fluctuations resulting in more rapid development during the earlier summer when water was warmer (based on the 2019 water temperature trend) and evacuation from the pond by later summer when water temperature had dropped below that of most other ponds still supporting larvae. More research is needed to investigate such preliminary observations of possible temperature dependent plasticity in long toed salamanders, and would be highly relevant to the topic of species persistence in the face of climate change.

5.2 Biotic impacts on populations

5.2.1 Other amphibians

I observed Columbia spotted frogs (*Rana luteiventris*) and western toads (*Bufo boreas*) in the survey ponds. Although amphibian biodiversity was higher in the Kananaskis (where most ponds had two other species) than in Bow Valley ponds (where most had one or less other species), most individuals belonging to other amphibian species were captured in the Bow, following a similar trend to long toed salamanders. While it may be intuitive to expect that suitable habitat for one amphibian species would also be good for others, this raises the question of interspecific competition. The magnitude and outcome would depend on the species involved as well as other factors, such as abundance of limiting resources. The only other amphibians I observed were frogs and toads. Because long toed salamander larvae are predatory (Fukumoto 1995) and Anuran tadpoles are herbivorous (Arribas et al. 2015), competition for food resources is unlikely in this case. This dietary niche differentiation could allow compatible amphibian species to coexist when other habitat features, such as water temperature, are favourable. It is likely that amphibians would respond similarly to a given environment and that their coexistence would be more coincidental than dependently determined. I suggest that the potential for amphibian species to be used as a predictor indicator for the presence and relative abundance of other species will depend on the species in question, habitat variables, and specific habitat preferences, as these are expected to differ among species (Dubos et al. 2020).

5.2.2 Fish

Minnow trapping confirmed fish presence in only two ponds: Quarry and Sheppard's. Although Quarry Pond had not been stocked with arctic grayling since 2013 and there was no record of stocking other species (Alberta Fish Stocking Reports), I observed fish feeding near the surface during multiple visits in both 2019 and 2020. I captured one individual, which I tentatively identified as a brook trout (*Salvelinus fontinalis*), in a minnow trap in the 2019 season. As fish stocking had ceased at this location for a duration of seven years and the only inflow observed was a small meltwater stream, this indicated that fish were able to overwinter in this pond, even though it was estimated to be only a couple of meters deep. Two anglers I interviewed at Quarry Lake on separate occasions stated that they remembered when long toed salamanders were abundant in and around this pond before stocking began.

Sheppard's Pond showed no record of being stocked with fish in years prior (Alberta Fish Stocking Reports), although I caught two individuals in traps, which I tentatively identified as white suckers (*Catostomus commersonii*), during the 2020 season. Their presence in the pond appeared to have been by means of an under-road culvert providing occasional connectivity with other nearby ponds. During the same year, I captured long toed salamanders during every sampling period, confirming a viable population. I did not, however, observe signs of any other amphibians there. Although cohabitation may be atypical for these ponds, as salamanders may have been rare in Quarry Pond and fish rare in Sheppard's, they do illustrate its possibility. In most cases, cohabitation is aided by pond heterogeneity and sufficient vegetative cover (Kenison et al. 2016). In both ponds, I observed variation in pond depth, substrate, and plant growth along various parts of the shore, indicating that this may have been an important reason for the observed individuals' survival, especially in Quarry Pond, where Arctic grayling would be likely predators. The gape limitation as well as different foraging niche of the much smaller fish I captured in both ponds would make an indirect threat such as competition for food more likely (Pearson 2004).

5.3 Impacts of roads on populations

The correlations between the road type and distance to nearest road with individuals captured were, somewhat surprisingly, not significant. Incidentally, the fact that roads and trails were on average closer to Bow Valley ponds than in the Kananaskis may be a contributor to the higher mean levels of turbidity I observed in the Bow Valley. Roads can contribute to the runoff of foreign materials into ponds, some of them bringing toxic compounds such as salts with them (Snodgrass et al. 2007; see also section 5.1.2 above for more discussion of these points).

Although I had expected roads to be a more obvious predictor of population abundance, I note that there was potential for unintentional bias in the ponds selected for survey. Although my selection was based on those with confirmed historical populations (Graham and Powell 1999), and historical ponds appear to have been selected at random (or at least haphazardly), accessibility likely played a role in which ponds were located and surveyed. Indeed, most survey ponds had at the very least a pre-existing access trail and, in many cases, a nearby road. I suggest that a more robust test of the effects of roads on population abundance would include more ponds located at greater distance from the nearest trail or road.

5.4 Potential for metapopulations

Given the arrangement of ponds on the landscape, as well as indicators of anthropogenic development in the area, I expected to observe signs of population turnover as an important vector for species persistence. My literature review suggests that, while little is known about metapopulations in long toed salamanders specifically, they are known in this species as well as multiple other amphibian species (Marsh & Trenham 2008, Corser & Dodd 2004, Smith & Green 2005). Potential for population turnover in long toed salamanders was also observed in the data I collected in the study area, while nearly half of the populations were not stable across both years, possibly indicating a blinking in and out of local populations even during long term species persistence in the area.

5.5 Limitations

As with most field studies conducted over brief time periods, one limitation of this study was relatively small sample sizes. Because the historical data were collected at a time prior to widespread use of GPS and location directions were at times very scant, I was limited to ponds I could find. This sample size was further reduced by the drying of ponds by the later seasonal stage at which I was granted permission for field work in 2020 due to COVID-19 restrictions. This small sample size may have contributed to a lack of statistical significance for pond features I had expected to be associated with variation in abundance, such as roads and fish stocking. Especially in the case of the latter, the number of ponds with fish was small, eliminating the possibility of robust conclusions. I acknowledge the risk of applying false negatives/positives as population statuses for both amphibians and fish, and the uncertainty of conclusions regarding longer term trends such as population turnover from only two years of data, however this would be a risk best mitigated by longer term studies beyond the time limitations of this study.

5.6 Summary and General Conclusions

Former literature shows that anthropogenic development negatively affects long toed salamander populations by altering their habitats and removing effective migration corridors (Funk & Dunlap 1999, Government of Alberta 2017). A clear gap exists in our knowledge of specific indicators of these disturbances and how they interact with populations. This gap is challenging to close because many of the variables we can observe are likely to be correlated, presenting a difficulty in teasing them apart and identifying effects of each on its own. In this research my goal was to investigate the effects of some of the potentially harmful indicators of human development on local populations, and to begin an exploration into whether population turnover could be an important means for species survival in the case of declining populations. Although it was difficult to make any concrete conclusions due to the limitations I discuss in section 5.5, overall, I was able to cautiously conclude that 1) there are fewer viable populations in the survey area than there were two decades ago, 2) limited measures of environmental variables I considered do not seem to accurately predict variation in population abundance, 3) future researchers and monitoring programs should be aware of the possibility of population turnover. These conclusions are not consistent with my hypothesized decrease in individual abundance in the presence of indicators of human disturbance, since dissolved oxygen was the only variable found to affect population abundance and is not necessarily associated with anthropogenic disturbance. They are consistent with my predictions of loss of present populations over the years, as well as the possibility for population turnover which may be able to provide a positive, at least partial, compensation. From a conservation perspective, this indicates that it is not only the breeding pond that is important,

but also the landscape between ponds that is used for vital migration and dispersal. More in-depth research on how metapopulations function in long toed salamanders and how they can be better facilitated could be relevant to the question of long-term species persistence in altered landscapes.

In the case of fish stocking, although it is likely a relationship of exclusion, this is not entirely supported by my findings, as not all ponds lacking long toed salamanders had fish, and not all ponds with seemingly healthy salamander populations were entirely fishless. Although this may have to do with the fish I observed being smaller in size and not a species of stocked fish (Alberta Fish Stocking Reports), further investigation into cohabitation with small native fish species could be useful in understanding the question of whether all fish, or only stocked fish, present a problem to salamanders. In broad terms, my research was able to at very least support the necessity for long-term monitoring and attention to conservation of this species. Future research might explore options of other less obvious habitat factors at play and take a closer look at the associations between variables that may be correlated or even combine to intensify with mixed effects. Although acute events such as the draining of a breeding pond or sudden increase in its toxicity tend to gain more attention, it may be equally important to incorporate monitoring of longer-term effects of more chronic exposure to less obvious disturbance, such as a slow build-up of turbidity from ground disturbance caused by a nearby trail or changing temperatures affecting smaller breeding ponds disproportionately. Additionally, future research could continue to explore the possibility for the effects of potential threats observed currently to increase with future environmental pressure, such as the timing of seasonal drying of ephemeral ponds and how this could become an increasing issue driven by climate change.

This thesis highlights the importance of long-term monitoring for long toed salamanders, as well as ongoing observation of habitat needs and disturbance and to better understand how to conserve breeding habitat and population connectivity for this sensitive species.

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TABLES

Table 2.1. Field sampling schedule for 2019 and 2020. Population presence/absence and abundance was determined on minnow trapping days, and water temperature, turbidity, dissolved oxygen, and pH were measured on water sampling days.

Year	Location	Minnow trapping dates	Egg counting dates	Water sampling dates
2019	Bow	June 21, 25, 29	May 8, 14, 20	July 13
	Kananaskis	July 11, 12, 13	May 23, June 10, 24	July 13
2020	Bow	August 12, 13, 14	NA	August 12
	Kananaskis	August 12, 13, 14	NA	August 12

Table 3.1. Individual and egg values are raw counts taken at three replicates each year (2019 only for eggs). Highest values (underscored) were used in analysis. Body size and developmental stage are the means of three replicate sampling periods in 2020. Habitat variables are the means of 3 replicate measurements each year (except pH in 2020). Pond abbreviations identify valley pre hyphen: BV=Bow Valley, KV= Kananaskis Valley. Post hyphen abbreviations identify ponds as follows: BV=Bow Valley Pond, SP=Sheppard’s Pond, JR=JamaR, QP=Quarry Pond, AP=Arrow Pond, KP=Kuhn’s Pond, ML1=Mount Lorrette#1, ML2=Mount Lorrette #2, FJ=Fortress Junction, WG1=Wintergate#1, WG2=Wintergate#2, WW=William Watson, LK=Lower Kananaskis.

2019																		
Pond	Location	Surface area (m ²)	Depth (m)	Elevation (m)	Eggs	Individuals	Presence/absence	Other amphibian species	Fish	Body length (mm)	Larval growth stage	Temperature (°C)	pH	Turbidity (FNU)	Dissolved Oxygen (ppm)	Roads (distance)	Roads (type)	Aquatic vegetation (1=low, 2=med, 3= high)
BV-BV	51.0884461, -115.0839131	50	0.67	1,285	155, 220, 200	<u>8</u> ,8,7	+	<i>Bufo boreas</i> , <i>Rana luteiventris</i>	-	NA	NA	24.4, ±0.21	7.92, ±0.46	4.61, ±0.49	4.76, ±0.12	200	1	2
BV-SP	51.0536261,-115.2626806	2500	3	1,299	33, 0, 0	0,0,0	-	NA	-	NA	NA	24.8, ±0.5	8.33, ±0.10	1.02, ±0.29	4.37, ±0.31	30	4	3
BV-JR	51.0544619, -115.2656502	2000	2	1,296	0,0,0	1,0, <u>3</u>	+	NA	-	NA	NA	24.6, ±0.91	8.59, ±0.17	1.95, ±0.46	5.71, ±0.14	20	4	3
BV-QP	51.0759609,- 115.3809376	8000	1.5	1,382	0,0,0	0,0,0	-	<i>Bufo boreas</i>	+	NA	NA	20.7, ±1.07	8.23, ±0.38	12.73, ±0.13.93	4.67, ±0.35	5	3	1
BV-AP	51.0803860, -115.3794872	60	1	1,368	0,0,0	0,0,0	-	<i>Bufo boreas</i>	-	NA	NA	21, ±1.62	7.78, ±0.47	13.6, ±12.78	3.85, ±0.26	6	1	3
BV-KP	51.0801189, -115.3838508	200	1	1,375	55, 0,0	<u>2</u> , <u>5</u> ,2	+	<i>Bufo boreas</i> , <i>Rana luteiventris</i>	-	NA	NA	17.7, ±0.38	8.12, ±0.03	2.27, ±0.52	3.31, ±0.27	300	1	1
KV-ML1	50.9682665, -115.1135213	80	2	1,407	0,0,0	0,0,0	-	NA	-	NA	NA	8.3, ±0.15	7.99, ±0.03	0.56, ±0.08	5.33, ±0.74	30	2	1
KV-ML2	50.9666664, -115.1070250	150	2	1,477	0,0,0	0,0,0	-	NA	-	NA	NA	16, ±2.12	7.83±0.11	0.57, ±0.48	5.20, ±0.24	2	2	3
KV-FJ	50.7844499, -115.1639435	225	1	1,570	0,0,0	0,0,0	-	NA	-	NA	NA	13.4, ±0.1	7.61, ±0.07	0.17, ±0.11	2.49, ±0.25	5	4	2
KV-WG1	50.7162677, -115.1075356	5000	2	1,716	0,0,0	2,1, <u>3</u>	+	<i>Bufo boreas</i> , <i>Rana luteiventris</i>	-	NA	NA	18.3, ±0.1	8.43, ±0.04	0.26, ±0.08	5.31, ±0.52	80	4	2
KV-WG2	50.6608486, -115.0869162	2400	1	1,764	0,0,0	0, <u>1</u> ,0	+	<i>Bufo boreas</i> , <i>Rana luteiventris</i>	-	NA	NA	17.2, ±0.95	8.18,±0.35	0.4, ±0.08	4.65, ±0.67	400	4	2
KV-WW	50.6674890, -115.1253123	10000	2	1,711	0,0,0	0,1, <u>2</u>	+	<i>Bufo boreas</i> , <i>Rana luteiventris</i>	-	NA	NA	20, ±0.80	7.96, ±0.08	0.46, ±0.58	4.01, ±0.27	10	2	1
KV-LK	50.6359925, -115.1193779	30000	3	1,682	0,0,0	4,1, <u>7</u>	+	<i>Bufo boreas</i> , <i>Rana luteiventris</i>	-	NA	NA	19.7, ±0.56	8.87, ±0.03	0.4, ±0.32	4.37, ±0.75	20	1	2

2020																		
Pond	Location	Surface area (m ²)	Depth (m)	Elevation (m)	Eggs	Individuals	Presence/absence	Other amphibian species	Fish	Body length (mm)	Larval growth stage	Temperature (°C)	pH	Turbidity (FNU)	Dissolved Oxygen (ppm)	Roads (distance)	Roads (type)	Aquatic vegetation (1=low, 2=med, 3=high)
BV-BV	51.0884461, -115.0839131	50	0.67	1,285	NA	0,0,0	-	<i>Bufo boreas</i> , <i>Rana luteiventris</i>	-	NA	NA	10.93, ±0.31	NA	2.69, ±0.29	5.54, ±0.17	200	1	2
BV-SP	51.0536261, -115.2626806	2500	3	1,299	NA	5,4,1	+	NA	+	63.5, ±4.20, n=10	17.30, ±14.81, n=10	14.73, ±1.02	NA	0.78, ±0.04	4.84, ±0.08	30	4	3
BV-JR	51.0544619, -115.2656502	2000	2	1,296	NA	4,3,8	+	NA	-	60.93, ±7.71, n=15	17.27, ±0.59, n=15	12.27, ±0.21	NA	3.66, ±3.08	5.01, ±0.56	20	4	3
BV-QP	51.0759609, -115.3809376	8000	1.5	1,382	NA	0,0,1	+	<i>Bufo boreas</i>	+	45.0, n=1	15.0, n=1	13.87, ±0.51	NA	6.83, ±1.19	5.21, ±0.13	5	3	1
BVAP	51.0803860, -115.3794872	60	1	1,368	NA	0,0,0	-	<i>Bufo boreas</i>	-	NA	NA	12.37, ±0.46	NA	2.73, ±0.58	4.74, ±0.34	6	1	3
BV-KP	51.0801189, -115.3838508	200	1	1,375	NA	60,68,98	+	<i>Bufo boreas</i> , <i>Rana luteiventris</i>	-	46012, ±7.74, n=226	16.63, ±0.72, n=226	12.90, ±0.53	NA	4.74, ±0.63	4.57, ±0.27	300	1	1
KV-ML1	50.9682665, -115.1135213	2400	2	1,407	NA	0,0,0	-	NA	-	NA	NA	10.97, ±2.45	NA	0.83, ±0.22	4.54, ±0.22	30	2	1
KV-ML2	50.9666664, -115.1070250	10000	2	1,477	NA	1,1,1	+	NA	-	58.33, ±2.08, n=3	17.0, ±0, n=3	12.13, ±0.06	NA	0.44, ±0.23	5.28, ±0.15	2	2	3
KV-LK	50.6359925, -115.1193779	30000	3	1,682	NA	11,8,18	+	<i>Bufo boreas</i> , <i>Rana luteiventris</i>	-	62.70, ±5.79, n=37	17.60, ±0.48, n=37	16.81, ±0.06	NA	0.42, ±0.26	4.87, ±0.06	20	1	1

Table 3.2. The means of seven environmental variables used in statistical analysis, by valley and by year. Abbreviations are as follows: BV19 = Bow Valley during 2019, BV20 = Bow Valley during 2020, KV19 = Kananaskis Valley during 2019, KV20 = Kananaskis Valley during 2020. Values for water temperature, pH, turbidity, and dissolved oxygen are the means of 3 replicate measurements taken each year (except pH in 2020). Surface area, depth, and elevation were not expected to show notable change between years therefore the same values were used after being measured in 2019.

Variable	BV19	KV19	BV20	KV20
Water temperature (°C)	22.20, ±2.88, n=6	16.13, ±4.13, n=7	13.23, ± 2.25, n=6	14.47, ±3.13, n=7
pH	8.21, ±0.29, n=6	8.15, ± 0.42, n=7	NA	NA
Turbidity (FNU)	8.13, ±5.52, n=6	0.40, ±0.15, n=7	3.75, ± 2.10, n=6	0.43, ±0.23, n=7
Dissolved oxygen (ppm)	4.38, ±0.82, n=6	4.34, ± 1.01, n=7	4.87, ±0.35, n=6	5.08, ±0.37, n=7
Surface area (m ²)	6.29, ±2.14, n=6	7.23, ±2.30, n=7	6.29, ± 2.14, n=6	7.23, ±2.30, n=7
Depth (m)	1.53, ± 0.86, n=6	1.86, ± 0.69, n=7	1.53, ±0.86, n=6	1.86, ± 0.69, n=7
Elevation (m)	1,334.17, ±45.19, n=6	1,618.14, ±135.63, n=7	1,334.17, ±45.19, n=6	1,618.14, ±135.63, n=7

Table 3.3. Principal component analysis loadings and eigenvalues by year for seven pond features measured in 2019 and six in 2020. PC1 and PC2 explained 38 and 25 percent of variation respectively in 2019, and 51 and 20 percent in 2020.

2019							
VARIABLE	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Water temp	-0.13	0.60	-0.33	0.25	-0.47	0.42	0.23
pH	-0.49	0.20	-0.05	-0.12	-0.21	-0.78	0.22
Turbidity	0.28	0.47	-0.34	-0.46	0.57	-0.06	0.21
Dissolved oxygen	-0.26	0.28	0.66	-0.57	-0.11	0.29	-0.07
Surface area	-0.53	0.02	-0.41	-0.11	0.18	0.14	-0.70
Depth	-0.49	0.05	0.24	0.45	0.59	0.17	0.35
Elevation	-0.28	-0.54	-0.34	-0.42	-0.90	0.29	0.50
Eigenvalues	2.67	1.73	1.04	0.62	0.46	0.40	0.08
Proportion of variance	0.38	0.25	0.15	0.09	0.07	0.06	0.01
2020							
VARIABLE	PC1	PC2	PC3	PC4	PC5	PC6	NA
Water temp	0.5	-0.32	0.01	-0.08	0.78	0.02	NA
Turbidity	-0.32	-0.48	0.65	0.12	0.12	-0.47	NA
Dissolved oxygen	-0.06	-0.68	-0.67	0.13	-0.18	-0.20	NA
Surface area	0.45	-0.39	0.36	-0.13	-0.56	0.42	NA
Depth	0.53	0.16	-0.37	-0.42	-0.15	-0.71	NA
Elevation	0.41	0.15	0.05	0.88	-0.07	-0.17	NA
Eigenvalues	3.08	1.19	0.95	0.59	0.17	0.02	NA
Proportion of variance	0.51	0.20	0.16	0.10	0.03	0.0	NA

FIGURES

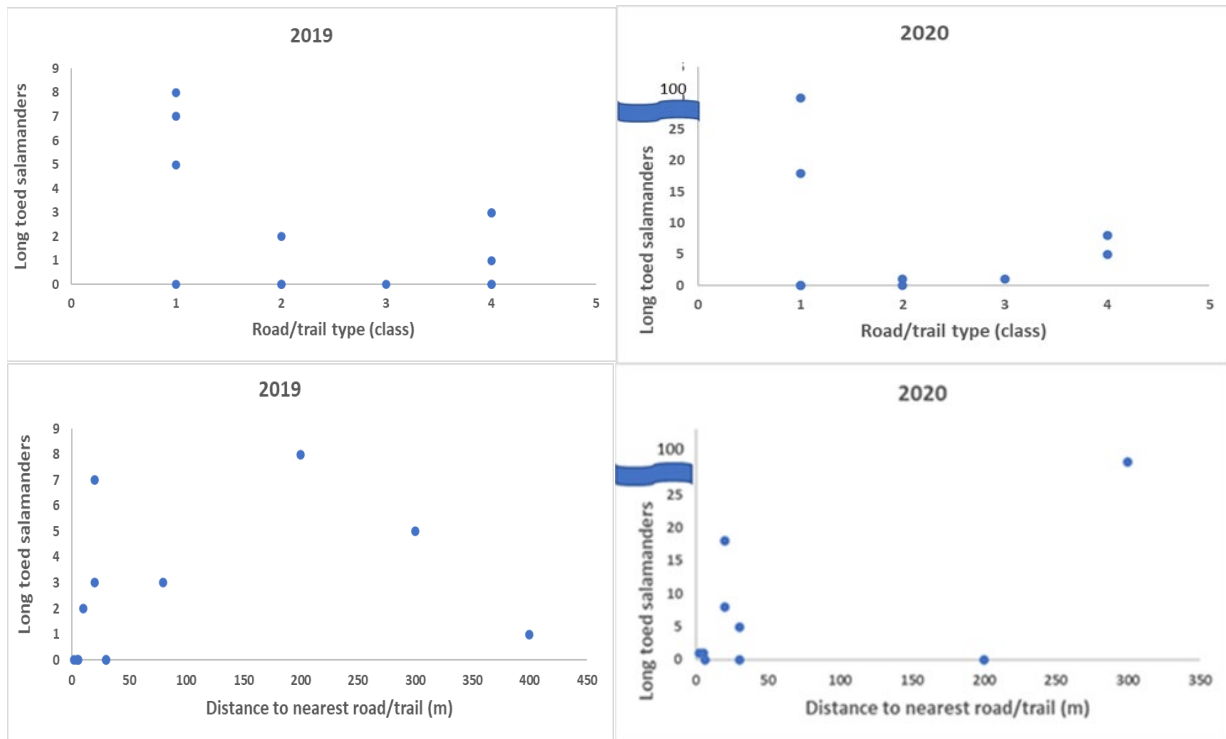
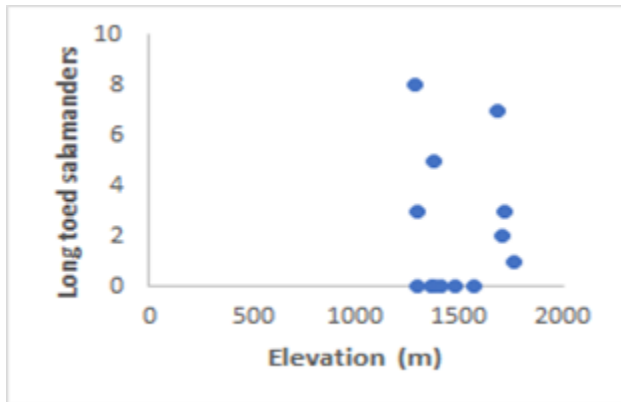
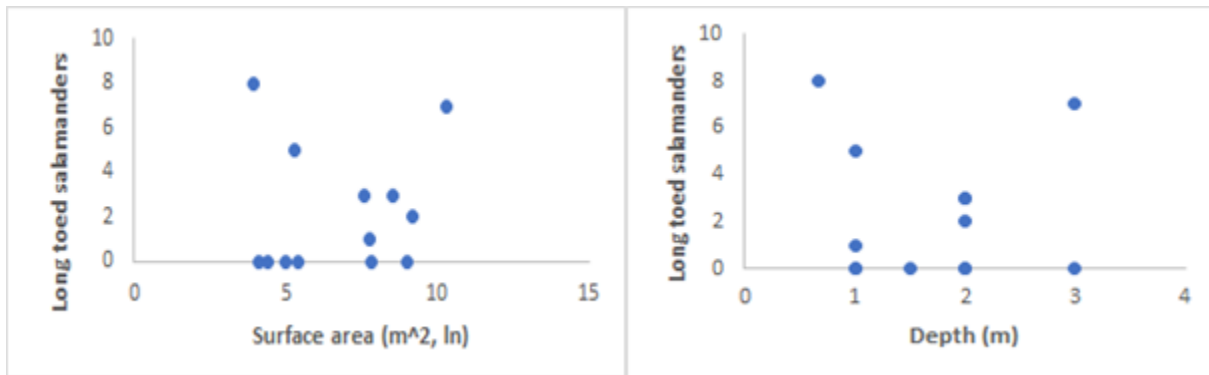
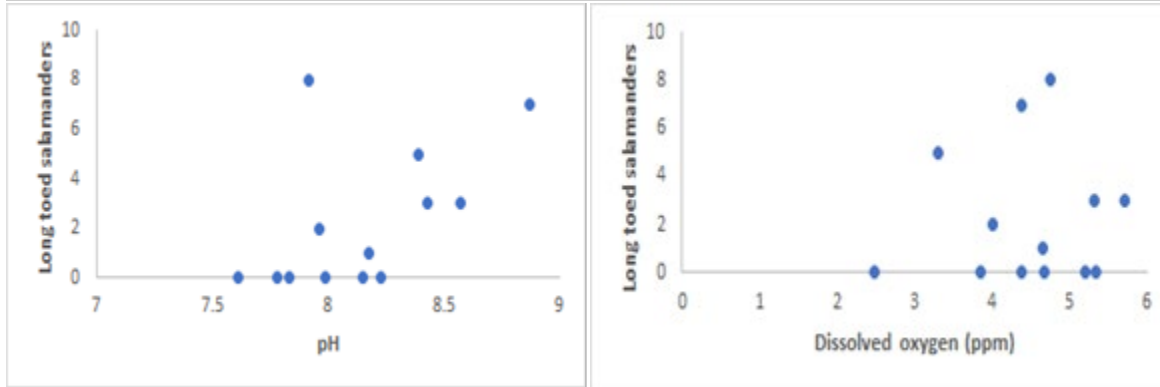
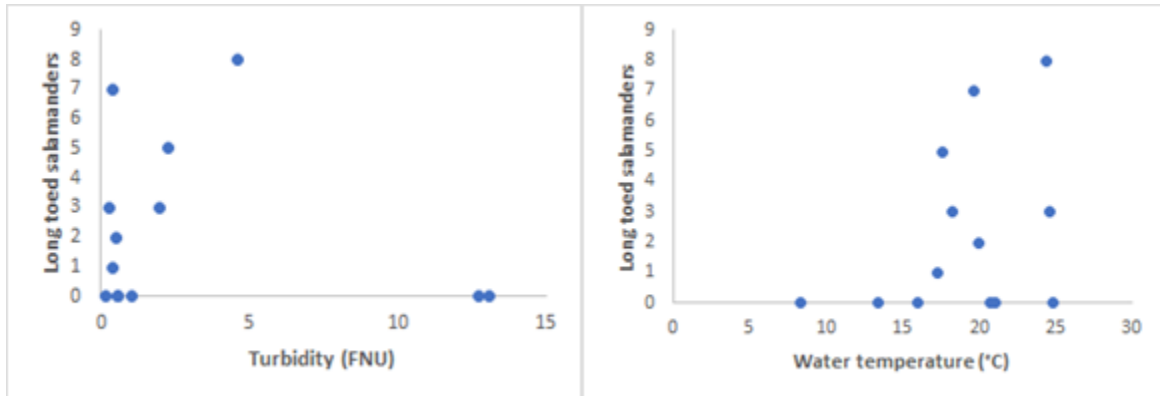


Figure 3.1. Effects of road type (perceived traffic volume value) and distance to nearest road (m) for 13 sample ponds in 2019 and nine in 2020. Individual values for long toed salamanders are the highest raw counts from three capture period replicates from 2019 and 2020 respectively. Road type is a class and distance is a raw value in meters. Both were measured in 2019 and not observed to change in 2020.

2019



2020

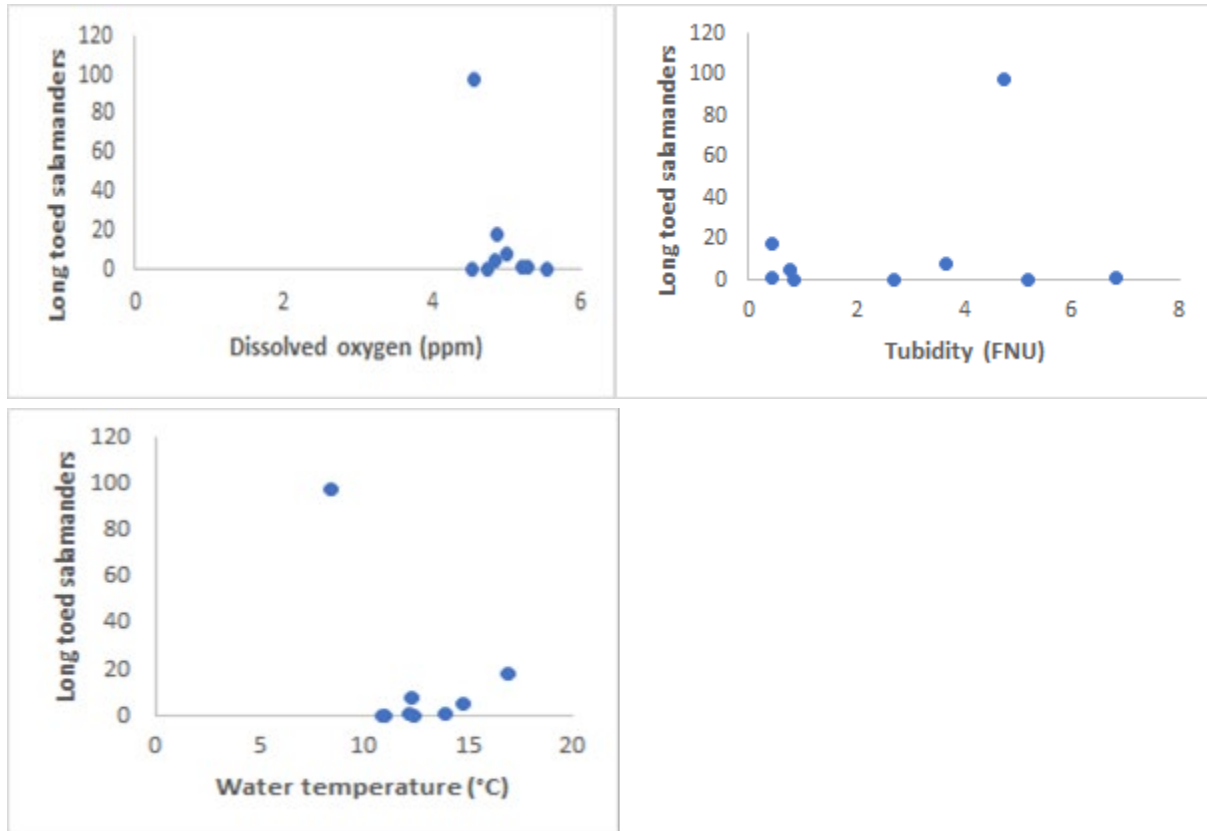


Figure 3.2. Scatterplot of the relationship between habitat features and number of individuals captured from 13 ponds in 2019 and nine ponds in 2020. Independent variables are the means of three replicates each year and values for individuals are representative of the highest capture in a single period for each year. Features of ponds that showed no notable change between years are shown for 2019 only, while pH could not be measured in 2020.

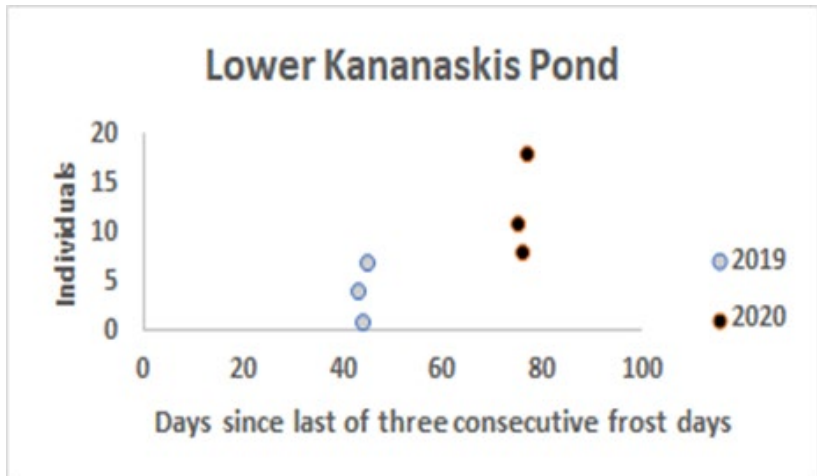
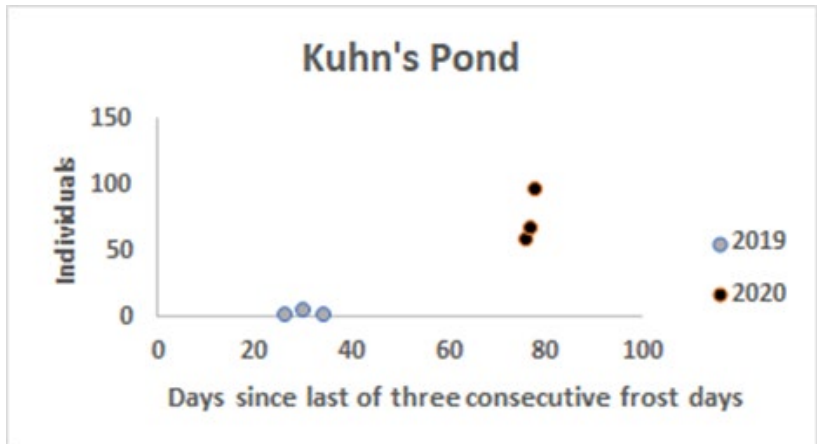
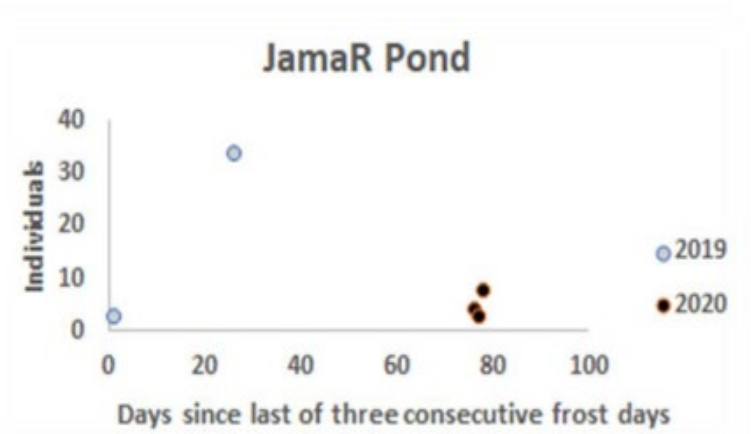


Figure 3.3. Population fluctuations for three ponds that maintained constant populations and could be surveyed during both years. Raw values for individuals are shown, and were recorded at three replicate visits each year, frost days were determined using the Government of Alberta Weather Station Data Viewer (2020) for Bow and Kananaskis weather stations.

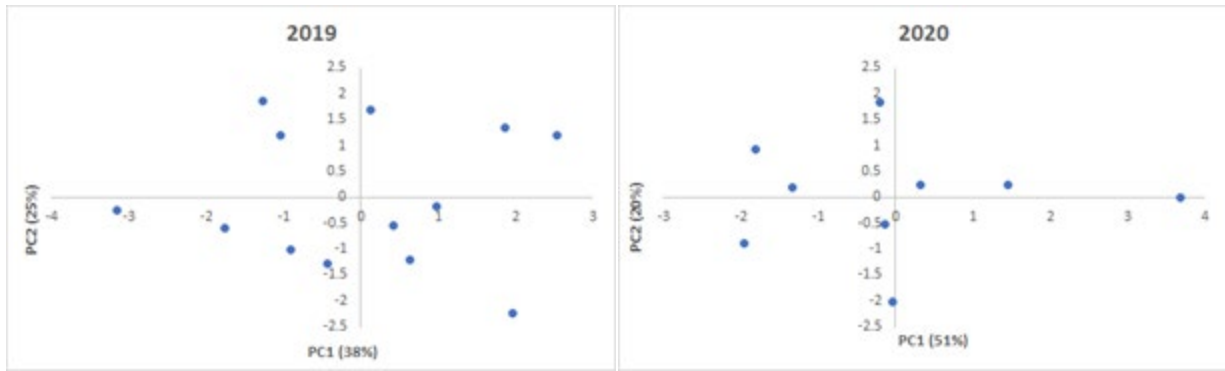


Figure 3.4. Principal components analysis results showing percent of variation explained by PC1 versus PC2 by year.

APPENDIX A: MAP OF SURVEY LOCATIONS

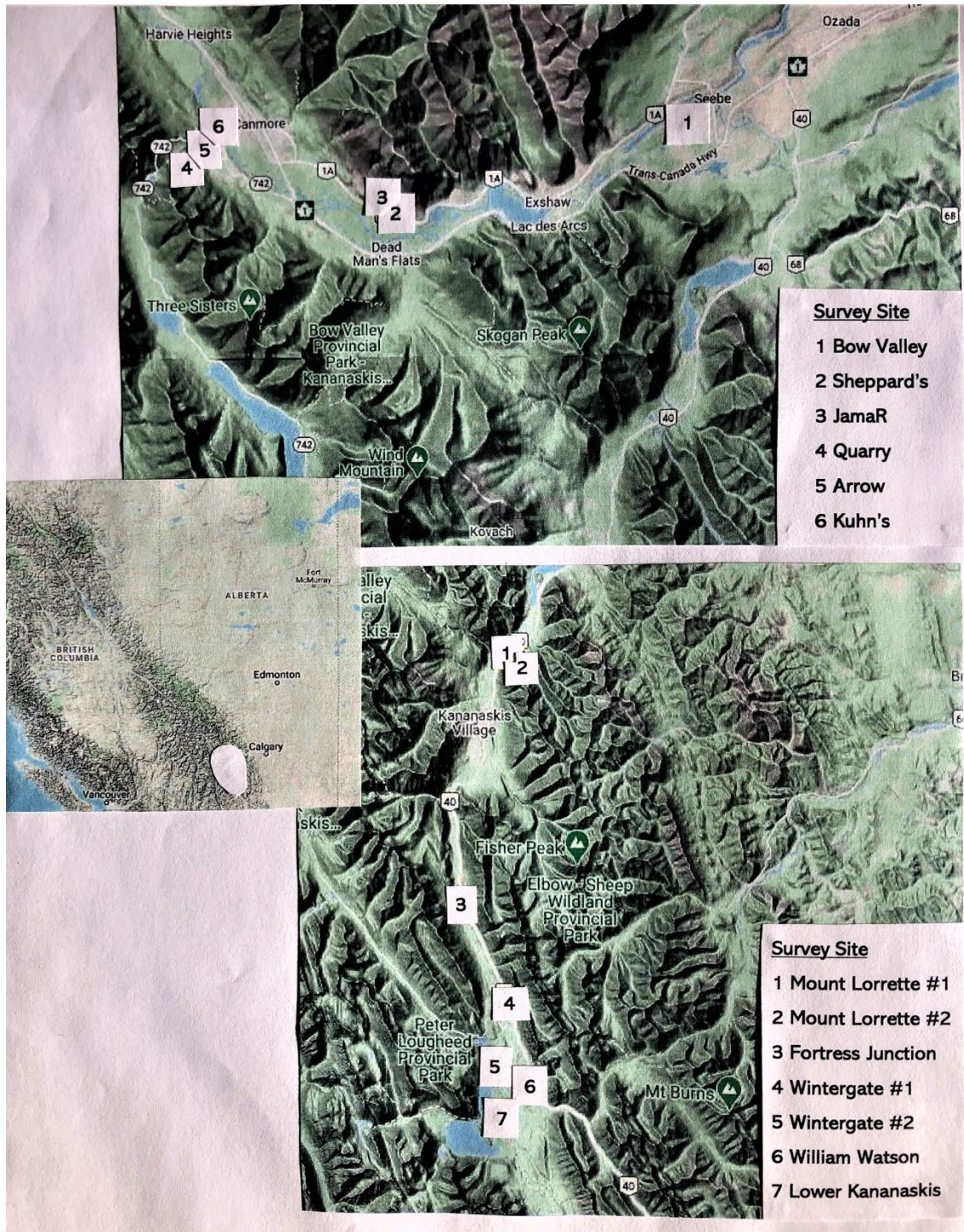


Figure A.1. Maps showing survey sites. Top map shows sites in the Bow Valley, bottom map shows sites in the Kananaskis Valley. Small middle map shows Alberta with whitened area highlighting the general location of both valleys. Maps were created using GoogleMaps.ca.