

**MOVEMENTS OF ADULT WESTERN TOADS, *Bufo boreas*,
IN A MANAGED FOREST LANDSCAPE AND THE
INCIDENCE OF A DISEASE IN SOUTHWESTERN
BRITISH COLUMBIA**

by

Isabelle Emiola Deguise

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ABSTRACT

Amphibians are declining dramatically around the globe, due primarily to two major threats: habitat destruction and emerging infectious diseases. The western toad, *Bufo boreas*, is an IUCN red-listed species thought to be affected by both these factors. The objectives of this thesis were to (1) determine how forest fragmentation affects western toad movement behaviour; and (2) determine if there is any evidence of the infectious disease, the chytrid fungus (*Batrachochytrium dendrobatidis*), in this species in British Columbia.

I used radio-telemetry to follow daily movement patterns of 23 adult male toads in a fragmented landscape near Vancouver, BC, composed of forest patches and small, recent clear-cuts. Movement parameters were analyzed using a Geographic Information System (GIS) and mixed effects models. Results showed that toads in forests were able to perceive clear-cut edges from as far as 150 m. Greater than 60% of toads released in forest patches actively chose to enter the clear-cuts from adjacent forests, indicating high boundary permeability. In addition, toad movement parameters were not significantly reduced in these disturbed environments, suggesting that clear-cuts do not restrict movement. Although toads appear to favour these disturbed environments, further research is required in larger, more realistically sized clear-cuts.

To investigate the prevalence of the chytrid fungus in southwestern British Columbia, I tested a breeding population of western toads in a protected area. Results showed a 28% infection rate, although no toads showed any visual signs of the disease. These results provide the first evidence of the chytrid fungus in western toads in British

Columbia and suggest that the disease is potentially more widespread than currently documented.

The results of my research suggest that the western toad may not be affected by small scale forest harvesting. In addition, my results support the hypothesis that western toads are reservoirs for the chytrid fungus. Although most populations in Canada appear to be faring well, long-term population monitoring and further testing for chytrid prevalence are required. To minimize the likelihood of western toads experiencing the dramatic declines that are presently taking place in the United States, protecting vital habitats and preventing the spread of chytrid through public awareness are required.

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CO-AUTHORSHIP STATEMENT

Chapter 2 is being prepared for submission in a scholarly journal under the same title as given here. The co-author of this chapter is Dr. John Richardson. I.E. Deguise conducted the research, performed all the data analysis and wrote the manuscript. The co-author helped design the study and improve the manuscript.

Chapter 3 is being prepared for submission as a research note in a scholarly journal under the same title. The co-author of this chapter is Dr. John Richardson. I.E. Deguise conducted the research and wrote the manuscript. The co-author provided funding and improved the manuscript.

CHAPTER 1: INTRODUCTION

More than 80% of Earth's land surface is currently being modified by human activity (Sanderson et al. 2002). Biodiversity is rapidly decreasing and global extinction rates have accelerated to mass extinction proportions, estimated at 4 times the natural background rate (Vitousek et al. 1997, May & Tregonning 1998). Species are disappearing at alarming rates, even in protected areas with very little human impact (Sanderson et al. 2002, Stuart et al. 2004).

One of the vertebrate taxa suffering most from changes to the planet are amphibians (Stuart et al. 2004). Over one-third of all amphibian species are globally threatened with extinction, more so than birds (12%) and mammals (23%) (Baillie et al. 2004, Stuart et al. 2004). In recent decades, numerous amphibian species have experienced dramatic population declines, range reductions or have disappeared completely (Alford and Richards 1999, Houlahan et al. 2000, Pounds et al. 2006). While some of these declines can be attributed to natural population fluctuations, the vast majority are undoubtedly anthropogenic. Amphibians are highly vulnerable to extinction because of their relatively low vagility, narrow habitat tolerances, and high susceptibility to pathogens (Cushman 2006).

There are numerous hypotheses that address the big question: what is causing amphibian declines? Factors such as alien species, over-exploitation, UV radiation and climate change have all been suggested (reviewed in Alford and Richards 1999 and Collins and Storfer 2003). However, scientists generally agree that the greatest threat to amphibians is habitat loss and fragmentation (Blaustein and Kiesecker 2002). Despite this, species are also declining in areas with little human impact and the most plausible

explanation for these declines is emerging infectious diseases (Berger et al. 1998, Pounds et al. 1997, Lips et al. 2006).

Habitat Loss and Fragmentation

The best-documented cause of amphibian declines is due to landscape alteration (Alford and Richards 1999). Numerous studies have found that amphibian abundance and diversity decrease in areas directly affected by habitat loss (e.g. Petranka et al. 1993, Semlitsch 1998). Although direct losses of amphibians from physical loss of habitat are obvious, the implications of landscape fragmentation also play a key role in population persistence and this topic is now a major focus of recent amphibian research. Habitat destruction and habitat conversion result in the formation of suitable habitat patches isolated from each other by areas of unsuitable habitat, often termed the 'matrix'. Metapopulation theory has emerged as a useful concept for conservation biologists studying habitat fragmentation (Marsh and Trenham 2001, Akçakaya et al. 2004, Smith and Green 2005). Much of the recent literature on habitat fragmentation focuses on the resistance provided by these landscapes to movement and their impacts on dispersal between suitable habitat patches (e.g. Rothermel and Semlitsch 2002, Marsh et al. 2005, Rothermel 2004, Rittenhouse and Semlitsch 2006, Stevens et al. 2006). Understanding how specific organisms move as a result of habitat fragmentation furthers our knowledge of how landscape structure influences animal movements (Stevens et al. 2004). Studying movement behaviour in fragmented landscapes contributes to both species conservation and theory development, helping bridge a gap between landscape ecology and metapopulation biology (Berggren et al. 2002, Fahrig 2003).

Emerging Infectious Diseases

A diversity of pathogens affect amphibians, including viruses, bacteria, protists, metazoan parasites and fungi (e.g. Blaustein et al. 1994, Kiesecker and Blaustein 1997, Daszak et al. 1999, Longcore et al. 1999). Diseases can affect all amphibian life stages, from eggs to adults, and cause direct mortality or sublethal damage, such as deformities. Some diseases have been around for many years, such as *Saprolegnia* water moulds and ranaviruses, whereas others have recently appeared and are increasing in incidence, virulence or geographic range. This group of pathogens is known as emerging infectious diseases (Daszak et al. 1999, 2003). A key representative of this group is chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (Daszak et al. 2003). The chytrid fungus has received significant attention in the recent literature because it has been associated with mass mortality events in amphibian populations around the globe (Berger et al. 1998, Bosch et al. 2001, Carey 2000, Muths et al. 2003, Lips et al. 2006). Massive population declines apparently caused by the chytrid fungus have been reported in areas of little human impact, including protected areas and remote tropical rainforests (Berger et al. 1998, Lips et al. 1999, Lips et al. 2006). Understanding the extent of disease ranges and infection prevalence contributes to our overall understanding of disease ecology and their role in population persistence.

Study Species

These two major threats to global amphibian populations are also having an impact at a regional scale in the Pacific Northwest. The western toad, *Bufo boreas* (Baird and Girard 1852), is one of the best documented cases of a species undergoing population declines in western North America and it has recently been listed on the World

Conservation Union's (IUCN) red-list of threatened species. The two leading causes for the disappearance of western toads are hypothesized to be habitat alteration and disease (Carey 1993, Muths et al. 2003, Hammerson et al. 2004).

Study Objectives

The overall objectives of this thesis were to investigate the impacts of two major threats on the western toad. Specifically, my goals were: (1) to determine how forest fragmentation affects western toad movement behaviour; and (2) to determine if there is any evidence of the emerging pathogenic chytrid fungus in this species in British Columbia.

In Chapter 2, I describe research on habitat-specific movement behaviour of western toads in a fragmented landscape; specifically looking at toad perceptual ability, landscape connectivity and boundary permeability. I used radio-telemetry to follow daily movement patterns of toads placed in different landscape contexts and determined if small, recent clear-cuts are barriers to movement.

In Chapter 3, I test for evidence of the chytrid fungus in a breeding population of western toads from a relatively pristine provincial park in British Columbia. This disease has been having significant negative impacts on western toads in the southern part of its range, yet there are no reports of its presence north of the Canada-US border.

In Chapter 4, I summarize the results of my research and discuss their implications for the field of amphibian conservation. I also comment on the strengths and weaknesses of my thesis and make suggestions for future research in this area. Lastly, I make recommendations for the management and conservation of western toad populations in British Columbia.

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CHAPTER 2: MOVEMENT BEHAVIOUR OF ADULT WESTERN TOADS, *Bufo boreas*, IN A FRAGMENTED LANDSCAPE¹

Introduction

As the human footprint continues to expand around the world, habitat loss and fragmentation continue to increase (Vitousek et al. 1997, Sanderson et al. 2002). The resulting landscape is patchy, with areas of suitable habitat isolated by areas of less suitable habitat, known as the 'matrix'. In order to persist, species must disperse between suitable areas and recolonize patches following local extinctions (Fahrig and Merriam 1994). The connectivity between suitable patches has major implications for population viability, since the probability of local extinction commonly increases with the extent of fragmentation, degree of isolation, and limitations on dispersal (Fahrig and Merriam 1985, Fahrig 2002, Stevens et al. 2004). Alterations to landscapes that modify or restrict organism movement will have potentially negative consequences on long-term survival (Fahrig and Merriam 1994, Fahrig 2003).

As a result of these fragmented environments, metapopulation theory is commonly applied to model populations in altered landscapes (Hanski and Gilpin 1997). A key to this metapopulation framework is an organism's ability to move through the matrix, which will be a result of the interaction between the landscape structure and the organism's movement behaviour (Goodwin and Fahrig 2002). Three major components will determine if an organism can successfully persist in these disturbed landscapes and thus how severely they will be affected by habitat fragmentation. First, the perceptual range of an individual will determine if it can perceive suitable habitat patches (Zollner

¹ A version of this chapter will be submitted for publication. Deguise, I. and Richardson J.S. Movement patterns of adult western toads, *Bufo boreas*, in a fragmented landscape.

and Lima 1997). Second, the individual must be able to cross the boundary between two landscape types, known as boundary permeability (Stamps et al. 1987, Stevens et al. 2006). Thirdly, the matrix habitat can facilitate or impede an individual's movement, referred to as landscape connectivity or patch resistance (Taylor et al. 1993, Stevens et al. 2006). However, all habitats are not equivalent and the influence of the matrix will often be species specific (Ricketts 2001, Stevens et al. 2004). Understanding how specific organisms move through fragmented landscapes is crucial for both species conservation and theory development, in addition to furthering our knowledge of how landscape structure influences movement (Berggren et al. 2002, Stevens et al. 2004).

Maintaining connectivity between remnant habitat patches is especially vital for amphibians, since many amphibian species exist in metapopulations which rely on successful dispersal for persistence (Gill 1978, Marsh and Trenham 2001). At a landscape scale, individual amphibians are exchanged between ponds, whereas at the local scale, dispersal between critical habitat elements (aquatic and forest) plays a crucial role in amphibian life histories (Rothermel 2004). Restriction of these movements would have dire consequences for both local amphibian subpopulations and the overall maintenance of regional populations (Hecnar and M'Closkey 1996, Cushman 2006). Much recent literature has assessed landscape connectivity and matrix permeability in a variety of animals, such as insects (e.g. Goodwin and Fahrig 2002, Haynes and Cronin 2006), birds (e.g. Bélisle and St. Clair 2002, Castellón and Sieving 2006) and small mammals (e.g. Bowman and Fahrig 2002, Selonen and Hanski 2003). However, studies involving amphibians are lacking despite their important role in terrestrial and aquatic

ecosystems and their declining populations worldwide (for some exceptions see Chan-McLeod 2003, Stevens et al. 2004, Stevens et al. 2006, Rittenhouse and Semlitsch 2006).

In the Pacific Northwest of North America, a significant portion of habitat fragmentation occurs through forest harvesting, including clear-cut logging and associated forest practices (such as road creation). Clear-cut logging accounts for some of the most destructive forms of habitat modification, yet little is known on how clear-cuts impact movement patterns on a landscape scale (Pawson et al. 2006). Changes in both the abiotic environment and biotic communities can be dramatic following clear-cutting (Chen et al. 1999). Clear-cuts are often hostile environments for amphibians and many species are negatively impacted by this land-use practice (Knapp et al. 2003). Despite this, little research has focused on the consequences of fragmentation caused by forest harvesting on amphibians.

In this study, I take a single-species approach to investigate the impacts of forest fragmentation on movement patterns in a generalist amphibian, the western toad, *Bufo boreas*. I used translocation and radio-telemetry to examine individual movement behaviour within patches of forested habitat and recent clear-cuts (or matrix, terms used interchangeably). I tested three main hypotheses: (1) Given that toads have the ability to orient themselves towards vital habitats, such as natal breeding ponds, I hypothesized that toads will be able to perceive suitable forest patches from within non-suitable patches. To do so, I looked at initial movement directions for evidence of strong affinities towards specific habitat types. (2) Forest/clear-cut boundaries will be permeable for western toads. As an indicator of boundary permeability, this question was assessed by examining the ability of toads to cross this habitat edge. (3) Given the complexity and potential

inhospitability of recent clear-cuts, I hypothesized that this matrix habitat will restrict toad movement through the landscape and I expect toads to avoid these environments. This study compared movement patterns between habitat types and patch exit time as indicators of landscape resistance. Overall, I determined if recent clear-cuts were acting as movement barriers.

Methods

Study Species

The western toad is distributed throughout western North America ranging from Baja California to southeastern Alaska (Stebbins 1985). *Bufo boreas* is a generalist species, common in a variety of habitats including forests, wetlands, open meadows and occasionally in disturbed areas such as clear-cuts, ditches and gravel pits (Bartelt et al. 2004, Wind and Dupuis 2002). Breeding occurs in the spring when toads congregate along the shallow margins of small water bodies. Western toads are considered explosive breeders, with females laying an average of 12,000 eggs in less than a week (Olson et al. 1986). They are also highly philopatric, since individuals generally return to the same breeding pond every year (Davis 2000, Muths et al. 2006). Toads are known to travel long distances, primarily by climbing and crawling - dispersal distances of up to 7.2 km from the breeding site have been recorded and home ranges vary between 0.1 ha and 4 ha, depending on habitat condition (Davis 2000, Muths 2003, Bartelt et al. 2004). Bartelt et al. (2004) and Muths (2003) measured movement distances from May to October and found that female toads moved up to 2.6 times further than males, with greatest distances for males around 0.94 km and females 2.44 km from the breeding pond.

Recently, western toads have declined dramatically throughout much of their range in the United States and although they appear to be faring well in Canada, they have been listed as Near Threatened on the IUCN red-list of threatened species (Carey 1993, Fisher and Shaffer 1996, Wind and Dupuis 2003, Hammerson et al. 2004, Muths and Nanjappa 2005). Declines have been primarily associated with disease (largely chytridiomycosis), UV radiation and habitat loss (Carey 1993, Blaustein et al. 1994a, Blaustein et al. 1994b, Muths et al. 2003, Hammerson et al. 2004). However, evidence for the impacts of fragmentation on movement dynamics in this species remains scarce and studies looking at the effects of forestry practices are nonexistent.

Study Sites

This study was conducted from May to June 2006 in the University of British Columbia's Malcolm Knapp Research Forest (49° 16' N, 122° 34' W), approximately 60 km east of Vancouver, British Columbia, within the Coastal Western Hemlock biogeoclimatic zone. The research forest is highly fragmented and contains patches of various habitats, including second growth forest and recent clear-cuts. Four study patches were selected as release sites for this translocation experiment, two forested patches (Maple Ridge Forest (2.87 ha) and Wood Lot Forest (4.91 ha)) and two recently harvested patches (CC03 (3.06 ha) and CC05 (3.23 ha), Figure 2.1). The two forest patches were second growth forest (>40 years) composed primarily of Western hemlock (*Tsuga heterophylla*), along with Douglas-fir (*Pseudotsuga menziesii*) and Western red cedar (*Thuja plicata*), with an understory of Salmonberry (*Rubus spectabilis*) and ferns (primarily Sword fern, *Polystichum munitum*). The two clear-cut patches were less than 3 years old and vegetation consisted primarily of young Red alder (*Alnus rubra*) and

invasive Himalayan blackberry (*Rubus discolor*). Slash from both sites had been moved into piles along the western and southern edges of the patches. The four patches were surrounded by continuous forest, except to the north of patch CC05. This area was fragmented by a hydroline right-of-way and other clear-cut patches. In addition, there were numerous forest roads in the area and the forest gradually increased in elevation to the north.

Radio-telemetry

Thirty-two male toads were captured from Edith Lake near Squamish, British Columbia (49° 46' N, 123° 06' W), and transported in individual containers to the MKRF. Toads were kept in large plastic containers containing moss, natural cover objects and water bowls, and fed crickets. All toads were measured with calipers (snout-to-vent length) and weighed using a field scale, then fitted with radio-transmitters (BD-2G, Holohil Inc, Carp, Ontario) attached with polyethylene belts. The harnesses were constructed and attached using a method devised by Bartelt and Peterson (2000), which found little impact on toad behaviour. A Telonics TR-4 receiver and Yagi directional antenna were used to locate toads every 1-3 days. A Garmin GPSMap60CSx was used to obtain toad locations to within 3 m accuracy in the clear-cuts and with less than 5 m error in the forests. At each location, 100 measurements were taken and the average was used to obtain a position in lat/long WGS 84.

All toads were tested for the chytrid fungus, *Batrachochytrium dendrobatidis*, before their introduction into the MKRF and only toads that did not test positive were used in this study (23 of 32 toads). Twenty-three toads were randomly assigned to sites (n = 6 for the Wood Lot, CC03 and CC05 sites and n = 5 for the Maple Ridge site) and

released one meter from each other along a north-south transect. Individual toads were placed in a bucket that was then flipped carefully up side down at the release site. Toads were given 2 minutes to adjust and then the bucket was removed from a distance using a long string. The observer stood at least 2 m away to minimize disturbance to the toads. Initial headings were recorded for each individual, along with GPS coordinates and habitat descriptions. All toads were given one full day to acclimatize to their new environments before radio-tracking began. The position of every toad was determined every 1 to 3 days for a total of three weeks (trial 1). At the end of three weeks, all toads were captured and returned to the same enclosures. This time frame was decided upon after some of the toads started to travel long distances into difficult terrain or through areas with active logging. Toads were kept in captivity for two nights, after which they were randomly assigned to one of the two sites of the opposite treatment and released using the same procedures as described above. Once again, toads were given one day to acclimatize and then their positions were determined every 1 to 3 days for another three weeks (trial 2). At the end of the study, all remaining toads were collected from the research forest and returned to their breeding site. Conducting two experimental trials increased my overall sample size and determined whether movement patterns were related to initial starting habitat. During this study, five toads were lost, presumably to predation. Five radio-transmitters from these toads were recovered and three had evidence of chewing. Data points for these individuals were truncated to the last confirmed sighting of the toads.

GIS Analysis of Movement Paths

All GPS data were imported into ArcMap (Environmental Systems Research Institute [ESRI] 2005) and projected into UTM Zone 10N. The straight-line distance between each toad location was deemed a step length and the joining of each step length formed the movement path. Using Hawth's Analysis Tools for ArcGIS (Beyer 2004), I extracted step lengths, total distances, turning angles and net displacements from each toad movement path. Only turning angles associated with step lengths greater than 5 m were used in the analyses. Step lengths smaller than 5 m have high errors associated with their turning angles and cannot be accurately associated with movement events due to limitations of GPS accuracy (Jerde and Visscher 2005). Since the number of days between toad relocations differed, measurements of total distance were divided by the number of days a toad was tracked, which provided an estimate of movement rate in meters per day.

Given that the majority of toads left their initial release patches and moved in different habitat types, movement paths had to be divided by their occurrence in three habitat classes (forest, matrix and road) before I could analyze the effects of habitat type on movement patterns (Turchin 1998, Goodwin and Fahrig 2002). Forest habitat was classified as areas with a closed canopy and trees greater than 15 m in height (see previous forest site descriptions for more detail). Roads in the Malcolm Knapp Research Forest are all gravel and single lane with culverts on either side. Matrix habitat was defined as any area that had experienced a recent disturbance and had vegetation no higher than 5 m. The majority of this habitat consisted of clear-cut patches, including the two clear-cut sites used as release patches in this study. An additional 7.8% of toad

movement locations occurred in areas that could not clearly be classified as forest, clear-cut or road. All of these ambiguous observations occurred in a hydroline right-of-way, which was last cleared in 2003 and contained primarily shrubs and young willow trees. Due to the small number of locations in this area, I grouped them with locations in clear-cuts (matrix habitats) to minimize the division of movement paths. For each movement path in each of the three habitat types, I measured movement rate (calculated as meters moved per day), maximum single movement (the largest movement made over a single two day period) and net displacement (straight-line distance between the release point and final recapture point). When possible, I also extracted the mean vector length, r , a measure of the straightness of the movement path, where an r value of 1 indicates a completely straight line.

Weather Data

Data for daily precipitation and air temperatures were taken from a meteorological station located at the entrance of the Malcolm Knapp Research Forest, approximately 1 km NW from the study site.

Research Permits

A provincial wildlife use permit (SU06-21053) and University of British Columbia animal care permit (A03-0196-A001) were obtained for this research project.

Statistical Analysis

Movement Patterns

I used circular statistics to calculate mean angles for each toad movement path and overall directionality. Rayleigh's test was used to determine if toad movement

directions were statistically different from random (Batschelet 1981). A modified version of Rayleigh's test, the V-test, was used to test whether initial movement angles of toads released in forest patches were oriented towards the clear-cuts. This test provides increased power by specifying a hypothetical direction and determining if observed angles are statistically clustered around this hypothesized angle (Batschelet 1981, Zollner 2000). In my case, this presumed direction was the mean angle towards the clear-cuts from the forest patches (Maple Ridge = 0°, Wood Lot = 90°, Figure 1). Watson's U^2 test was used to compare directions between toads in the two trials.

I analyzed the differences in movement patterns of toads in the three habitat types (forest, matrix and road) as separate, mixed-effects models for each of the three dependent variables: movement rate, maximum single movement and net displacement (all log transformed to meet assumptions of normality). Using mixed effects models allowed me to analyze the two trials together since these models accommodate correlated error structures associated with non-independence of data points that exist not only between repeated measurements of movement for individual toads but also between the two trials (Buckley et al. 2003). Habitat type was included as a fixed effect in the model, while trial was included as a random effect. The effect of individual toads was incorporated in the error term. To account for the repeated measures used in this experiment and the unequally time spacing, I specified a SP(POW) spatial power covariance matrix structure (SAS Institute Inc. 1999). However, certain assumptions had to be made given that every toad was not in the experiment for the same duration, nor did every toad participate in a second trial. Likelihood ratio tests were used to test the significance of the random factor (differences between the two experimental trials).

Bonferroni corrections were made to adjust for multiple comparison tests. I ensured that the assumptions of normality and equal variance were met by examining fixed-effect residuals from each model using normal probability plots and residual versus predicted plots.

Simple linear regression was used to test whether toad movement behaviour was related to the change in mass of an individual toad within each trial (mass at completion of trial – mass upon release into trial) and multiple regression was used to compare daily average distances to mean air temperature and precipitation in the preceding 48 hours. Distance data for the multiple regression was cube-root transformed to meet assumptions of normality and equal variance of residuals. Paired t-tests were used to compare the proportion of time (arcsine square root transformed) spent in forest and matrix habitats. Only toads that completed the trials were included in these analyses.

Days to Exit Release Patches

Translocating toads into forest and clear-cut patches was performed to assess the ability of toads to move through these habitats. Comparing the number of days it took for a toad to exit these patches could give an indication of an individual's ability to move and in turn, provide information on the potential resistance of the landscape to movement (Castellón and Sieving 2006). To test the hypothesis that the number of days to exit a release patch would differ between the treatments, I also used mixed models. I looked for significant differences in the days to exit release patches between the two trials and between the two treatments within trials. The same models were used as described in the previous section with the number of days to exit the habitat as the response variable.

Cox's regression was used to compare exit times from release patches between the two treatments. This type of regression is appropriate for any "time to event" analysis, in my case, days before exiting the release patch (Castellón and Sieving 2006). Habitat type was used as an independent class variable, along with three other landscape variables that were predicted to influence exit times: patch size, distance to the nearest edge and percent forest cover in a 100 m buffer around the release site. The two experimental trials were analyzed separately to maintain the assumption of independence between data points. Before conducting the analyses, Spearman rank correlations were calculated to verify that none of the predictor variables were strongly correlated ($r > 0.7$).

All circular statistics were performed using R v 2.2.1 (CircStats package, copyright 2005, The R Foundation for Statistical Computing). Mixed effect models (PROC MIXED) and Cox's regression (PROC TPHREG) were conducted in SAS v 9.0 (SAS Institute, Cary, USA) and all other analyses were done in S-PLUS (Insightful Corp., Seattle, USA).

Results

Twenty-three toads were initially translocated to the Malcolm Knapp Research Forest where a total of 474 locations were recorded. Toads were radio-tracked for an average of 21.26 ± 0.1 (SE) days in trial 1 and for 17.61 ± 1.3 days in trial 2. Toads ranged in size from 72.1 to 95.9 mm (mean \pm SE = $83.83 \text{ mm} \pm 1.2$) and body mass varied from 40.62 to 103.3 g. The mean body mass of the toads upon release into their respective treatments was $65.31 \text{ g} \pm 2.97$ for trial 1 and $60.11 \text{ g} \pm 3.46$ for trial 2. Upon capture and removal from the experiment, the average mass was $60.98 \text{ g} \pm 3.34$ for trial 1 and $65.74 \text{ g} \pm 3.98$ for trial 2.

Movement Patterns

Toads spent proportionately more time in matrix habitat (mean \pm SE = 0.64 ± 0.05 days) than in forested habitat (0.36 ± 0.05 days). The same pattern emerged when the two trials were analyzed separately, yet these differences were not significant in trial 1 (paired $t = -1.414$, $df = 18$, $p = 0.174$, Figure 2.2) but were in trial 2 (paired $t = -2.4$, $df = 17$, $p = 0.028$, Figure 2).

Toad movement directions over the entire course of this study were not statistically random ($r = 0.529$, $n = 42$, $p < 0.001$) and were oriented around a mean angle of 44.84° (circular variance = 0.471). Comparing the two trials, the mean directions were still oriented to the northeast (trial 1: mean = 54.92° , $r = 0.487$, $n = 19$, $p = 0.007$; trial 2: mean = 36.01° , $r = 0.653$, $n = 18$, $p < 0.001$; Figure 2.3) and these directions were not statistically different ($U^2 = 0.089$, $n = 42$, $p > 0.05$).

Initial post-release movement directions (first 5 days) for toads released in the forest sites were statistically oriented towards the clear-cuts (Maple Ridge site: mean direction = 44.25° , $r = 0.412$, $n = 9$, $p = 0.04$; Wood Lot site: mean = 76.77° , $r = 0.548$, $n = 10$, $p = 0.006$; Figure 2.4). Within clear-cut sites, Rayleigh's test showed that initial movements were not statistically different from random (CC03: mean = 26.04° , $r = 0.246$, $n = 9$, $p = 0.593$; CC05: mean = 23.62° , $r = 0.316$, $n = 10$, $p = 0.379$; Figure 2.5). For these initial directions, movement was more linear in forest sites (mean vector length $r = 0.512$, $n = 19$) than in the clear-cut sites ($r = 0.282$, $n = 19$).

Irrespective of habitat type and trial, the greatest movement rate by an individual was 121.6 m/day and the lowest was 3.1 m/day (during one trial). For all toads, most bouts of movement were between 0 and 40 m/day (Figure 2.6). One individual moved a

total of 1999.3 m over 19 days whereas another only moved 65.9 m in 21 days. The highest single movement event in a period of approximately 24 hours was 982.1 m. The largest net displacement by a single toad was 1482.9 m and the smallest was 17.1m.

Averages of movement rate, maximum single movement and net displacement were all higher in forest habitat ($33.72 \text{ m} \pm 12.11$, $150.34 \text{ m} \pm 28.95$, $209.23 \text{ m} \pm 39.41$, respectively) than in matrix habitat ($20.19 \text{ m} \pm 4.1$ and $134.57 \text{ m} \pm 27.13$, $187.03 \text{ m} \pm 39.74$, respectively, Figure 2.7). These differences were not significantly different for maximum single movement ($F_{1,15} = 2.91$, $p = 0.109$) and for net displacement ($F_{1,15} = 2.76$, $p = 0.118$), but they were for movement rate ($F_{1,15} = 5.46$, $p = 0.034$). However, for 9 of 43 (21%) toad movement paths, a portion of the path occurred on forest roads. The average movement rate ($126.8 \text{ m} \pm 48.58$), average maximum single movement ($375.73 \text{ m} \pm 98.31$) and average net displacement ($431.94 \text{ m} \pm 106.51$) on roads were all higher than those in forest and matrix habitat (Figure 2.7). Movement rate and maximum single movement differed significantly between matrix and roads but not for net displacement (Table 2.1). Differences between forest and road were not statistically different for maximum single movement and net displacement, but there was a difference in movement rate (Table 2.1). In all cases, there was no significant difference between the two trials (movement rate: $F_{1,37} = 3.40$, $p = 0.073$; max single move: $F_{1,37} = 1.08$, $p = 0.306$; net displacement: $F_{1,37} = 0.83$, $p = 0.368$).

Toad movement patterns were not associated with body size or climate. There was no relationship between daily movement rates and the change in body mass of individual toads (trial 1: $R^2 = 0.078$, $F_{1,17} = 1.453$, $p = 0.245$; trial 2: $R^2 = 0.165$, $F_{1,16} =$

3.154, $p = 0.095$). Daily movement was also unrelated to both temperature and precipitation in the preceding 48 hours ($R^2 = 0.035$, $F_{2,35} = 0.628$, $p = 0.539$, Figure 2.8).

Days to Exit Release Patches

Of the 23 toads initially released, 20 were followed for the duration of the first trial, of which 15 (75%) left their release patches and dispersed into the surrounding landscape. In trial 2, 18 toads completed the experiment, of which 17 (94.4%) dispersed from their initial patches. Over both trials, mean (\pm SE) time to exit the release patch was longer in the clear-cut treatment (11.38 days \pm 1.92) than in the forest treatment (5.53 days \pm 1.2) and this difference was statistically significant ($F_{1,15} = 18.34$, $df = 15$, $p < 0.001$, Figure 2.9). These patterns were consistent in both trial 1 (clear-cut = 17.11 days \pm 1.68, forest = 7.5 days \pm 2.03) and trial 2 (clear-cut = 5.4 days \pm 2.05, forest = 3.33 days \pm 0.75). Within trial 1, there was a significant difference in times to exit between the two treatments ($F_{1,17} = 12.93$, $p = 0.002$, Figure 2.9) but not in the second trial ($F_{1,16} = 0.94$, $p = 0.347$, Figure 2.9).

Spearman rank correlations found a significant negative association between habitat type and the percent forest cover in a 100 m buffer at each site ($r = -0.89$, $p < 0.001$), so the forest cover variable was dropped from the models. All other correlations were not strongly associated with habitat type ($r < 0.7$). Cox's regression showed that the habitat type of the release patch (forest or clear-cut) was a significant predictor of time to exit in trial 1 (Wald $X^2 = 8.92$, $p = 0.003$) but not in trial 2 (Wald $X^2 = 0.719$, $p = 0.396$). Patch area was also significant in predicting time to exit in trial 1 ($X^2 = 7.19$, $p = 0.007$) but the nearest edge was not ($X^2 = 2.15$, $p = 0.142$). In trial 2, none of the predictor variables were significant in predicting time to exit.

Discussion

Overall, these results indicate that small, recent clear-cuts did not act as movement barriers for adult western toads. I showed that a toad's perceptual range may reach at least 150 meters and that the boundary permeability between forests and clear-cuts was quite high. My results indicated that movement was not significantly restricted in this matrix habitat and therefore landscape connectivity for the western toad may not be seriously affected by forest harvesting.

Contrary to expectation, adult toads seemed to show an attraction for clear-cuts and actively chose to enter these habitats from neighbouring forested environments. The affinity for clear-cuts was evident by the initial post-release movements of toads in forested sites, in which the majority of toads directed themselves towards the forest/clear-cut edge. Even though the clear-cut sites had slash piles along the forest/clear-cut edges, greater than 60% of toads released in the forest sites did eventually enter the matrix habitat, although this was not proportional to habitat availability. Thus, I conclude that the permeability of the forest/clear-cut edge was high, even with the increased resistance provided by the slash piles. To my knowledge, this is the first study to show that western toads chose to enter and stay in recent clear-cuts (< 5 years), apparently favouring this habitat over adjacent second-growth forests.

These results also contribute to knowledge on the perceptual range of adult western toads, possibly the first to document any such measurements for this species. Toads released in forest sites did not orient themselves as expected by chance alone and therefore I conclude that they were able to perceive the clear-cut edge from up to 150 m away. However, toads released in clear-cut sites did not orient themselves in a specific

direction. Although it is well known that amphibians can orient themselves toward their native ponds (Sinsch 1990), experimental examinations of their perceptual abilities in relation to terrestrial habitat characteristics are scarce (Rothermel 2004). Rothermel (2004) concluded that juvenile salamanders did not exhibit target-oriented behaviour, even as close as 5 m to a forest edge. In contrast, Mazerolle and Desrochers (2005) found that adult frogs were able to orient back to a pond from 70 m away and Rittenhouse and Semlitsch (2006) demonstrated that adult salamanders could orient themselves towards forested habitat during migratory movements. Even though toads in my experiment did show significant orientation towards specific habitat types, I cannot dismiss the possibility of random walk events given the small size of habitat patches.

In terms of landscape resistance, the majority of movement patterns were not significantly reduced in the matrix habitat. Although all movement parameters were lower in the matrix than in forested habitat, toads were able to move through this environment, indicating that the permeability of clear-cuts was relatively high for western toads. If clear-cuts significantly restricted movement rates, I would have expected toads to take much longer to leave these release patches (Castellón and Sieving 2006). Although toads did in fact take longer to exit, this observation was likely due to the toad's preference for this environment and not an inability to move through the matrix. For both movement rates and the number of days to exit release patches, there were cases where specific toads moved through the matrix at rates equal and even greater than those recorded in forested habitats and on roads, providing evidence that lower movement rates and longer exit times in the matrix was not necessarily due to reduced permeability.

Many recent studies have tested movement behaviour in various matrix habitats, with some showing decreased movement ability and others showing no difference, solidifying the claim that matrix permeability is species specific (Ricketts 2001). Although few studies have looked at matrix permeability in amphibians, this claim appears to hold true. Similar to my results, Patrick et al. (2006) found that clear-cuts did not present greater resistance and were not significant barriers to juvenile wood frogs (*Rana sylvatica*). Stevens et al. (2004) found that the movement ability of juvenile natterjack toads (*Bufo calamita*) was best on surfaces mimicking sandy soils and roads, whereas performance was poorest in forest environments. On the contrary, Chan-McLeod (2003) found that clear-cuts acted as barriers to movement for adult red-legged frogs (*Rana aurora*). Furthermore, Mazerolle and Desrochers (2005) concluded that anthropogenically-disturbed areas, especially those devoid of cover, increased landscape resistance for adult northern green frogs (*Rana clamitans melanota*) and northern leopard frogs (*Rana pipiens*). However, in this study, toad movement was not significantly inhibited in the matrix habitat and I thus conclude that small, recent clear-cuts provide little resistance to movement and that sandy and graveled roads facilitated movement.

Forest fragmentation also occurs through road construction and a major by-product of forest harvesting practices is the creation of logging roads to access clear-cuts. Through both direct effects, such as road kill, and indirect effects, like the facilitation of predator movement and ingress of invasive species and diseases, roads are rarely shown to have a positive effect (Forman and Alexander 1998). For amphibians, diversity and abundance is known to decline in relation to proximity to roads (Fahrig et al. 1995, Marsh et al. 2005), even on low traffic and abandoned logging roads (Semlitsch et al.

2007). However, I suggest that low traffic and abandoned logging roads may actually be beneficial for western toads. Logging roads may serve as movement corridors for toads and assist movement through the landscape. All movement parameters measured in this study were higher on logging roads, suggesting that they were facilitating movement ability, possibly encouraging dispersal and helping maintain connectivity in the landscape. Every toad that encountered a road would stay on or near the road, where they would either bask along its edges and burrow in the ditches, or use the roads to move long distances. The longest movement bouts recorded during this study occurred on roads, including one individual that moved 982 m in approximately 24 hours. Other studies have found that amphibians use ditches for movement and prefer cement substrates when given a choice (Mazerolle 2004, Stevens et al. 2006). In this study, toads were observed using the roadside culverts or verges for movement and not the actual road surface. Since road mortality in toads and other amphibians is generally high (Hels and Buchwald 2001), it is uncertain whether the cost of mortality would outweigh the benefits of increased movement through landscapes and better connectivity between populations.

Although the western toad is often thought of as a forest associated species, it has been found in a variety of habitats (Bartelt 2000, Davis 2000). Most toad species are known to enjoy basking and burrowing in sandy soils. Recent clear-cuts may potentially present a very suitable environment for this species, with ample sunlight, sandy soils, many potential refugia, and a high abundance of invertebrates (Niemela et al. 1993, Pawson et al. 2006). Davis (2000) found that adult toads seemed to prefer young regenerating forests created by logging (~10-15 years) to closed canopy forests and Bartelt et al. (2000) found that both males and females selected sites in clear-cuts 10-15

years old. Tocher et al. (1997) found four frog species migrating into and colonizing matrix habitats following logging in an Amazonian forest. Other studies have found that certain amphibians do not avoid heavily disturbed areas, such as the California red-legged frog, *Rana draytonii* (Bulger et al. 2003), and American bullfrogs, *Rana catesbeiana* (Patrick et al. 2006). Although toads are less sensitive to desiccation than other amphibians and can tolerate the higher temperatures and lower moisture in clear-cuts (Stebbins and Cohen 1995), I believe clear-cuts may be favourable during the early summer months only. Later in the season, this habitat could potentially be very inhospitable when temperatures are extremely high and there is very little precipitation, resulting in a significant risk of desiccation for toads. A temperature logger placed in a clear-cut site (CC05) for the duration of the study recorded temperatures above 35°C for four consecutive days in July 2006 and study sites received only 44 mm of rain during July and August combined. Davis (2000) did find western toads to be relatively inactive in the dry, late summer, remaining in moist terrestrial microhabitats or in water. Since my study did not follow toads for the duration of the summer and I did not consider physiological states (e.g. dehydration) of toads during their time spent in the clear-cuts, further research is needed before any definitive conclusions can be made in terms of the suitability of clear-cuts for western toads.

Amphibian movement is often associated with environmental conditions, such as precipitation and humidity (Preest and Pough 1989). Here, I found no relationship between movement rates and daily mean temperatures and precipitation levels. In contrast, other studies have found that precipitation and temperature are strong determinants of anuran movement in disturbed landscapes (Mazerolle 2001, Johnston and

Frid 2002, Chan-McLeod 2003). However, Rothermel (2004) found that juvenile American toad (*Bufo americanus*) movements were not constrained by rainfall. Several studies have shown that bufonids are more resistant to desiccation, and may therefore have a greater tolerance to the climatic conditions found in recent clear-cuts (Todd and Rothermel 2006).

The majority of studies on the ecological impacts of clear-cut harvesting occur at spatial scales of less than 10 ha (Pawson et al. 2006) and this experiment is no exception. In British Columbia, clear-cuts are on average about 23 ha (BC Market Outreach Network 2004), whereas the clear-cuts used in my study were all less than 5 ha. Toads showed the ability to move 500 m in a short period of time (1 to 2 days) and were easily able to cross the small clear-cuts used in this study. However, large clear-cuts typically found in British Columbia may pose a much greater obstacle for toads and would require extended periods of time in this matrix habitat. At this scale, clear-cuts are predicted to be much less permeable and represent significant barriers to movement. There is the possibility of a clear-cut threshold size, under which the ecological impacts of clear-cuts are limited (Pawson et al. 2006), yet this has not been investigated. Overall, results at small spatial scales are unlikely to be applicable at larger scales.

Despite this limitation, these study results suggested that clear-cuts may not be movement barriers to adult western toads and may actually provide suitable habitat for this species. I showed that edge and clear-cut permeability was high and as such, forest fragmentation may not be interfering with landscape connectivity. Data on movement abilities in these different habitats can be used to parameterize spatially explicit movement models and metapopulation models (e.g. least-cost analysis, RAMAS-GIS).

My study contributes to the overall understanding of how disturbed habitats influence movement behaviour, which ultimately leads to a better understanding of the impacts of habitat fragmentation on population persistence.

Table 2.1. Summary of the differences in movement behaviours based on least squares means analyses for the three habitat types (forest, matrix, road). Applying a Bonferroni correction for multiple comparisons (3), the significance level is 0.017.

| Response Variable | Comparison | DF | t-value | p-value |
|--------------------------|-------------------|-----------|----------------|-------------------|
| Movement Rate | Forest vs. Matrix | 21 | 2.65 | 0.015 |
| | Forest vs. Road | 21 | -2.70 | 0.014 |
| | Matrix vs. Road | 21 | -4.82 | < 0.001 |
| Maximum Single Movement | Forest vs. Matrix | 21 | 2.09 | 0.049 |
| | Forest vs. Road | 21 | -1.35 | 0.193 |
| | Matrix vs. Road | 21 | -2.98 | 0.007 |
| Net Displacement | Forest vs. Matrix | 21 | 1.93 | 0.068 |
| | Forest vs. Road | 21 | -0.97 | 0.345 |
| | Matrix vs. Road | 21 | -2.47 | 0.022 |

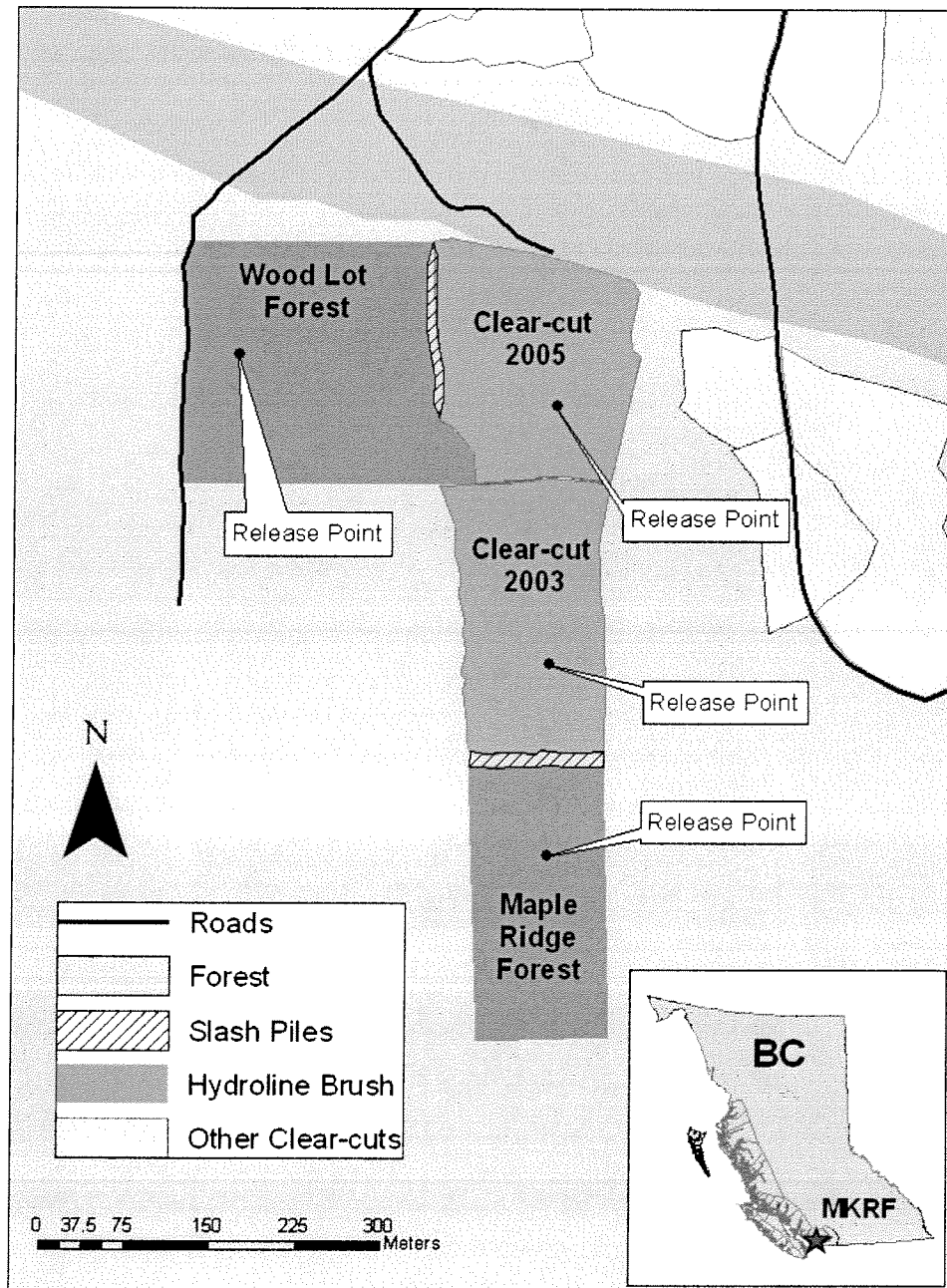


Figure 2.1. Map of the Malcolm Knapp Research forest in SW British Columbia, Canada, showing the location of the four experimental patches and release sites for toads.

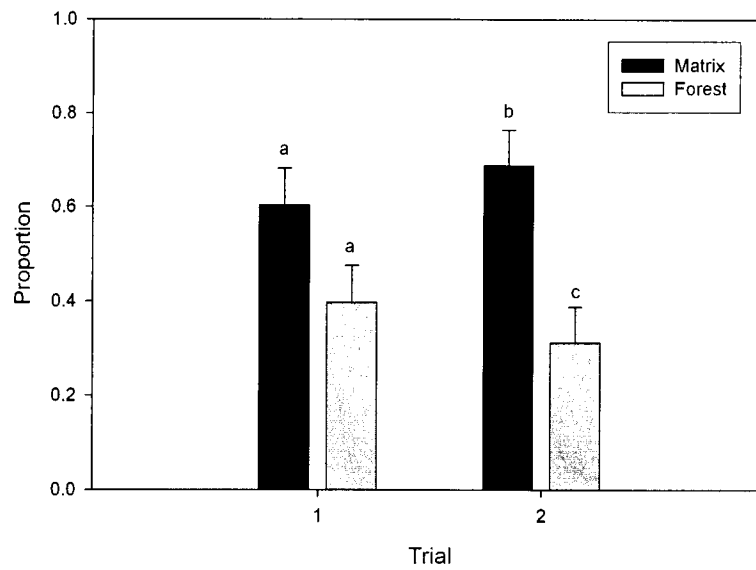


Figure 2.2. Proportion of time spent by radio-marked toads in the two habitat types in trial 1 (n = 19) and trial 2 (n = 18).

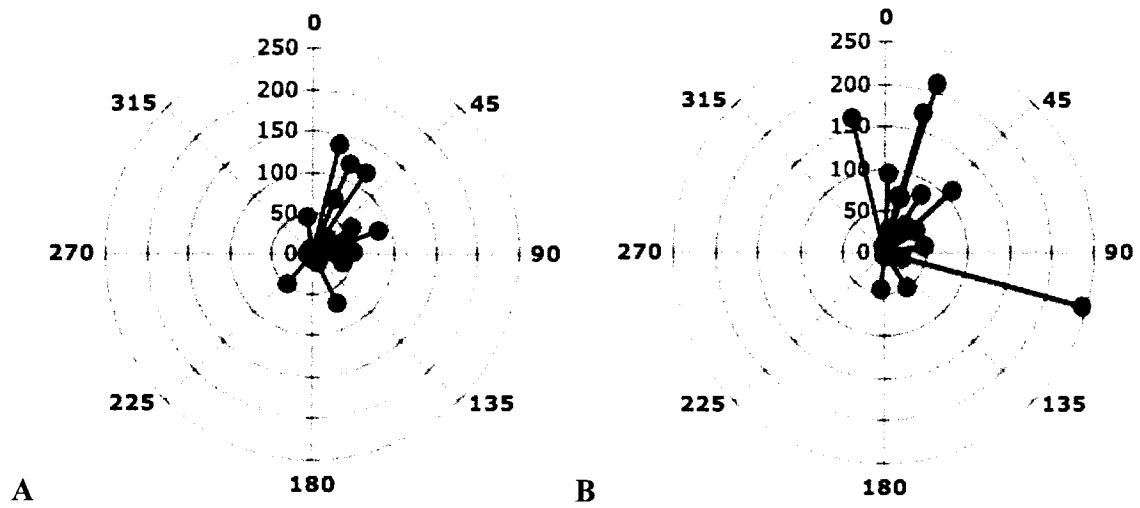


Figure 2.3. Average distances and directions for toads released in either forests or clearcuts in trial 1 (A, $n = 19$) and trial 2 (B, $n = 18$). Each dot represents an individual toad and lines are the average distance moved from its release point regardless of habitat type.

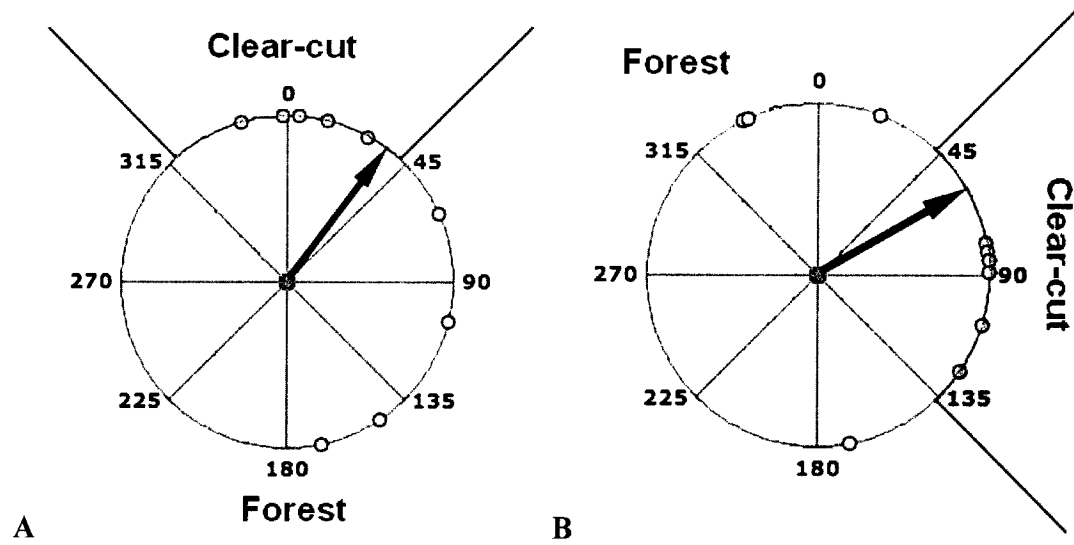


Figure 2.4. Initial directions (first 5 days) of toads released in forest sites: A) Maple Ridge ($n = 9$), B) Wood Lot ($n = 10$). The centre point represents the release point for individual toads (A = 90 m from edge, B = 150 m from edge) and the arrow represents the mean angles of movement (Maple Ridge = 44.25° , Wood Lot = 76.77°).

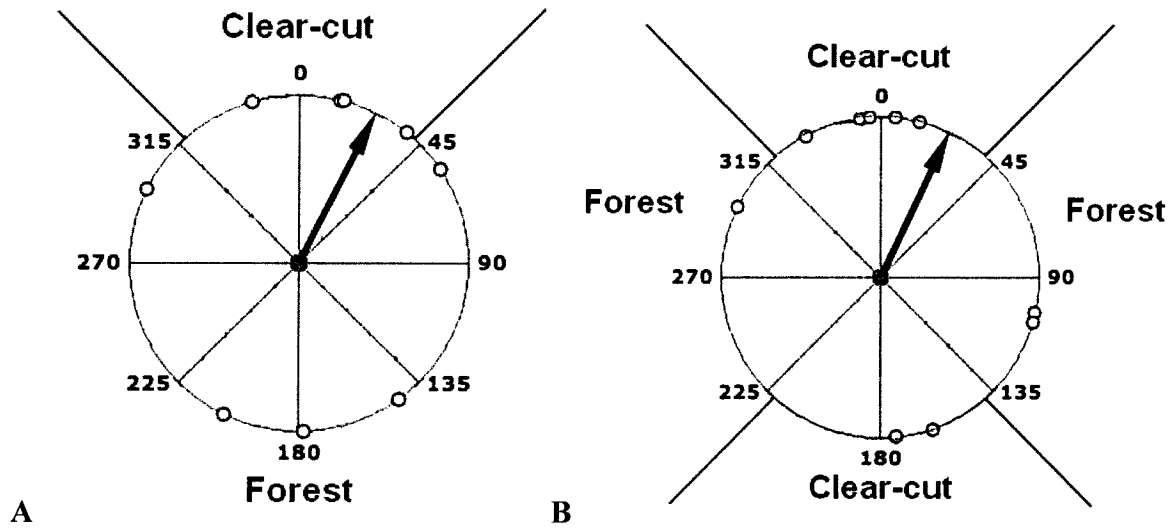


Figure 2.5. Initial directions (first 5 days) of toads released in clear-cut sites: A) CC03 ($n = 9$), B) CC05 ($n = 10$). The centre point represents the release point for individual toads (A = 60 m from nearest edge, B = 60 m from nearest edge) and the arrow represents the mean angles of movement (CC03 = 26.04° , CC05 = 23.62°).

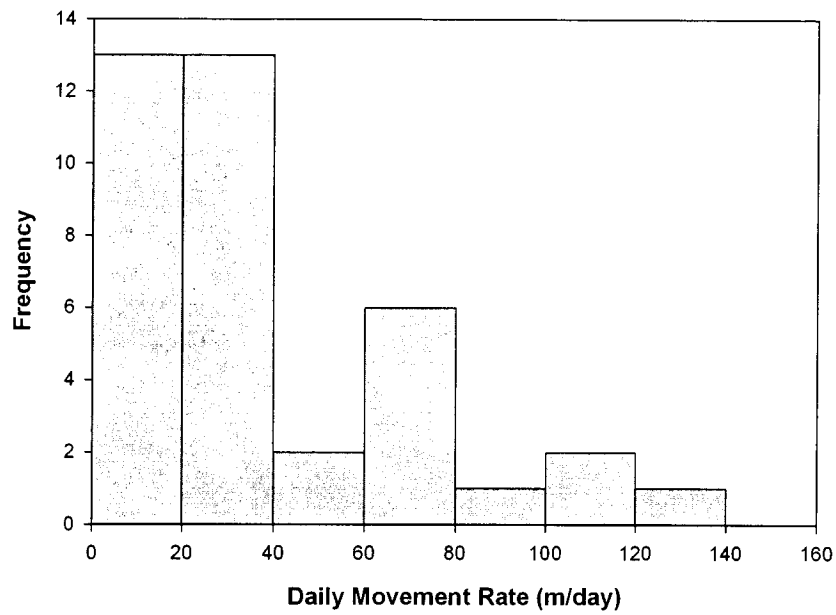


Figure 2.6. Distribution of average daily movement rates for all toads in the entire experiment ($n = 38$).

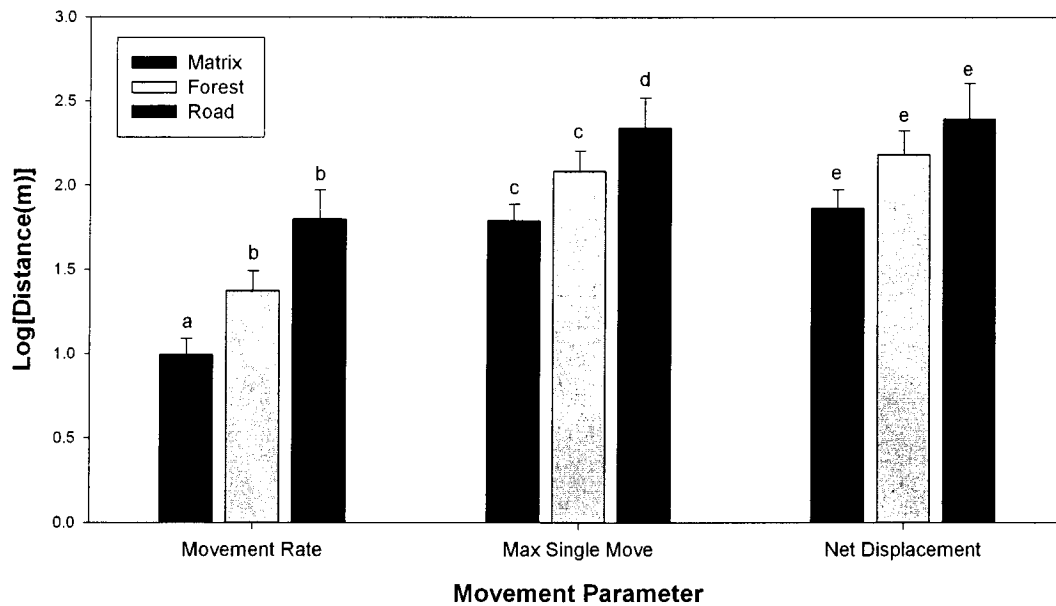


Figure 2.7. Average movement rate, maximum single movement and net displacement (± 1 SE) for the three habitat types, matrix ($n = 32$), forest ($n = 20$) and road ($n = 9$). The two experimental trials are combined and data are log transformed. Each movement parameter was analyzed separately from the others, so letters represent significant differences within parameters only.

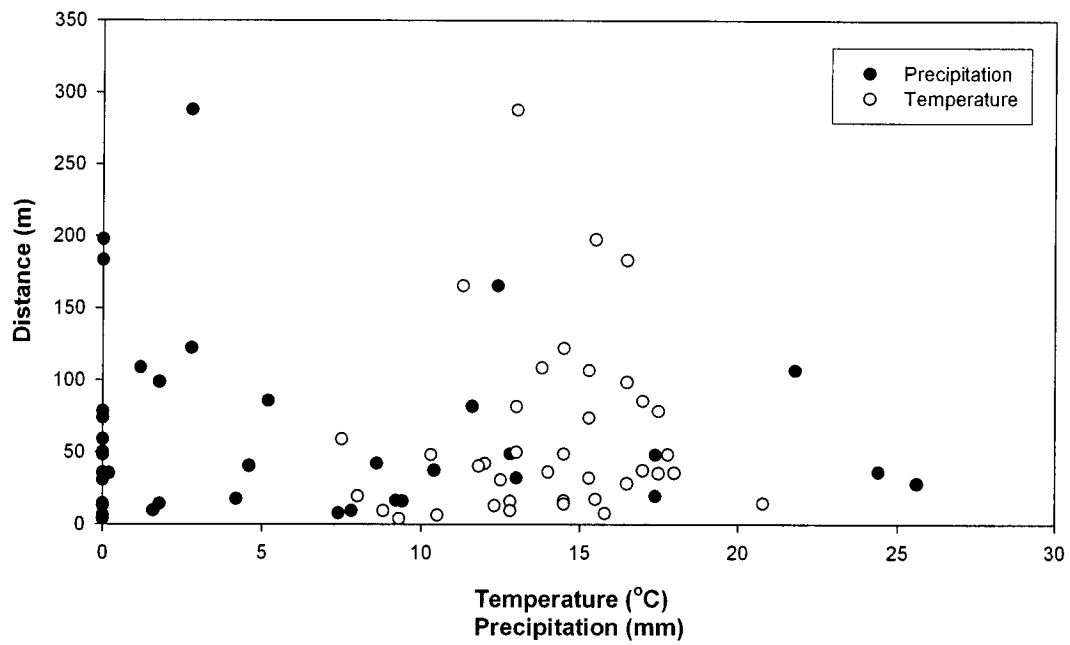


Figure 2.8. Relationship between the average daily distance, average daily air temperature ($^{\circ}\text{C}$, open circles) and total precipitation (mm, closed circles) in the previous 48 hours between May 4th and June 16th, 2006.

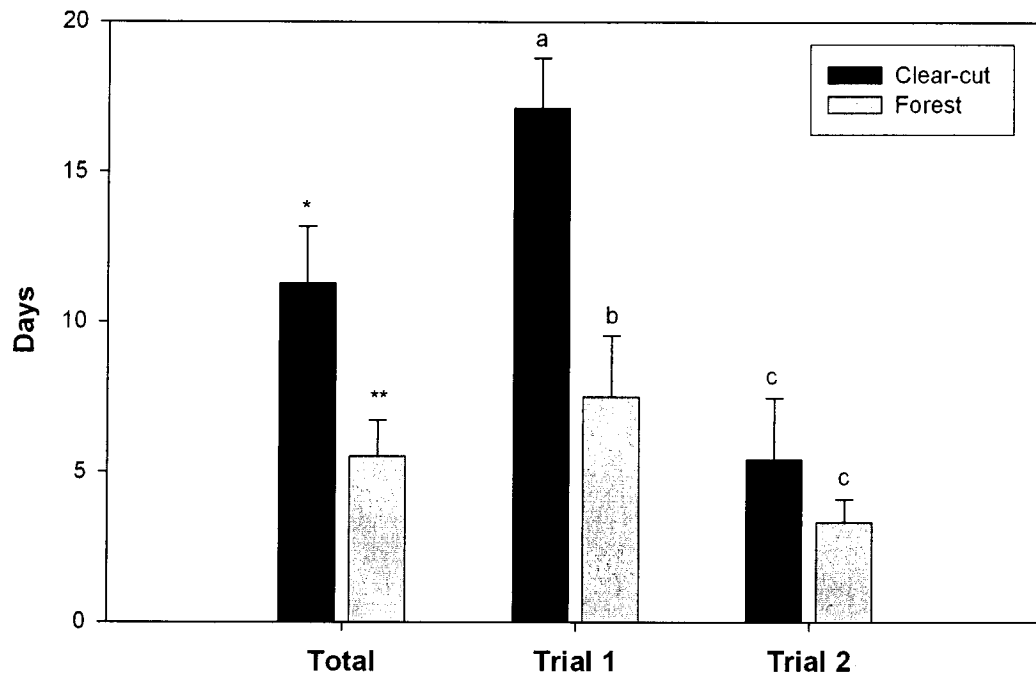


Figure 2.9. Number of days toads spent in their release patches (trial 1: $n = 19$, trial 2: $n = 18$) before dispersing into surrounding landscape (means \pm 1SE). Data for the two trials were combined ('Total') and then analyzed separately by trials. Letters represent significant differences within each separate analysis.

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CHAPTER 3: PREVALENCE OF THE PATHOGENIC CHYTRID FUNGUS (*Batrachochytrium dendrobatidis*) IN WESTERN TOADS (*Bufo boreas*) FROM A PROVINCIAL PARK IN BRITISH COLUMBIA, CANADA²

Introduction

Amphibian populations have been declining around the globe and recent research suggests this phenomenon is more serious than originally reported (Alford and Richards 1999, Houlahan et al. 2000, Stuart et al. 2004). Nearly half of all amphibian species are experiencing some form of population decline, making amphibians one of the most threatened groups of vertebrates, more so than even birds and mammals (Stuart et al. 2004). Species are even declining in relatively undisturbed habitats and protected areas around the world (Pounds et al. 1997, Berger et al. 1998, Lips et al. 1998, Bosch et al. 2001, La Marca et al. 2005, Lips et al. 2006).

Although significant amphibian losses have resulted from familiar and obvious threats such as habitat loss and invasive species, disease has recently emerged as a suspected major contributor to global declines (Daszak et al. 1999, Collins and Storfer 2003, Stuart et al. 2004). Specifically, the emergence of a pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*, and its associated pathology, chytridiomycosis, has been attributed as the proximate cause of several mass mortality events (Berger et al. 1998, Bosch et al. 2001, Carey 2000, Muths et al. 2003, Lips et al. 2006).

Chytridiomycosis is a cutaneous infection caused by a zoosporic chytrid fungus. The fungus reproduces via waterborne zoospores and attacks keratinized cells of the

² A version of this chapter will be submitted for publication. Deguise, I. and Richardson J.S. Prevalence of the pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*) in Western toads (*Bufo boreas*) from a provincial park in British Columbia, Canada.

amphibian epidermis, both in post-metamorphic amphibians (skin) and larvae (mouthparts) (Longcore et al. 1999).

One of the species that is known to be susceptible to chytrid fungus infection is the western toad, *Bufo boreas*. Although widely distributed from Baja California up to Alaska, the western toad has suffered severe population declines in the southeastern part of their range (Carey 1993, Muths et al. 2003). *B. dendrobatidis* has been identified in western toads in Colorado, Wyoming, Oregon and Washington (Muths et al. 2003, S. Wagner, pers. comm.) and mass mortality events have been attributed to chytridiomycosis (Muths et al. 2003, Scherer et al. 2005). Blaustein et al. (2005) found that larval western toads were highly susceptible to *B. dendrobatidis* and experienced increased mortality. However, there are no reports of chytrid infection in western toads in Canada.

In this study, I tested a population of breeding western toads from a protected area in southwestern British Columbia, Canada, for the presence of *B. dendrobatidis*. My objective was to determine if the chytrid fungus was present in toads in this relatively pristine area. In addition, I wanted to test the hypothesis that body condition of adult toads is related to infection prevalence (Woodhams and Alford 2005).

Methods

Study Site

This study took place in Alice Lake Provincial Park (Figure 3.1), near Squamish, BC (49° 46' N, 123° 06' W, elevation 275m) within the Coastal Western Hemlock biogeoclimatic zone. The park is 396 ha and composed primarily of Western hemlock (*Tsuga heterophylla*), along with Douglas-fir (*Pseudotsuga menziesii*) and Western red

cedar (*Thuja plicata*). Toads were captured at Edith Lake, a location accessible only by hiking trails and a private access road (C. Platz, pers. comm.). The lake is approximately 25 ha, with a mean depth of 6.1 m and maximum depth of 13 m. In 1950, the lake was stocked with Rainbow trout and with Brook trout in 1965 (B.C. Ministry of Environment Fisheries Inventory Data). Toads were found breeding in the northern corner of Edith Lake, in a sandy shallow area approximately 2 m² and 0.3 m deep. Riparian and aquatic vegetation included salal (*Gaultheria shallon*), sedges (*Carex* spp.), and young willows (*Salix* spp.).

Testing Procedures

A total of 32 adult male toads were removed from Edith Lake and kept in individual containers to avoid cross-contamination. All individuals were thoroughly checked for visual signs of chytridiomycosis, including lethargy, redness on the legs and sloughing skin (Berger et al. 1998, Ouellet et al. 2005). Toe clips were taken from each toad using a simple numeric system (Hero 1989) and stored in 70% ethanol in individual 2 ml screw-cap tubes. Scissors were sterilized in 70% ethanol and flamed between toe-clipping of each toad to minimize the chance of disease transfer between toads and destroy any residual DNA. Gloves were also used by the handler and changed after every clip. Toe clips were transported in a cooler to the British Columbia Animal Health Centre in Abbotsford, BC. Toe clips were tested for the presence of *B. dendrobatidis* using established PCR techniques. All equipment was thoroughly disinfected in a 5% bleach solution.

Statistical Analysis

Upon capture, all toads were weighed to the nearest gram and snout-to-vent length was measured to the nearest mm. The mass to length ratio was used as a measure of body condition (Woodhams and Alford 2005). A t-test was used to look for significant differences between mass and infection status of toads. To determine the relationship between infection status and body condition, a logistic regression was conducted. PROC TTEST and PROC LOGISTIC in SAS v 9.0 was used for statistical analyses (SAS Institute, Cary, USA).

Results

A total of 32 toads were tested in this study, of which 9 individuals (28.1%) tested positive for the presence of *B. dendrobatidis* (Table 3.1). None of the toads showed any visual signs of infection and appeared healthy. Toads who tested positive for chytrid had a lower mean mass ($n = 9$, mean = 59.7, SE = 3.2) than those that tested negative ($n = 23$, mean = 60.8 ± 2.9) but this difference was not statistically significant ($t = 0.203$, $df = 30$, $p = 0.84$). The mean body condition for toads that tested negative for the chytrid fungus was only slightly higher ($n = 23$, mean = 0.719, SE = 0.025) than those who tested positive ($n = 9$, mean = 0.701 ± 0.029 , Figure 3.2). Logistic regression showed no significant relationship between body condition and infection status ($r^2 = 0.005$, Wald $X^2 = 0.164$, $df = 1$, $p = 0.6851$).

Discussion

These results provide the first evidence for the presence of chytrid fungus in western toads in Canada. Given a 28% infection rate in a relatively pristine provincial

park, the prevalence of this disease in western toads is likely more widespread than currently reported. The chytrid fungus has been documented as the proximate cause of declines in other *B. boreas* populations (Muths et al. 2003). Yet, numerous studies suggest that a complexity of causative agents is responsible for the decline in amphibian populations (Alford and Richards 1999, Ouellet et al. 2005, Garner et al. 2006). Given that toads have been present at this site for numerous years but that no mass mortality events have been reported (L. Dupuis, pers. comm.), these results lend support to the hypothesis that chytrid is naturally occurring in wild amphibian populations and that lethal outbreaks are triggered by other factors (Blaustein & Kiesecker 2002).

It is not entirely surprising that the chytrid fungus is present in western toads in Canada. Ouellet et al. (2005) found that chytrid infection was widespread in various amphibians across Canada and the United States. They document infection in 13.1% of amphibian individuals from 12 common species but did not find any evidence of infection in *B. boreas* (Ouellet et al. 2005). Garner et al. (2006) found very high prevalence levels of *B. dendrobatidis* in bullfrogs (*Rana catesbeiana*) on Vancouver Island, BC. Therefore, further testing of toads throughout its range is needed to properly document the extent of chytrid infection in this vulnerable species.

The vulnerability of amphibians to infections can be related to body condition, a proxy for individual health (Woodhams and Alford 2005). In this study, body condition was not significantly different between infected and uninfected toads, and there was no relationship between body condition and infection prevalence. These results are similar to those by Woodhams and Alford (2005), who found no correlation between infection status and body condition. However, since body condition is known to affect

susceptibility levels to various stressors (Brown & Brown 1998), this hypothesis warrants further investigation.

Although western toads appear to be faring well in Canada, accurate accounts of population trends are unavailable and declines have been noted in areas where toads historically existed (Wind and Dupuis 2002, I. Deguise and S. Wood, unpublished data). Given the susceptibility of *B. boreas* to chytrid infection (Blaustein et al. 2005) and the fact that the prevalence of chytridiomycosis can increase very rapidly (Muths et al. 2003, Lips et al. 2006), the population of western toads at Edith Lake and surrounding areas should be monitored closely. The status of *B. boreas* is not well known in Canada (Wind and Dupuis 2002), and systematic surveys are needed to document not only the presence of this species but its health in our ever-changing environment.

Table 3.1. Summary of testing results for western toads from Edith Lake, B.C. Infection status of 1 indicates a chytrid-positive PCR test, 0 is negative.

| Toad | Mean Mass (g) | Body Condition | Infection Status |
|------|---------------|----------------|------------------|
| 1 | 57.86 | 0.68 | 0 |
| 2 | 37.50 | 0.52 | 0 |
| 3 | 44.10 | 0.57 | 1 |
| 4 | 50.95 | 0.61 | 1 |
| 5 | 62.51 | 0.73 | 1 |
| 6 | 55.11 | 0.66 | 0 |
| 7 | 60.34 | 0.72 | 0 |
| 8 | 52.75 | 0.65 | 0 |
| 9 | 67.31 | 0.74 | 1 |
| 10 | 93.32 | 0.97 | 0 |
| 11 | 58.50 | 0.72 | 1 |
| 12 | 50.25 | 0.61 | 0 |
| 13 | 68.50 | 0.84 | 0 |
| 14 | 100.35 | 1.08 | 0 |
| 15 | 54.81 | 0.69 | 0 |
| 16 | 59.25 | 0.72 | 0 |
| 17 | 78.70 | 0.86 | 0 |
| 18 | 61.39 | 0.72 | 1 |
| 19 | 56.12 | 0.69 | 0 |
| 20 | 53.25 | 0.70 | 0 |
| 21 | 56.90 | 0.67 | 0 |
| 22 | 57.40 | 0.71 | 0 |
| 23 | 68.35 | 0.73 | 0 |
| 24 | 53.20 | 0.65 | 0 |
| 25 | 66.97 | 0.76 | 0 |
| 26 | 54.50 | 0.65 | 1 |
| 27 | 59.71 | 0.69 | 0 |
| 28 | 77.31 | 0.87 | 1 |
| 29 | 61.10 | 0.71 | 1 |
| 30 | 54.32 | 0.68 | 0 |
| 31 | 52.01 | 0.66 | 0 |
| 32 | 50.75 | 0.61 | 0 |

This figure has been removed due to copyright reasons. The original map can be found on the British Columbia Parks website:
http://www.env.gov.bc.ca/bcparks/explore/parkpgs/alice_lk/alicelake.pdf

Figure 3.1 Location of toad breeding site in Alice Lake Provincial Park, British Columbia.

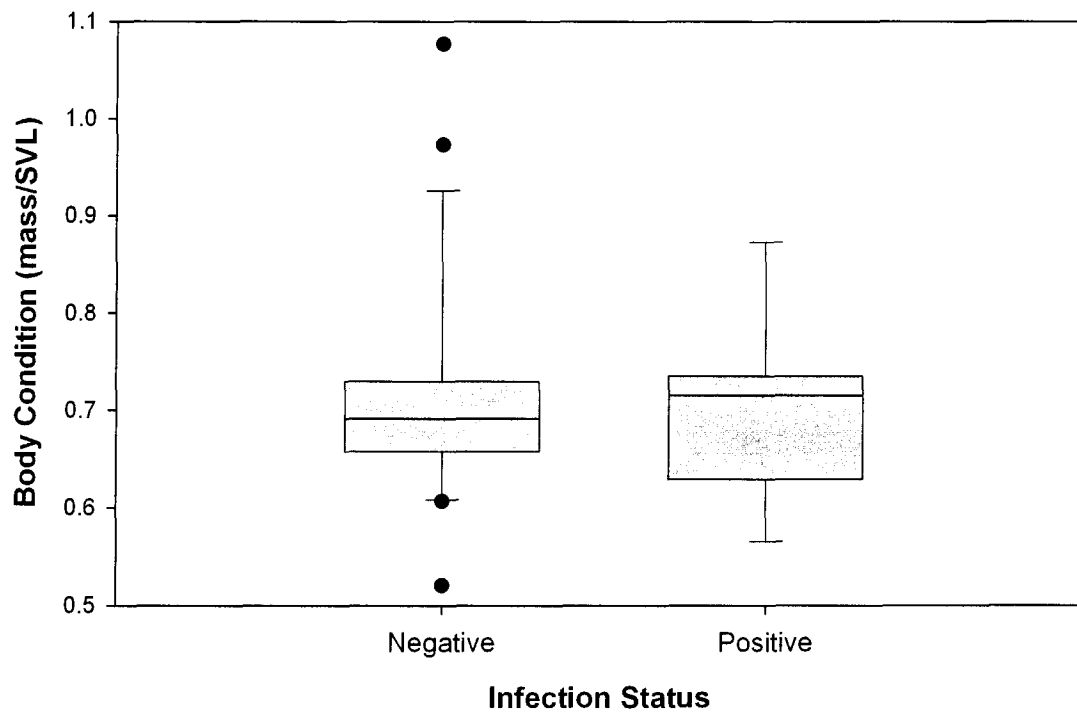


Figure 3.2 Box plot showing body condition of toads that tested negative for the chytrid fungus ($n = 23$) or positive ($n = 9$). The center line in each box represents the median, lines are the first and third quantiles, while dots are outliers.

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CHAPTER 4: GENERAL DISCUSSION AND CONCLUSIONS

Thesis Summary

Habitat fragmentation and disease are considered to be two of the major threats facing amphibian populations around the globe (Alford and Richards 1999, Collins and Storfer 2003). Population declines due to these two factors have been well documented for a North American species, the western toad, in the southern portion of its range, from Washington State to New Mexico (Carey 1993, Muths et al. 2003, Hammerson et al. 2004). Although approximately half of the range of this species is in Canada, little research has been conducted in British Columbia (Wind and Dupuis 2002). In this thesis, I examined movement behaviours in different habitat types and tested for the prevalence of an infectious disease in a breeding population within a provincial park.

Habitat Fragmentation

My original objective was to determine how forest fragmentation influences movement behaviour in adult western toads. I found that movement patterns, such as movement rate, were not significantly different in disturbed areas versus forested areas. Small, recent clear-cuts did not act as movement barriers for adult western toads, made apparent by the ease with which toads entered and moved within clear-cuts. Therefore, I conclude that these disturbed areas are unlikely to reduce landscape connectivity for toads and that movement between suitable habitat patches or populations can still occur. In addition, I found that edges were highly permeable to toad movement and that roads facilitated movement through the landscape. Logging roads are likely acting as movement corridors for amphibians, as long as direct mortality caused by vehicular

traffic does not out-weigh the benefits of increased movement through landscapes. Lastly, I conclude that these small, young clear-cuts are favoured habitat for adult western toads during the early summer. These environments provide warm and abundant food, as well as ample refugia.

As mentioned in my introduction, metapopulation dynamics are often applied when studying the impacts of habitat fragmentation and establishing conservation measures (Hanski 1998, Marsh and Trenham 2001, Smith and Green 2005). A critical component of metapopulation dynamics is the ability of individuals to move between isolated habitat fragments and these dispersal abilities are often assumed to be poor in amphibians (Marsh and Trenham 2001). In addition, the metapopulation framework assumes that the matrix habitat interferes with movement between suitable habitat patches (Ricketts 2001, Goodwin and Fahrig 2002). However, neither of these factors appears to hold true for western toads. Results from this study found toads to be quite mobile and able to move over 1 km in a couple of days, including through the matrix habitat. Given these findings, it appears that western toads may not be as restricted by habitat fragmentation as other amphibian species. However, further research needs to take place before any affirmative statements can be made.

Chytrid Fungus in BC

The chytrid fungus has received widespread attention in the recent literature due to its involvement in massive amphibian declines around the globe (Berger et al. 1998, Bosch et al. 2001, Carey 2000, Muths et al. 2003, Lips et al. 2006). As the western toad has been found to be highly sensitive to infection (Muths et al. 2003, Blaustein et al. 2005), a second major objective of my thesis was to look for evidence of this pathogen in

toads in British Columbia. Results of tests from a population in a protected area showed a 28% infection rate. To my knowledge, I am the first to report the presence of *Batrachochytrium dendrobatidis* in western toads in Canada. These results imply that the chytrid fungus is more geographically widespread in this species than currently documented.

There are two broad groups of hypotheses related to disease-mediated amphibian population declines. The first suggests that observed pathogens are novel and the second claims that other factors are triggering disease emergence (Blaustein and Kiesecker 2002). Although some researchers have claimed that chytridiomycosis is a novel disease (Morell 1999), it appears more likely that cofactors are involved in its recent emergence. Surveys of museum specimens detected chytrid in amphibians before widespread population declines occurred (Ron & Merino 2000, Ouellet et al. 2005). In addition, Pounds et al. (2006) found a strong relationship between global warming and disease emergence. Contaminants in the environment have also been suggested as an indirect cause of declines (Carey 1993). These factors could be weakening amphibian immune systems, making them more susceptible to infection by diseases.

Given that none of the toads studied here showed any physical signs of the disease and that evidence of massive die-offs have not been reported (C. Platz, pers. comm.), it seems likely that chytrid is naturally occurring in western toads in British Columbia. If cofactors are indeed triggering the emergence of chytridiomycosis, it may be only a matter of time before we begin to see mass mortality events in Canada.

Implications

Western toad populations have been experiencing significant declines in the wild and it is clear no single cause can explain these lethal outbreaks (Carey 1993, Blaustein et al. 1994, Muths et al. 2003, Scherer et al. 2005, Carey et al. 2005). Although I have not shown a direct negative impact of habitat fragmentation, it does not mean that this stressor is not affecting overall populations. Obviously, habitat loss is an important contributor to population declines. In conjunction with disease, this species is facing numerous threats and the synergistic effects are not promising for the long-term persistence of this species.

Recommendations for Future Research

Demographic modeling of amphibian populations has shown that juvenile survival in *B. boreas* is the most important life stage for population persistence (Vonesh and De la Cruz 2002). In addition, juveniles are responsible for most interpond dispersal in many amphibian species (Berven and Grudzien 1990, Sjogren-Gulve 1994, Sinsch 1997). However, little research has focused on the effects of habitat fragmentation on juveniles and how they move in various habitat types (for exceptions see Rothermel and Semlitsch 2002, Stevens et al. 2004, Stevens et al. 2006). Given that juveniles experience higher rates of evaporative water loss because of their smaller size (Spight 1968, Spotila 1972), they are likely more susceptible to harsh climatic conditions in recent clear-cuts. Likely, juveniles would not fare as well as adult western toads in the clear-cuts used in this experiment and this hypothesis provides an interesting avenue for future research.

In addition, female western toads have been found to move significantly farther than male toads, often twice the distance (Muths 2003, Bartelt et al. 2004). They are also

larger in size than males, and larger adult toads have displayed higher locomotor performances than smaller individuals (Goater et al. 1993). Studying sexual differences in movement patterns in heterogeneous landscapes would provide further insight on impacts of fragmentation for amphibians. Given that male movement behaviour was not significantly altered in disturbed habitats, I predict that females will have similar ease moving in and through recent clear-cuts.

In addition to studies on juveniles and female toads, research needs to occur in more realistic settings. The sizes of clear-cuts used in this experiment were significantly smaller (mean = 3.5 ha) than the average size of clear-cuts found in British Columbia (mean = 23 ha, BC Market Outreach Network 2004). Further research should explore the impact of larger clear-cuts on movement dynamics in amphibians. I predict that such large disturbances would be significant barriers to amphibian movement and would result in declined habitat connectivity and increased mortality.

Given that the chytrid fungus has already been detected in Canada in several other amphibian species (Ouellet et al. 2005, Garner et al. 2006), it is no surprise that the disease was detected in *B. boreas*. But given toad susceptibility to the disease and the declines already attributed to the fungus, immediate testing for chytrid is needed throughout the province to further evaluate the extent of this disease. The chytrid fungus has been found to spread very rapidly (Muths et al. 2003, Lips et al. 2006), so populations need to be closely monitored and management plans adapted accordingly.

Lastly, research programs need to move away from single factor causes of amphibian population declines and look at the interactions between numerous potential causes (Blaustein and Kiesecker 2002). Recent research has shown that amphibian

declines are often context dependent and the result of a dynamic environment, not a static one. The interactions between complex stressors, both biotic and abiotic, should be emphasized to better comprehend amphibian declines (Blaustein and Kiesecker 2002, Collins and Storfer 2003).

Western Toad Conservation in Canada

Given the severe and rapid declines of western toads in the southern portion of its range, individuals in Canada represent an important source for recolonization. Although toads are believed to be widespread and common, very little systematic monitoring or baseline data collection has occurred in British Columbia and there are no published reports on population trends (Davis 2000, Wind and Dupuis 2002). The dramatic declines witnessed in portions of the United States demonstrate the vulnerability of this species (Wind and Dupuis 2002) and actions to slow these declines, and ideally reverse them, are required immediately. Long-term monitoring programs need to be established and immediate federal protection of this species is required. Since toads have been shown to move large distances in terrestrial environments, protecting aquatic breeding habitats will not suffice. Awareness for preventing the spread of infectious diseases by thoroughly disinfecting boots and other equipment needs to occur, both at the public and private level. Although certain large-scale threats, such as climate change, may be uncontrollable, local initiatives to conserve this species can maintain populations for generations to come.

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