LANDSCAPE GENETICS OF HABITAT ALTERATION ACROSS MULTIPLE SPATIAL AND TEMPORAL SCALES IN THE ANURAN GENUS *ASCAPHUS*

By

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Abstract
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Habitat alteration is considered the single greatest threat to the conservation of natural communities and thus understanding population response to habitat change is critical. In particular, studies are needed that address the effects of disturbance at multiple temporal and spatial scales. In this dissertation, I investigated landscape influence on population genetic structure in the two species of tailed frogs (genus *Ascaphus*), a pair of stream-associated amphibians that are hypothesized to be especially reliant on mesic forest conditions. This dissertation had three objectives: 1) examine the effect of timber harvest on coastal tailed frog gene flow across a relatively mild climate, 2) investigate patterns of genetic structure in response to both fire and timber harvest in the Rocky Mountain tailed frogs and 3) understand what landscape and environmental factors facilitated colonization of the Mount St. Helens blast zone by coastal tailed frogs. In Chapter 1, I demonstrated that tailed frog populations had high overall genetic connectivity that occurred terrestrially, but loss of forest cover and high solar radiation impeded gene flow in both protected and managed areas. The managed landscape also led to declines in effective population size. In Chapter 2, I detected high gene flow across severely burned areas. However, population connectivity in intensively harvested forests was best explained by movement through riparian corridors that avoided the terrestrial environment. This shift to stream corridors was not seen in coastal tailed frogs in Chapter 1, and suggests an ecological difference between the coastal and Rocky Mountain species. Finally, in Chapter 3, we found high gene flow throughout the Mount St. Helens blast zone and inferred colonization from multiple sources in the outside intact forest. However, environmental influence on gene flow differed among disturbance types, as there was reduced landscape influence on genetic structure in the unmanaged blast zone, but connectivity in the managed blast zone was best described by the effects of several environmental variables. Collectively, this dissertation research demonstrates that dispersal is an important component of tailed frog population structure, and the extent of landscape influence on genetic connectivity is dependent on disturbance type.

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Dedication

This dissertation is dedicated to my wife, Denim Jochimsen.

Thanks for supporting and being there for me through the long hours of research,

not to mention the heat waves and high water in the field.

INTRODUCTION

Land use change is considered the single greatest threat to the conservation of natural communities (Sanderson *et al.* 2002, Foley et al. 2005). Nearly every habitat has been disturbed or fragmented to some degree by humans, and most of the world's species are consequently challenged with anthropogenic disturbances. Furthermore, anthropogenic disturbance can alter the ecological consequences of natural disturbances (Paine et al. 1998). Thus, in the current situation, it is critical to our fundamental understanding of organismal biology to study ecological and evolutionary population responses to habitat alteration (Ferrière *et al.* 2004). A key aspect of population dynamics influenced by land use change is dispersal, which affects nearly every ecological and evolutionary process (Dieckmann *et al.* 1999). Increased dispersal and consequent gene flow across altered landscapes could increase the potential for evolutionary change due to the introduction of new genetic variation and reduction of inbreeding (Holt & Gomulkiewicz 2004). Conversely, increased movement rates may be detrimental if dispersal through a fragmented habitat matrix leads to high disperser mortality (Gibbs 1998; Fahrig 2001; Baguette & Van Dyck 2007). The above results suggest successful dispersal across fragmented environments is dependent on two main factors: (1) how strongly different landscape variables facilitate or inhibit movement; and (2) the spatial and temporal scale of disturbance.

There is a critical need to conduct investigations across multiple spatial and temporal scales to determine how dispersal varies across disturbed environments. Generally, studies of dispersal are only conducted at one scale within a single disturbance type and will only be applicable at that scale. To develop a complete model of dispersal for any species, we must study dispersal at all the relevant scales. However, studying actual dispersal rates is very difficult (Dieckmann *et al.* 1999), particularly rare, long-distance movements that are often important for connectivity. Gene flow often occurs through dispersal events and, as a result, is highly correlated with dispersal (Bohonak 1999). A particularly suitable approach is landscape genetics, which can be used to understand the effect of landscape variables, composition or configuration on population genetic structure across a variety of scales (Holderegger & Wagner 2006; Storfer *et al*. 2007). Landscape genetics offers more insight into how individuals disperse than traditional population genetics methods based on isolation-by-distance alone. For example, studies have identified specific habitat features that explain observed differences in genetic structure within the same species (Hitchings & Beebee 1997; Jacquemyn 2004; Banks *et al*. 2005). Therefore, landscape genetic analyses can serve as tools for understanding the dynamics of populations in disturbed landscapes.

I used a landscape genetic approach to understand the genetic response of tailed frogs of the genus *Ascaphus* in disturbed forests in the Pacific Northwest of the United States. Tailed frogs are the most widespread stream-breeding amphibians in the Northwest. They require forested areas and have among the lowest desiccation and thermal tolerances known in amphibians (Claussen 1973; Brown 1975). As a result, tailed frogs may require closed forest for persistence, and so could be highly susceptible to environmental change (Welsh 1990). Tailed frogs currently consist of two species, the coastal tailed frog (*Ascaphus truei*) and the Rocky Mountain tailed frog (*A. montanus*), that are disjunct and separated by the Cascade Mountain rain shadow of the Pacific Northwest (Nielson et al. 2001). There are distinct climatic differences between inland and coastal areas, with inland areas characterized by less precipitation, higher temperatures in summer, and colder winters (Western Regional Climate Center). The inland region is also characterized by a more frequent disturbance regime due to large fires.

This dissertation consists of three population genetic studies in two species that integrates the effects of recent fragmentation from human disturbance, ongoing natural disturbance and natural catastrophic change. I examined the influence of timber harvest on coastal tailed frogs in an environment lacking a frequent natural disturbance regime (Chapter 1), compared the dual effects of wildfire and timber harvest on the genetic connectivity of Rocky Mountain tailed frogs (Chapter 2) and examined recolonization of coastal tailed frogs across areas with different levels of disturbance after the eruption of Mount St. Helens (Chapter 3).

OVERVIEW OF CHAPTERS

Chapter 1: Landscape genetic structure of coastal Tailed Frogs (Ascaphus truei) in protected versus managed forests

In this study, I had three questions regarding the effects of timber harvest on population connectivity of coastal tailed frogs across the Olympic Peninsula of Washington: 1) Does timber harvest alter patterns of genetic structure relative to protected forests; 2) does timber harvest lead to declines in effective population size in this species; and 3) what natural landscape or environmental factors must contribute to observed patterns of tailed frog population connectivity?

Coastal tailed frogs are restricted to the forests of the Pacific Northwest coast and the Cascade Mountains. This restricted range is at least partially due to physiological limitations, as individuals require continual moisture and cool temperatures (Claussen 1973, Brown 1975). Historically, the Olympic Peninsula of Washington has been characterized by old-growth forest stands and limited broad-scale disturbances such as fire (Agee 1993). However, recent, ongoing timber harvest disturbs forested habitats by altering landscape configuration. Fragmentation effects of timber harvest can be complex, as the process creates a landscape matrix that is a mixture of forested and open areas at different stages of re-growth due to harvest rotation. As a result, managed forest landscapes are dynamically changing, which may constrain the effectiveness of evolutionary response in these systems (Singer & Thomas 1996). It is likely that source-sink processes (Pulliam 1988) predominate in these systems, and therefore connectivity among populations in a patchy, harvested environment might be necessary for population persistence.

There has been relatively little research done on the population biology of *A. truei*, and there has been no work on the genetic connectivity of populations at a fine-scale level (i.e. a few kilometers). In general, it has been assumed that dispersal and gene flow are low, due to the physiological limitations of the frogs and their association with stream habitats (Adams & Pearl 2005). However, mark-recapture studies using pitfall traps have found individual frogs away from streams at distances of 100 meters to 1 kilometer (Corn & Bury 1989; Wahbe *et al.* 2004). These observations, in addition to the favorable moist microclimate of old-growth forests, indicate that there is probably at least some gene flow between streams that occurs terrestrially and that landscape processes are important for understanding population structure.

Chapter 2: The influence of both anthropogenic and natural forest disturbance on extent and pattern of gene flow in a stream-associated amphibian, the Rocky Mountain tailed frog (Ascaphus montanus)

In Chapter 2, I addressed three main questions about how Rocky Mountain tailed frog populations respond to an environment that includes both frequent natural disturbance and anthropogenic disturbance. These included 1) Are there differences in extent of genetic connectivity of frogs across burned areas and harvested areas, 2) how do natural environmental features modify patterns of gene flow in response to both fire and harvest and 3) Has the exposure of Rocky Mountain tailed frogs to a more frequent natural disturbance regime led to differences in population genetic response as compared to coastal tailed frogs?

In forests of the northern Rockies, fire and timber harvest are the most widespread and frequent perturbations (Agee 1993). Both fire and harvest result in spatial heterogeneity of habitat types (Thiollay and Meyburg, 1988, Turner et al., 2003) and can remove large expanses of standing cover. However, a major difference is that timber harvest usually removes vegetative cover from the site, whereas large fires leave much of the vegetation onsite in the form of biological legacies such as coarse woody debris (Lindenmayer and McCarthy, 2002, Turner et al., 2003). Therefore, the two types of disturbance might result in different impacts on populations of species that rely on forested environments (Felton et al. 2008). Furthermore, the frequent fire regime of the northern Rockies is in contrast to the relatively stable coastal forests that comprises the majority of the coastal tailed frog's range. Therefore, Rocky Mountain tailed frogs may have evolved to tolerate less cover and drier microhabitats. Thus, a study of Rocky Mountain tailed frogs will address whether the two species require different forest management practices, and furthermore will give insight into the genetic response of frogs to both timber harvest and fire, and may highlight important ecological differences between the two disturbances.

Chapter 3: Colonization and gene flow of coastal tailed frogs (Ascaphus truei) at Mount St. Helens: Population Response across Disturbance Gradients

Chapter 3 addresses three questions regarding the population genetic response to a major volcanic eruption. These include 1) Are current populations residing within the impact zone of Mt. St. Helens descendants of individuals that survived the blast or colonists from the surrounding forest, 2) Are there differences in population isolation or genetic diversity across the different disturbance categories within the blast zone, and 3) if there is any recent genetic connectivity within the blast zone, which landscape or genetic factors allow for movement across the landscape?

The eruption of Mount St. Helens, WA, in 1980 provided biologists a "natural laboratory" in which to study the ecological response to a catastrophic disturbance that led to local extinctions and landscape fragmentation (Dale *et al.* 2005). The eruption leveled approximately 600 km^2 of forest surrounding the mountain (Crisafulli *et al.* 2005). However, the severity of disturbance was not uniform, shaping the habitat within a few kilometers of the mountain into four main categories: naturally recovering blast zone, blast zone replanted by humans, standing forest with eruption ash-fall, and undisturbed standing forest.

The eruption locally extirpated tailed frogs and other organisms within the area of forest blown down by the blast. However, tailed frogs began to re-colonize some streams just five years post-eruption, and currently occur in several streams in the blast zone (Crisafulli *et al.* 2005). There are two potential hypotheses regarding the source of the tailed frog populations currently residing in the eruption zone. The first is that individuals re-colonized sites via dispersal from surrounding populations not impacted by the blast. However, this would require movement across terrestrial areas with limited cover. An alternative hypothesis is that colonists were individuals that survived the eruption within the blast zone. This may be plausible because the eruption occurred in May, when snow was still on the ground and amphibians were likely still hibernating.

Furthermore, this type of study has relevance to population management as well. This is because certain aspects of the eruption are similar to more routine anthropogenic and natural forest disturbances. The monument blast zone is similar to a windstorm or stand-replacing fire because of the presence of dead blown-down or standing trees. The managed blast zone was extensively salvage logged and replanted with conifers, and as such is similar to twenty-five years after clear-cut timber harvest. Therefore, quantifying genetic structure across each of these disturbance zones will elucidate how populations will respond to more common landscape impacts.

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CHAPTER FORMATS AND ATTRIBUTION

This dissertation is the combination of three chapters intended for separate publication. Thus, the chapters are not all formatted consistently, and are instead formatted to meet the guidelines and restrictions of the focal journal. I was the main contributor to all chapters and was primarily involved in all study design, field and data collection, data analysis, and writing. However, I had two additional collaborators as authors on the papers. My committee chair, Andrew Storfer, contributed ideas for study design and analysis, as well as manuscript revision, for all chapters and is therefore a co-author on each. Charlie Crisafulli strongly contributed to study design and provided information about the Mount St. Helens area and eruption to assist with conclusions in Chapter 3. Chapter 1 is currently published in Molecular Ecology, and is thus formatted for that journal. Chapter 2 is formatted for Biological Conservation. I am currently undecided regarding where to submit Chapter 3, and thus I have formatted consistently with Chapter 2.

CHAPTER 1: Landscape genetic structure of Coastal Tailed Frogs (*Ascaphus truei*) in protected versus managed forests

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ABSTRACT

Habitat loss and fragmentation are the leading causes of species' declines and extinctions. A key component of studying population response to habitat alteration is to understand how fragmentation affects population connectivity in disturbed landscapes. We used landscape genetic analyses to determine how habitat fragmentation due to timber harvest affects genetic population connectivity of the Coastal Tailed Frog (*Ascaphus truei*), a forest dwelling, stream-breeding amphibian. We compared rates of gene flow across old-growth (Olympic National Park) and logged landscapes (Olympic National Forest) and used spatial autoregression to estimate the effect of landscape variables on genetic structure. We detected higher overall genetic connectivity across the managed forest, although this was likely a historical signature of continuous forest before timber harvest began. Gene flow also occurred terrestrially, as connectivity was high across unconnected river basins. Autoregressive models demonstrated that closed forest and low solar radiation were correlated with increased gene flow. In addition, there was evidence for a temporal lag in the correlation of decreased gene flow with harvest, suggesting that the full genetic impact may not appear for several generations. Furthermore, we detected genetic evidence of population bottlenecks across the Olympic National Forest, including at sites that were within old-growth forest but surrounded by harvested patches. Collectively, this research suggests that absence of forest (whether due to natural or anthropogenic changes) is a key restrictor of genetic connectivity and that intact forested patches in the surrounding environment are necessary for continued gene flow and population connectivity.

INTRODUCTION

Anthropogenic land use change is the greatest threat to the conservation of biodiversity (Sanderson *et al.* 2002). Habitat fragmentation and loss challenge the majority of the world's species at the population level and it is thus central to organismal biology to investigate their response (Ferrière *et al.* 2004; Ewers & Didham 2005). On one hand, frequent disturbance of environments may select for increased dispersal ability as individuals try to locate more suitable habitat (Holt & McPeek 1996; Parvinen 2004). Conversely, dispersal may lead to population declines if movement through fragmented habitat leads to high disperser mortality or decreased fitness (Gibbs 1998; Casagrandi & Gatto 1999; Fahrig 2001). Thus, studies that estimate dispersal rates alone may lack sufficient insight into the evolutionary potential of fragmented populations. However, dispersal studies that assess the biotic and abiotic factors that influence dispersal can provide valuable predictions regarding which types of habitat alteration will maintain or reduce population connectivity, thereby influencing population genetic structure. Gene flow often occurs through dispersal and successful breeding and, as a result, is highly correlated with dispersal (Bohonak 1999). Comparative landscape genetic studies across fragmented and continuous landscapes will yield important insights into the habitat variables most important for facilitating or inhibiting dispersal and consequent gene flow.

A major form of habitat alteration is timber harvest. Fragmentation effects of timber harvest can be complex, resulting in a landscape matrix composed of a mixture of forested and open areas at different stages of re-growth (Thiollay & Meyburg 1988). As a result, harvested landscapes may constrain evolutionary responses of forest species by reducing gene flow or genetic diversity (Singer & Thomas 1996). Connectivity among populations in a patchy, harvested environment is often necessary for population persistence due to the predominance of source-sink processes (Pulliam 1988). Recently harvested areas may represent population sinks, and therefore individuals may need to successfully immigrate to surrounding forested patches to survive or reproduce. The harvested forests on the Olympic Peninsula of Washington in the Pacific Northwest of the US (see Fig. 1) are a well-studied example with regard to understanding the effects of fragmentation on the demographic dynamics of mammals (Lomolino & Perault 2001). Based on mark-recapture studies within a number of forest patches, species richness of mammals was positively correlated with both percentage of old-growth forest in the habitat matrix and distance to forested corridors among patches. Such studies suggest that landscape configuration and environmental variables are important for connectivity among patches, yet mark-recapture approaches cannot easily assess which specific factors are influencing population connectivity in fragmented landscapes.

A valuable approach to the problem of understanding the detailed effects of habitat fragmentation is to use landscape genetics, which integrates the fields of population genetics and landscape ecology to identify specific landscape variables that influence genetic structure (Manel *et al.* 2003; Storfer *et al.* 2007). Several landscape genetic studies have shown that least-cost paths based on resistance surfaces better describe gene flow than straight-line routes (Michels *et al.* 2001; Vignieri 2005; Cushman *et al.* 2006). Other studies demonstrate that genetic structure is different among different habitats or disturbance types within the same species (Hitchings & Beebee 1997; Jacquemyn 2004; Banks *et al*. 2005). For these reasons, a landscape genetics approach has great potential to address fundamental questions relating to connectivity of populations in fragmented habitat.

One species that serves as an excellent example for addressing effects of forest fragmentation is the Coastal Tailed Frog, *Ascaphus truei*. This species is restricted to the forests of the Pacific Northwest coastal mountains and the Cascade Mountains of the U.S. This limited geographic range is at least partially due to the physiological limitations of tailed frogs, as individuals require continual moisture, cool temperatures and low sedimentation in breeding streams (Claussen 1973; Brown 1975; Adams & Pearl 2005). Although no studies of fine-scale genetic connectivity of tailed frogs have yet been conducted, it has been assumed that dispersal and gene flow are low, due to the low desiccation tolerance and mark-recapture studies that documented individual frogs 100 m to 1 km from streams (Corn & Bury 1989; Wahbe *et al.* 2004). Due to these restrictions, harvest is expected to isolate frog populations because it is unlikely that frogs are able to disperse through habitat with reduced canopy cover and moisture.

In this study, we test three hypotheses of the effects of timber harvest on population connectivity of a forest-associated species: 1) genetic connectivity of tailed frog populations is positively correlated with extent of forested habitat across the landscape; 2) timber harvest should genetically isolate formerly connected populations, resulting in population declines and increased dispersal mortality; 3) while forest cover is hypothesized as an important variable affecting dispersal, other landscape variables will also modify impacts of timber harvest. Based on previous studies of the biology of tailed frogs, we predict that areas with low solar radiation, low slope and high precipitation should be positively correlated with genetic connectivity. Collectively, testing these predictions will not only yield insight into the current ecology of tailed frogs, but will also allow for inference of population response to future anthropogenic disturbance.

MATERIALS AND METHODS

Study Site and Field Sampling Methods

We selected localities within the northeast corner of Olympic National Park (NP) on the Olympic Peninsula of Washington, U.S. (Fig. 1) as unharvested old-growth sites for this study. Previous surveys indicated that this region contained the highest known prevalence of tailed frogs across NP (Adams & Bury 2002). Harvested study sites are located in the nearby southern area of Olympic National Forest (F), which lies within the largest continuous block of national forest on the Olympic Peninsula (Fig. 1). We chose these sites because spatial data are freely available (as opposed to privately-owned forests) and because of the presence of some remaining old-growth stands for comparison to the unharvested area within NP. Despite higher elevation across NP, there are not large differences in average slope between the two areas. Within both the old-growth and harvested areas, we used a stratified random sampling design, with stratification by two classes of solar radiation (low (0-0.5) and high (0.51-1)) and river drainage. We used solar radiation because of the reported negative influence of high temperature and desiccation on tailed frogs (Claussen 1973; Brown 1975). Solar radiation was estimated based on aspect and using the following equation: $(1-\cos((\pi/180)(\text{aspect}-30)))/2$ (Roberts & Cooper 1989). This creates a continuous variable from 0 to 1, with 1 representing highest solar radiation. Additionally, sampling streams in separate drainages was important for testing whether gene flow was restricted to occurring along stream corridors, as suggested by previous tailed frog studies showing close proximity of metamorphosed animals to streams. We obtained genetic material from 20-30 individuals per site non-lethally by collecting mouth swabs from adults (Goldberg *et al.* 2003) or tail clips from larvae. Samples were stored in 95% EtOH (tail clips) or a lysis buffer (mouth swabs).

DNA Extraction and Genotyping

We used Qiagen DNeasy™ 96 well plate kits (Qiagen Inc, Valencia, CA) to extract DNA from tissue or mouth swabs. Using polymerase chain reaction (PCR), we amplified 13 polymorphic microsatellite DNA markers developed for *Ascaphus truei* (Spear *et al.* 2008) to obtain indices of genetic diversity and gene flow. Specific PCR conditions for each locus are described in Spear *et al.* (2008) and negative controls were included within each PCR run. Microsatellite products from each PCR were run on an ABI 3730 automated sequencer (Applied Biosystems, Inc., Foster City, CA) at the Washington State University LBB1 core facility and genotyped using GeneMapper 3.7 software (Applied Biosystems, Inc.). Because larvae were primarily sampled, we identified potential family groups using the maximum likelihood algorithm in the program COLONY (Wang 2004). We used COLONY results to ensure that number of individuals per family group were equal at each site, thus minimizing influence of any particular family group on genetic structure.

Genetic Data Analysis

We tested for significant deviations from Hardy-Weinberg equilibrium and the presence of linkage disequilibrium using GENEPOP version 3.4 (Raymond & Rousset 1995). Allelic diversity and expected heterozygosity were calculated using FSTAT 2.9.3 (Goudet 2001). To investigate the extent of gene flow, we estimated the level of genetic differentiation among populations using G_{ST}' (Hedrick 2005). G_{ST}' is a standardized measure of genetic distance based on Weir and Cockerham's (1984) adjustment of F_{ST} that divides the estimated F_{ST} by the upper limit of F_{ST} (the value if the two populations were maximally differentiated (i.e. shared no alleles)). This correction is useful when there is high allelic diversity and the upper bound of F_{ST} is <1, as is common with microsatellites. We used RecodeData v. 0.1 (Meirmans 2006) to create a Fstat file with maximally differentiated populations, and ran both the original and recoded file in Fstat to calculate G_{ST}'. Other genetic distance measures (D_{ps}, Nei's D and chord distance) gave similar results as G_{ST}', but we chose G_{ST}' because it generally had greater support in models we tested.

We estimated population clusters based on two methods. The first was the Bayesian algorithm in the program Structure (Pritchard *et al.* 2000). We used the admixture model with correlated allele frequencies and for each potential *K* (number of clusters), we conducted 5 runs consisting of 1,000,000 simulations with a 100,000 burnin period (which was sufficient for convergence). We evaluated the most likely number of populations using the posterior probability of each *K* using the average value of the ln Pr (*X*/*K*) generated by Structure, as suggested by the program authors.

We also used a Bayesian clustering algorithm that included spatial information in the form of hidden Markov random fields (François *et al.* 2006) using Tess v. 1.1 (Chen *et al.* 2007). Hidden Markov random fields are used to model spatial dependence among individuals and therefore incorporate the *a priori* assumption that nearby individuals are more likely to have similar allele frequencies than more distant individuals. Tess was run for 50,000 simulations (10,000 burnin) to estimate *K*, as well as assign individuals to clusters. We chose 50,000 simulations because convergence was always reached at this level after 5 independent runs. As suggested by the manual, we used the parameters of no F model and no admixture. We used a spatial interaction value (which determines the degree of spatial dependence) of 0.6 (as suggested by the authors), but trials at other interaction parameters (0.3 and 0.9) produced consistent results. For both clustering methods, we assigned each site to the cluster that the majority of individuals at that site assigned to. Individuals were assigned to the cluster with the greatest proportion of membership, although we recognize that low membership probabilities may indicate weak structure or unsampled populations.

Three genetic tests for reductions in effective population size were implemented to determine whether timber harvest is leading to potential population declines. These included tests for heterozygosity excess relative to equilibrium expectations (Cornuet & Luikart 1996), shifted allele distributions (Luikart *et al.* 1998) and M-ratios relative to a threshold expected value (Garza & Williamson 2001). We used the program BOTTLENECK (Cornuet & Luikart 1996) to test for both heterozygosity excess and shifted allelic distributions. We assessed significant heterozygosity excess assuming a stepwise mutation model and using a Wilcoxon sign-rank test, with correction for multiple comparisons using the false discovery rate method (FDR; Benjamini & Hochberg 1995). Finally, the M-ratio is the ratio of k/r, with k representing number of alleles and r represents the allelic size range. As rare alleles are lost, k is reduced faster than r, and therefore a low M-ratio relative to a critical value indicates population declines. We used the critical value of 0.68 provided by Garza & Williamson (2001). All three tests were used because they may give insight into the timing of the population declines. For example, a study of tiger salamanders (*Ambystoma tigrinum*) found that the heterozygosity excess test was sensitive to only very recent disturbances, whereas shifted allele distributions and M-ratios should retain bottleneck signatures for a longer time (Spear *et al.* 2006).

Spatial Analysis

To test the influence of landscape and habitat variables on genetic structure, we used spatial autoregression with several potential paths of connectivity. The first path was topographic straight-line distance between sites, which would be expected if the population structure is due solely to distance rather than landscape characteristics. Second, we developed a least-cost path that maximized movement through intact, unharvested forest. Forest cover data for Olympic National Park was derived from a vegetation layer with 25x25 m resolution that was developed specifically for the park using both ground-truthed data and Landsat Thematic Mapper satellite imagery (National Park Sevice 1996). We used a polygon layer based on forest age class from a polygon database maintained by Olympic National Forest to identify harvested patches (Olympic National Forest 2001). This layer classified patches as one of six age classes (years 0-20, 21-40, 41-60, 61-80, 81-160 and 160+). We considered patches 160 years or older as unharvested forest, as these patches have no record of harvest by the U.S. Forest Service. We converted the polygon coverage to a grid with 10x10 m resolution. As the forest data were categorical, they were assigned cost values. Because we had no empirical data to guide cost assignment, we tested three different potential cost ratios (2:1, 10:1 and 100:1), with all non-forest (for NP) or non-forest/harvested (for F) patches assigned the higher cost and unharvested patches given a cost of 1. Additionally, within the F study area, to test whether there was a temporal lag in genetic response to land change (as demonstrated by Holzhauer *et al*. 2006), we created least-cost paths minimizing movement only through harvest older than 20 years (i.e. harvest less than 20 years was grouped with unharvested stands and given a cost of 1) and paths only avoiding harvested areas greater than 40 years old. We did not create least-cost paths based on only harvest greater than 60 years because the 61-160 age class made up a very small percentage of the total harvested area. As with the previous forest least-cost paths, we used the three different cost ratios to create the 20+ year and 40+ year least cost paths.

The next pair of least-cost paths minimized solar radiation and slope, respectively. Both slope and aspect were derived from a USGS digital elevation model (DEM) with 10x10 m resolution. For computational efficiency, we reclassified both solar radiation and slope into five categories with cutoffs between classes based on natural breaks. These categories were then assigned a cost value of 1-5, with 5 indicating the highest slope or solar radiation. However, these 5 categories were a simplification of a continuous gradient, and therefore we did not assign any alternative cost values (i.e. we assumed a linear relationship with cost to gene flow). Additionally, we created a least-cost path based on the multiplied effect of cover and solar radiation. As we were testing the hypothesis that solar radiation was primarily important in areas with reduced or disturbed cover (non-forest or harvest), we set the cost of all forested areas to "1", regardless of the solar radiation. Therefore the combined cover/solar radiation path was primarily influenced by solar radiation in patches without undisturbed forest. Our final least-cost paths tested whether gene flow primarily occurred along riverine corridors. However, because Olympic rivers primarily flow into salt water, it is impossible to connect all sites by rivers without moving across land. Therefore, we created a cost surface maximizing movement along rivers by giving a cost to terrestrial movements. Once again, we used the same three different relative cost ratios as with the forest paths, with rivers always at a cost of 1 and land the higher cost. We used county-wide stream layers available through the Washington Department of Natural Resources. All least-cost paths were created using the "cost distance" function in ArcGIS 9.2 (Environmental Systems Research Institute, Redlands, CA). This function calculates a single line between two sites that has the lowest cumulative cost value.

For each path of gene flow, we calculated several independent variables along the route. These included total topographic distance, topographic distance through non-forest patches created either by natural processes (NP) or harvest (F), and the weighted averages of solar radiation, slope and precipitation along the path. We calculated these averages by first multiplying each individual value by the percent of the overall route that passed through pixels with that value, and then adding individual calculations together to produce a weighted average. Precipitation data were taken from a data layer created by the PRISM Group (Oregon State University, http://www.prismclimate.org). Among the independent variables, there was no correlation of non-forest or harvest with any of the other variables. While there is some correlation (r^2 = 0.05-0.15) between solar radiation and slope and between slope and precipitation along some paths, this relationship is relatively weak and therefore, no independent variable strongly predicts the other.

To analyze the influence of the independent landscape variables described above on gene flow (estimated using G_{ST}') along each path, we used spatial autoregression, implemented in the program GEODA (Anselin 2004). Spatial autoregression is similar to ordinary least-squares (OLS) regression, except that autocorrelation among the dependent variable (common in gene flow measures, as each site is included in multiple paths) is explicitly incorporated into the regression equation as a spatially-lagged dependent variable (O'Loughlin & Anselin 1992). The spatially-lagged dependent variable measures how similar values of a variable are to nearby values. We expect that including a spatially-lagged variable accounts for the non-independence of pairwise genetic data because paths involving the same sites are likely to be close spatially. The spatial autocorrelation component is defined based on a spatial weighting matrix (Haining 2003), which is computed using a variable that is expected to lead to autocorrelation in the dependent measure (i.e. gene flow). We tested autocorrelation variables using several spatial weighting matrices based on drainage contiguity or distance between route midpoints. The midpoint for each route was the point halfway between the two sites involved in a comparison. Thus, we are testing the hypothesis that site pairs within the same drainage and path routes that are close to one another (and thus connect nearby sites) will have similar rates of gene flow. We created spatial weighting matrices for midpoint distance at six distance thresholds (1 km, 3km, 5km, 10km 15km and 20km). Any variable was only included in a particular least-cost path model if it was statistically significant through a stepwise procedure in which all variables are initially included and then excluded based on significance. We then evaluated the best regression model among the different least cost paths using three criteria suggested by the author of GEODA: r^2, log likelihood and Akaike Information Criterion (AIC).

RESULTS

Population Genetic Structure

We obtained sufficient numbers of genetic samples from 20 sites across NP (mean = 26) and 18 sites across F (mean = 28). All loci and populations were in Hardy-Weinberg equilibrium with the exception of site EL2 at locus 14A and site S1 at locus 4A. Additionally, only two pairs of loci (out of 78 pairwise comparisons) were significantly out of linkage equilibrium; this is no greater than at random using an $\alpha = 0.05$. There were very few sibling pairs detected at each site, and therefore, on average only one or two individuals were excluded per site (i.e. there were at least 20 family groups for every site). Overall, both genetic diversity and gene flow were high among sampling sites in both regions. Number of alleles was high (21 alleles/locus in NP, 25 alleles/locus in F) and heterozygosity was also very high (0.855 in NP, 0.912 in F). Without standardization, there was little apparent population subdivision in either area with a F_{ST} of 0.03 across NP and a value of 0.004 across F. However, the high heterozygosity of these markers reduced the maximal F_{ST} to a value far less than one (0.09-0.17). Standardizing these values yielded a G_{ST}' of 0.16 (0.11-0.22 95% c.i.) for NP and 0.04 (0.03-0.05 95% c.i.) for F. Therefore, there is higher overall genetic differentiation across NP relative to F.

The NP sites clearly separated into three genetic clusters, based on both STRUCTURE (Fig. 2; posterior probability = 1) and TESS results. While these three clusters were spatially continuous and tended to group by drainage, sites EL4, EL5 and EN1 all group with the Morse Creek sites (Fig. 4a). This suggests dispersal is not limited to stream corridors. Examination of pairwise G_{ST}' estimates are consistent with the clustering results, but also give insight into the degree of differentiation within and among clusters (Table 1). The Gray Wolf sites had the greatest divergence from the other two clusters, with all comparisons exhibiting high differentiation. Second, the cluster consisting of the majority of the Elwha sites appears to contain substructure between the northern and southern sites not detected through Bayesian clustering. Finally, the cluster dominated by Morse Creek sites has the greatest genetic connectivity despite the fact that it includes three separate drainages and has sites separated by up to 12 km. Overall, the greatest distance between two sites that were genetically connected was 24 km (between sites EL6 and EL10).

In contrast, across F, the two clustering approaches gave different results, although both methods show low genetic differentiation (Fig. 4b). STRUCTURE has the greatest likelihood for a single cluster that included all individuals (Fig. 3; posterior probability = 1). TESS indicated two clusters, but these two clusters were highly admixed, with several sites evenly split into two clusters. Even sites assigned to one of the two clusters only had 60-70% of individuals assigned to the cluster. The border of the two clusters lies within the western half of the study area. Interestingly, the pairwise distance measures, while supporting low differentiation, were not entirely consistent with the clustering results. The cluster represented by the squares (Fig. 4b) did display pairwise differentiation from most of the sites in the Satsop and Wynoochee cluster (represented by stars). However, sites H9 and S1 each demonstrated moderate differentiation with nearly all comparisons, yet these sites were included with other sites in the clustering results. The pairwise distance matrices indicate that these two sites might belong in individual clusters (Table 2). The maximum distance at which pairwise comparisons showed low differentiation was 30 km, between sites H8 and W2.

There was no evidence of recent declines in population size across NP (Table 3) as evidenced by lack of heterozygosity excess, lack of shifted allele distributions, and the fact that no M-ratio values were below the critical value. On the other hand, there were 7 of 18 sites across F that showed significant heterozygosity excess after correction for multiple comparisons (Table 4). However, all F allele distributions were normal, and all M-ratio values exceeded the critical value.

Spatial Analysis

Across NP, there was one model that alone explained the greatest variation in gene flow (Table 5). The model with the most support ($r^2 = 0.65$, AIC wt = 0.98) was a least-cost route that minimized travel through areas of non-forest and high solar radiation. This best model included significant spatial autocorrelation at a spatial scale of 3 km, as well as the variables of total topographic distance, slope and solar radiation. Distance, slope and solar radiation all had a positive relationship with genetic distance, and therefore were negatively correlated with gene flow. Although not a strongly supported model, the model with a 2:1 non-forest cost performed better than either the 1:10 or 1:100 ratio. Lastly, there was no evidence that gene flow primarily occurred along stream corridors based on the low support for these models, no matter the cost ratio.

There were three best supported models across F (Table 6) and they explained less variation than the NP models. Additionally, there was not significant spatial autocorrelation based on drainage contiguity or spatial proximity. Therefore, all F regression models are based on OLS regression. The three models with the strongest support (total AIC wt = 0.92) were two least-cost paths that minimized movement through harvested areas of all age classes with cost ratios of 1:2 and 1:10, and a third path based on the combination of harvest of age class 20+ years and solar radiation, and all included total topographic distance, slope and solar radiation (except for the third path, which excluded the latter). Topographical distance and solar radiation had a positive relationship with genetic distance. However, percent slope was negatively correlated with genetic distance across F, in contrast to NP. Overall, no single variable was present in every model, but a variable related to harvest was included in the least-cost path or as an independent variable in every tested model except one (Table 6). In general, different cost ratios produced models of similar support, with the exception of the 1:100 cost ratio for all harvest, which had the lowest support of any model. Additionally, there was some evidence for a temporal lag in the effect of timber harvest on gene flow. One of the best supported models was based on the interaction of solar radiation with harvest older than 20 years. Additionally, six of the models tested included distance through older harvested areas (either 20+ or 40+) as the most significant independent variable.

DISCUSSION

The results of this study revealed several insights of general importance to population genetic studies. These include the importance of incorporating landscape analyses in comparative genetic studies, the observed temporal lag in genetic response within harvested areas and the presence of population bottlenecks across the landscape. Additionally, this study provided new information regarding the population structure of tailed frogs and its implications for conservation and management.

Importance of Landscape Analysis

We demonstrated that a comparison of gene flow among different regions may produce misleading conclusions unless multiple landscape variables are specifically tested. Typically, studies exploring land use change compare only genetic diversity or gene flow between a continuous region and a fragmented region (examples include Hitchings & Beebee 1997; Millions & Swanson 2007; Noel *et al*. 2007). In our study, we found greater genetic subdivision across the unharvested region (NP) than in the harvested region (F); this could lead to a conclusion that harvest increased genetic connectivity for tailed frog populations, counter to our initial hypothesis. However, our landscape analysis strongly suggested that subdivision across NP was primarily due to the presence of non-forest (primarily high-elevation meadows) and high solar radiation. These alpine areas are entirely absent from the F study region. In fact, the only natural non-forest found within our F study sites is a lake and a few riparian areas, which account for less than 2% of the area (based on GAP land cover data). Additionally, we detected a negative correlation between gene flow and harvested patches, suggesting that timber harvest leads to decreased gene flow. As a result, our landscape analysis indicates that the difference between the genetic differentiation across NP and F is primarily due to the higher elevations found in NP and not due to human management. The historic condition of continuous late-successional forest across F would allow for extensive movement and likely explains the lack of spatial autocorrelation. Furthermore, as harvest is relatively recent in this area, there may have not been sufficient time to structure populations as seen across NP, and thus explains the weaker landscape correlation. Overall, this result supports our hypothesis that landscape features, particularly forest cover, have strong influence on tailed frog genetic population structure. However, despite the strong correlation of gene flow with forest, its absence does not seem to be an absolute barrier, as the higher cost of 100:1 always had weaker support compared to cost ratios of 2:1 or 10:1

Furthermore, we discovered that one variable, slope, had an inconsistent relationship with genetic distance between the two regions; percent slope is positively correlated with genetic distance within NP, but negatively correlated across F. This discrepancy may be explained by the differences in the spatial distribution of slope values between the two study areas. Several studies have documented a positive association of stream gradient with larval abundance (Corn & Bury 1989; Diller & Wallace 1999; Adams & Bury 2002). Therefore, tailed frogs likely emigrate from (and immigrate to) areas of higher slope to breed. While both NP and F have regions of higher slope, there is higher positive spatial autocorrelation (i.e. clustering) of slope across F (Moran's I = 0.14; $z = 201$) than NP (Moran's I = 0.02; $z = 30$). This suggests that an F frog is more likely to encounter higher slope habitat after leaving breeding areas, and thus may have no choice but to cross high slope habitat to locate breeding areas across F. In contrast, the negative relationship between slope and gene flow in NP may be due to greater proximity between high and low slope areas. If this explanation is accurate, it implies that tailed frogs prefer to move through gentler slopes if available, but that the frogs are capable of successfully moving through steeper slopes.

Temporal Lag in Genetic Response

Our data suggest that there is a temporal lag in full genetic response to timber harvest. Although only one of the three best supported models across F included a lag (20 years), this was the model that included an interaction with solar radiation, which was the best supported model across NP. Furthermore, whenever distance through harvest was included as an independent variable, it was always represented by 20+ or 40+ year age class. Therefore, we suggest that while recent harvest does have some initial effect on gene flow, it is not representative of the full response to harvest and its interaction with other variables such as solar radiation. Although other genetic studies have addressed temporal effects by testing variables separately (Keyghobadi *et al.* 2005, Holzhauer *et al.* 2006), our results are valuable in that they suggest that the interaction among landscape variables may not be immediately detected in the genetic response, even if the individual variables alone are.

The time lag observed suggests that timber harvest does not necessarily lead to immediate differentiation, but rather requires multiple generations to begin to change genetic population structure. Therefore, abundance studies may not accurately reflect the dynamics of the system. A study by Findlay and Bourdages (2000) illustrates this point. Species richness of several taxa (reptiles, amphibians, birds and vascular plants) was more strongly associated with historic road density than current road density. Ultimately, a lag may provide support for the theoretical idea of an “extinction debt” (Tilman *et al.* 1994), in which populations do not go extinct until years after the disturbance that led to the decline.

Therefore, long-term genetic monitoring (e.g., Schwartz *et al.* 2007) should be used to understand the viability of populations in harvested landscapes. While the full response of genetic structure to landscape change may take several generations to detect, a genetic monitoring program would be especially useful in determining whether connectivity is re-established following forest recovery. For example, in the future, if there is no longer any significant correlation with the older age classes, then this would strongly suggest renewed connectivity across regenerated forest.

Evolutionary Response to Forest Fragmentation

We have demonstrated that fragmentation due to loss of cover (either natural or anthropogenic) limits gene flow (and presumably dispersal) in a forest-associated species. However, it is unclear from the above result whether reduced gene flow is due to reduced movement or disperser mortality. Our detection of significant heterozygosity excess at 7 of the F sites suggests recent population bottlenecks across the region. Although we did not detect bottlenecks with either of the other two tests (M-ratios and allele frequency shifts), we believe that bottlenecks have occurred across F for two reasons. First, there were no indications of heterozygosity excess across NP. If the significant heterozygosity excess across F were due to some other factor, then we would expect significant excess at NP as well. Second, both the allele frequency distribution and M-ratio tests are strongly influenced by the number of alleles per locus. Our loci were highly variable, and therefore, a loss of a few rare alleles may not have greatly changed the frequency distribution or the M-ratio. The observed bottlenecks cannot be solely attributed to degradation of breeding habitat, as four of the sites with declines are located in intact old-growth forest. Instead, individuals dispersing from streams in closed forest into the surrounding secondary growth or clear-cut forest may be subject to higher mortality and/or there is an overall reduction in emigration from breeding sites across the entire area.

These results are consistent with a recent empirical study on an herbivorous insect that demonstrated the surrounding habitat matrix was a better indicator of individual emigration than internal patch quality (Haynes *et al.* 2007). Sites with successful reproduction (birth rate > death rate) were identified, but they had high emigration with limited immigration due to the inhospitable surrounding matrix. These types of patches have been called "sieves" (Thomas & Kunin 1999) and represent areas where stable populations are unlikely to exist as long as emigration is high. If timber harvest has indeed led to sites becoming sieves, then there should be selective pressure for lower dispersal rates from these patches. Accordingly, Baguette & Van Dyck (2007) suggest that reduced movement across fragmentation boundaries is an expected evolved response.

Further support for the hypothesis that organisms will evolve a tendency to avoid moving through inhospitable habitat comes from the results across NP. This area has patches of natural forest fragmentation due to the presence of alpine meadows. However, while the lack of forest reduced gene flow, there was no evidence of population size declines at any NP sites. Additionally, the significant spatial autocorrelation due to midpoint distance among gene flow paths across NP suggests that individuals are using similar routes across the landscape. This indicates either individuals are genetically predisposed to move in certain directions through continuous forest, or that behavior has been altered to avoid open areas.

Tailed Frog Population Structure and Conservation

Our results suggest that tailed frog gene flow is common and extensive through overland forested habitat, contrary to previous expectations of strong stream association with metamorphosed individuals. Surprisingly, population connectivity occurs at a scale of up to 25-30 km. This long-distance gene flow occurs terrestrially as the clustering algorithms group sites not connected by rivers or streams and there was little support for a least-cost path based on stream connectivity. Previous studies have differed in their conclusions regarding tailed frog movement. Daugherty and Sheldon (1982) reported very low movement in tailed frogs, but this study investigated the closely related Rocky Mountain Tailed Frog (*Ascaphus montanus*) across a drier environment. The authors found that juvenile frogs had the lowest recapture rates and highest degree of movement. In Coastal Tailed Frogs, there has been some evidence of longer movements, with frogs caught in pitfall traps up to 100 m from streams (although the average movement was only 14-37 m) (Wahbe *et al.* 2004) and were encountered up to 1 km from water (Corn & Bury 1989). Therefore, long distance movement likely occurs via a small number of individuals and may be difficult to track using mark-recapture techniques.

The regression analyses clearly supported our hypothesis that landscape and environmental variables had important influence on gene flow in this system. In particular, topography and land cover strongly affected population connectivity in both study regions. As expected, amount of forest cover, amount of solar radiation and degree of slope all significantly influenced gene flow. Precipitation was a significant variable in only one model that had low support, but this is probably due to a narrow gradient of precipitation change within the scale of each study region. Finally, the autocorrelation across NP suggested that paths of gene flow were most similar when in relatively close proximity (within 3 km). The narrow autocorrelation is somewhat unexpected, as genetic connectivity between some sites ranged up to 20 km. The strong similarity of genetic values among movement paths within a few km does suggest that tailed frog movement is highly non-random with corridor use through closed forest habitat. However, it must be noted that midpoints only represent a very small portion of the overall path and therefore we cannot make definitive conclusions about the scale of autocorrelation along the entire path.

Our study has several important implications for the conservation and management of tailed frogs and potentially for forest-associated species in general. Protection of breeding sites (such as through the use of stream buffers), while undoubtedly important for successful reproduction, may only be partially sufficient to maintain viable populations. It is also important to protect terrestrial corridor zones of appropriate habitat to allow for movement between sites. The weak subdivision observed within the managed forest suggests that differentiation due to harvest is beginning to occur. This pattern may eventually lead to loss of genetic diversity due to disperser mortality and genetic drift from isolation, potentially compromising evolutionary potential. However, if continuous patches of intact forest are maintained between streams, then we suspect that connectivity will be maintained across harvested forests. Our data also appear to be consistent with findings that forest-associated mammals are most common near corridors of intact forest (Lomolino & Perault 2001). Additionally, recent reduction in gene flow due to forest disturbance has occurred among capercaillie (grouse) populations in Europe (Segelbacher *et al.* 2008). Therefore, the implications for population genetic structure may not only be relevant to stream amphibians, but more generally to a taxonomic variety of forest-associated species.

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FIGURE LEGENDS

Fig. 1 Map of sampling sites across both study regions. Bold black line represents boundary of Olympic National Park. Inset indicates general location of study site on a map of the Pacific Northwest of the United States.

Fig. 2 Plot of ln Pr (*X*/*K*) versus K (number of populations) for STRUCTURE analysis of sites across Olympic National Park. The greatest ln Pr (*X*/*K*) (K = 3) has a posterior probability of 1.

Fig. 3 Plot of ln Pr (*X*/*K*) versus K (number of populations) for STRUCTURE analysis of sites across Olympic National Forest. The greatest ln Pr (*X*/*K*) (K = 1) has a posterior probability of 1.

Fig. 4 Population clustering results from STRUCTURE and TESS output. Labels next to symbols are site names. A) Sites within Olympic National Park; shapes represent different distinct clusters supported by both STRUCTURE and TESS. EL = Elwha River drainage, EN = Ennis Creek drainage, GW = Gray Wolf River drainage, M = Morse Creek drainage. Black patches represent non-forested areas. Thick gray border is park boundary. B) Sites within Olympic National Forest; squares and stars represent different clusters, triangles represent sites with equal membership in either cluster as identified by TESS. STRUCTURE grouped all F sites into one cluster. H = Humptulips River drainage, S = Satsop River drainage, W = Wynoochee River drainage. Background represents patches of harvest of the three age classes of 0-20, 21-40, and 41-60. White areas represent forest older than 60 years

Tables

Table 1. Pairwise G_{ST}' for sampling sites across Olympic National Park (NP). Site names are as in Fig. 4A. Bold G_{ST}' values indicate moderate differentiation (>0.05) and bolded and italicized values represent high differentiation (>0.15) (Wright 1978).

	EL1	EL2	EL3	EL4	EL5	EL6	EL7	EL8	EL9	EL10	EN1	GW1	GW2	GW3	GW4	M1	M2	M3	M4
EL2	0																		
EL3	**0.06**	0.01																	
EL4	0.04	0.03	**0.06**																
EL5	0.04	0.04	**0.09**	0.03															
EL6	**0.12**	**0.05**	**0.06**	**0.14**	**0.13**														
EL7	**0.11**	0.03	**0.08**	**0.14**	**0.12**	0													
EL8	**0.11**	0.03	0.04	**0.1**	**0.13**	0	0.02												
EL9	***0.18***	**0.12**	**0.12**	***0.17***	***0.21***	0.04	0.03	**0.06**											
EL10	***0.15***	**0.07**	**0.07**	***0.17***	***0.18***	0.03	0.02	-0.02	0.01										
EN1	**0.1**	**0.08**	**0.12**	0	**0.06**	***0.18***	***0.21***	**0.15**	***0.22***	***0.21***									
GW1	***0.3***	***0.28***	***0.27***	***0.25***	***0.28***	***0.33***	***0.34***	***0.31***	***0.36***	***0.38***	***0.29***								
GW2	***0.19***	**0.14**	***0.21***	***0.16***	***0.15***	***0.23***	***0.25***	***0.22***	***0.26***	***0.26***	***0.2***	0.05							
GW3	***0.31***	***0.26***	***0.3***	***0.23***	***0.23***	***0.34***	***0.35***	***0.33***	***0.33***	***0.34***	***0.25***	0.05	**0.05**						
GW4	***0.28***	***0.26***	***0.29***	***0.24***	***0.26***	***0.32***	***0.33***	***0.32***	***0.31***	***0.34***	***0.28***	**0.09**	**0.06**	0.02					
M1	**0.14**	**0.13**	***0.17***	0.03	**0.05**	***0.2***	***0.22***	***0.16***	***0.23***	***0.24***	-0.02	***0.21***	***0.14***	***0.17***	***0.22***				
M2	**0.07**	**0.09**	**0.11**	0.02	0.04	**0.15**	***0.18***	**0.13**	***0.23***	***0.22***	-0.01	***0.27***	***0.19***	***0.26***	***0.27***	0.03			
M3	**0.14**	**0.15**	**0.13**	0.03	**0.09**	***0.21***	***0.25***	***0.19***	***0.26***	***0.29***	0.03	***0.3***	***0.24***	***0.28***	***0.3***	0.01	0.05		
M4	**0.12**	**0.12**	***0.18***	0.03	**0.06**	***0.25***	***0.25***	***0.22***	***0.32***	***0.31***	0.05	***0.36***	***0.24***	***0.34***	***0.35***	0.02	0.03	0.01	
M5	**0.06**	**0.06**	**0.09**	-0.01	0.04	**0.13**	***0.19***	**0.13**	***0.21***	***0.2***	0	***0.25***	**0.14**	***0.24***	***0.28***	-0.01	-0.02	-0.03	-0.03

Table 2. Pairwise G_{ST}' for sampling sites across Olympic National Forest (F). Site names are as in Fig. 4B. Bold G_{ST}' values indicate moderate differentiation (>0.05) and bolded and italicized values represent high differentiation (>0.15) (Wright 1978).

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	S1	S2	S3	W1	W2	W3	W4
H2	-0.01																
H3	-0.01	0.01															
H4	0.04	-0.01	0.03														
H5	0.03	0.01	0	0.04													
H6	0.04	0.01	0.03	0.02	0.03												
H7	-0.02	-0.03	0.01	0.02	0	0.02											
H8	0	0.02	0.02	0.04	0	**0.08**	0										
H9	**0.09**	**0.08**	**0.1**	**0.1**	**0.11**	**0.1**	**0.1**	**0.12**									
H10	0.03	0.04	0.01	0.05	-0.01	0.03	0.01	-0.01	**0.11**								
S1	**0.06**	**0.07**	**0.11**	**0.11**	**0.1**	**0.1**	**0.06**	**0.11**	***0.24***	**0.12**							
S2	0.03	0.05	0.04	**0.09**	**0.06**	0.05	0.04	**0.07**	**0.12**	**0.06**	**0.06**						
S3	0.04	-0.03	**0.07**	**0.07**	**0.06**	**0.07**	0.02	**0.09**	***0.19***	0.05	0.02	0.02					
W1	0.02	0.04	0.03	**0.05**	0.05	0.03	0.02	**0.09**	**0.08**	**0.08**	**0.07**	0	0.01				
W2	-0.01	0.04	0.05	**0.1**	**0.07**	**0.1**	0.04	0.04	***0.18***	**0.1**	0.05	0.04	0.03	0.01			
W3	0.02	0.04	0.03	**0.05**	0.03	0.04	0.05	0.03	**0.14**	**0.05**	**0.07**	0.01	0.02	0	**0.05**		
W4	-0.01	0.01	-0.01	0.01	0.01	0.01	-0.01	-0.02	**0.1**	0.03	**0.1**	0	0.04	0.01	0.04	0.03	
W5	0.01	0.02	0.03	**0.08**	0.01	**0.06**	0.01	0.01	**0.13**	**0.06**	**0.08**	0.01	0.05	**0.05**	0.02	**0.05**	0.04

Table 3. Results from tests of population size reductions across NP sites. Sites are as in Fig. 4A. H_e - H_{eq} represents the difference between actual expected heterozygosity and expected heterozygosity under the stepwise mutation model and p-value estimates the probability of no heterozygosity excess. Allele distribution is either normal or shifted.

Site	**H_e - H_{eq}**	**p-value**	**Allele distribution**	**M-ratio**	**M-ratio variance**
EL1	-0.005	0.905	normal	0.826	0.014
EL2	-0.007	0.5	normal	0.819	0.015
EL3	-0.007	0.953	normal	0.824	0.023
EL4	-0.006	0.393	normal	0.78	0.022
EL5	-0.003	0.393	normal	0.793	0.035
EL6	-0.006	0.632	normal	0.755	0.024
EL7	-0.001	0.42	normal	0.697	0.031
EL8	-0.008	0.812	normal	0.755	0.031
EL9	-0.003	0.682	normal	0.775	0.022
EL10	-0.002	0.473	normal	0.762	0.015
EN1	-0.005	0.682	normal	0.763	0.02
GW1	-0.007	0.863	normal	0.712	0.045
GW2	-0.002	0.095	normal	0.787	0.03
GW3	-0.007	0.98	normal	0.796	0.034
GW4	-0.008	0.863	normal	0.779	0.026
M1	-0.012	0.905	normal	0.794	0.02
M2	-0.004	0.658	normal	0.814	0.021
M3	-0.012	0.847	normal	0.771	0.011
M4	-0.005	0.936	normal	0.816	0.02
M5	0.001	0.393	normal	0.713	0.028

Table 4. Results from tests of population size reductions across F sites. Sites are as in Fig. 4B. H_e - H_{eq} represents the difference between actual expected heterozygosity and expected heterozygosity under the stepwise mutation model and p-value estimates the probability of no heterozygosity excess. Bolded values indicate statistical significance after FDR correction. Allele distribution is either normal or shifted.

Site	**H_e - H_{eq}**	**p-value**	**Allele distribution**	**M-ratio**	**M-ratio variance**
H1	0.004	**0.011**	normal	0.874	0.015
H2	0.008	0.047	normal	0.862	0.017
H3	0.006	0.108	normal	0.881	0.007
H4	0.01	**0.011**	normal	0.806	0.025
H5	0.015	**0.002**	normal	0.837	0.02
H6	0.006	0.029	normal	0.745	0.012
H7	0.006	0.047	normal	0.838	0.015
H8	0.006	0.055	normal	0.796	0.018
H9	-0.004	0.916	normal	0.766	0.02
H10	0.006	0.095	normal	0.841	0.022
S1	-0.012	0.878	normal	0.811	0.021
S2	-0.001	0.207	normal	0.792	0.022
S3	0.009	**0.016**	normal	0.736	0.024
W1	0.008	**0.02**	normal	0.809	0.012
W2	-0.002	0.446	normal	0.825	0.02
W3	0.003	0.04	normal	0.788	0.017
W4	0.01	**0.001**	normal	0.81	0.023
W5	0.012	**0**	normal	0.768	0.024

Table 5. Spatial regression results for Olympic National Park models. Model refers to hypothesized route of gene flow and relative costs (see Methods). Variables are all significant parameters included in best model (3km midpt represents the spatially lagged dependent variable). We used the three criteria of r^2, log-likelihood, and AIC. AIC weights are also included for each model to demonstrate the comparative level of support. All variables had a positive relationship with the dependent variable. Bolded indicates best supported models.

Model	**Variables**	**r^2**	**Log-likelihood**	**AIC**	**AIC weight**
straight-line	3km midpt Distance Solar radiation	0.61	577.065	-1144.1	0
forest (2:1)	3km midpt Non-forest dist Solar radiaton Slope	0.63	583.773	-1155.5	0.02
forest (10:1)	3km midpt Non-forest dist	0.52	556.491	-1105	0
forest (100:1)	3km midpt Non-forest dist	0.53	557.533	-1107.1	0
forest/solar	**3km midpt** **Distance** **Slope** **Solar radiation**	**0.65**	**587.758**	**-1163.5**	**0.98**
Solar	3km midpt Distance Slope Solar radiation	0.62	580.47	-1148.9	0
Slope	3km midpt Distance Slope	0.63	581.412	-1152.8	0
stream (2:1)	3km midpt Non-forest dist	0.49	548.778	-1089.6	0
stream (10:1)	3km midpt Distance Slope	0.5	550.146	-1090.3	0
stream (100:1)	3km midpt Non-forest dist	0.51	550.187	-1092.4	0

Table 6. Spatial regression results for Olympic National Forest models. Model refers to hypothesized route of gene flow and relative costs (see Methods). Variables are all significant parameters included in best model. We used the three criteria of r^2, log-likelihood, and AIC. AIC weights are also included for each model to demonstrate the comparative level of support. All variables had a positive relationship with the dependent variable unless indicated by a (-) symbol. Bold indicates best supported models.

Model	Variables	r^2	Log-likelihood	AIC	AIC weight
straight	41-160 distance	0.18	636.41	-1266.8	0
harvest (1:2)	**Distance** **Slope (-)** **Solar radiation**	**0.27**	**645.062**	**-1280.1**	**0.37**
harvest (1:10)	**Distance** **Slope (-)** **Solar radiation**	**0.27**	**644.497**	**-1279**	**0.21**
harvest (1:100)	Distance Slope (-) Solar radiation	0.14	632.315	-1254.6	0
harvest 20+ (1:2)	Distance Slope (-)	0.22	639.741	-1271.5	0
harvest 20+ (1:10)	Distance Slope (-)	0.24	641.137	-1274.3	0.02
harvest 20+ (1:100)	Distance Solar radiation Slope (-)	0.24	641.406	-1272.8	0.01
harvest 40+ (1:2)	Distance Slope (-) Solar radiation	0.23	640.467	-1270.9	0
harvest 40+ (1:10)	Distance Solar radiation Slope (-)	0.23	640.747	-1271.5	0
harvest 40+ (1:100)	Distance Slope (-)	0.21	638.054	-1268.1	0
harvest/solar	Distance Slope (-)	0.22	639.823	-1271.6	0.01
harvest 20+/solar	**Distance** **Slope (-)**	**0.26**	**643.992**	**-1280**	**0.34**
harvest 40+/solar	41-60 length Slope (-)	0.21	638.732	-1269.5	0
Solar	Distance Solar radiation(-) Slope (-)	0.24	642.194	-1274.4	0.02
Slope	41-60 distance Slope (-)	0.21	639.209	-1272	0
Stream (1:2)	21-40 distance Slope (-) Solar radiation	0.23	640.818	-1271.6	0.01
Stream (1:10)	21-160 distance Precip Slope (-)	0.22	639.316	-1268.6	0
Stream (1:100)	41-160 distance Slope (-)	0.21	638.532	-1269.1	0

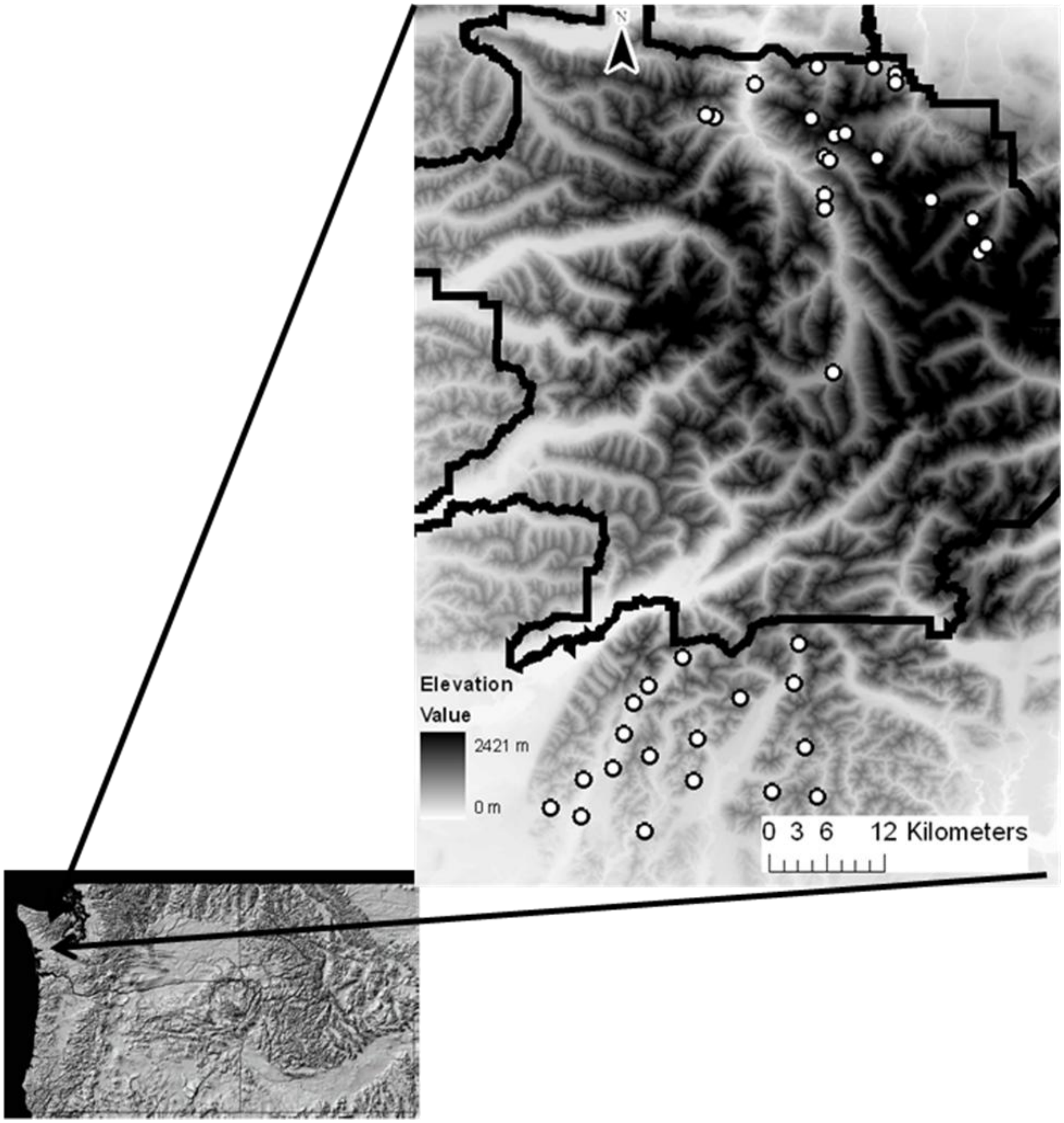


Figure 1

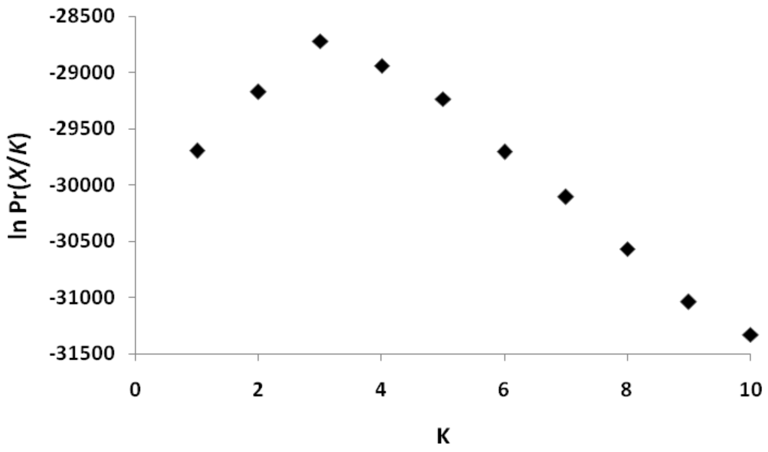


Figure 2

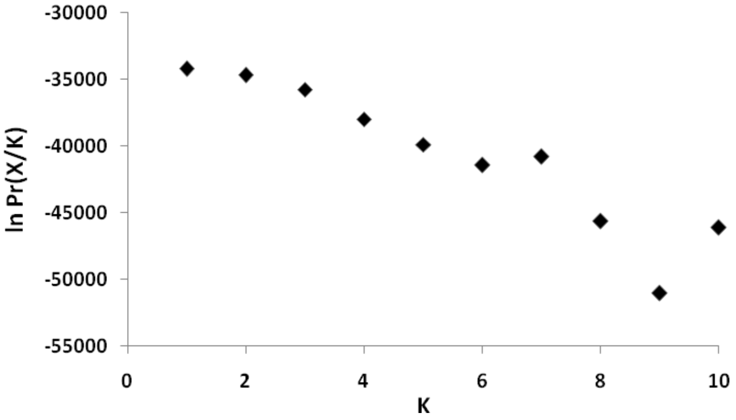
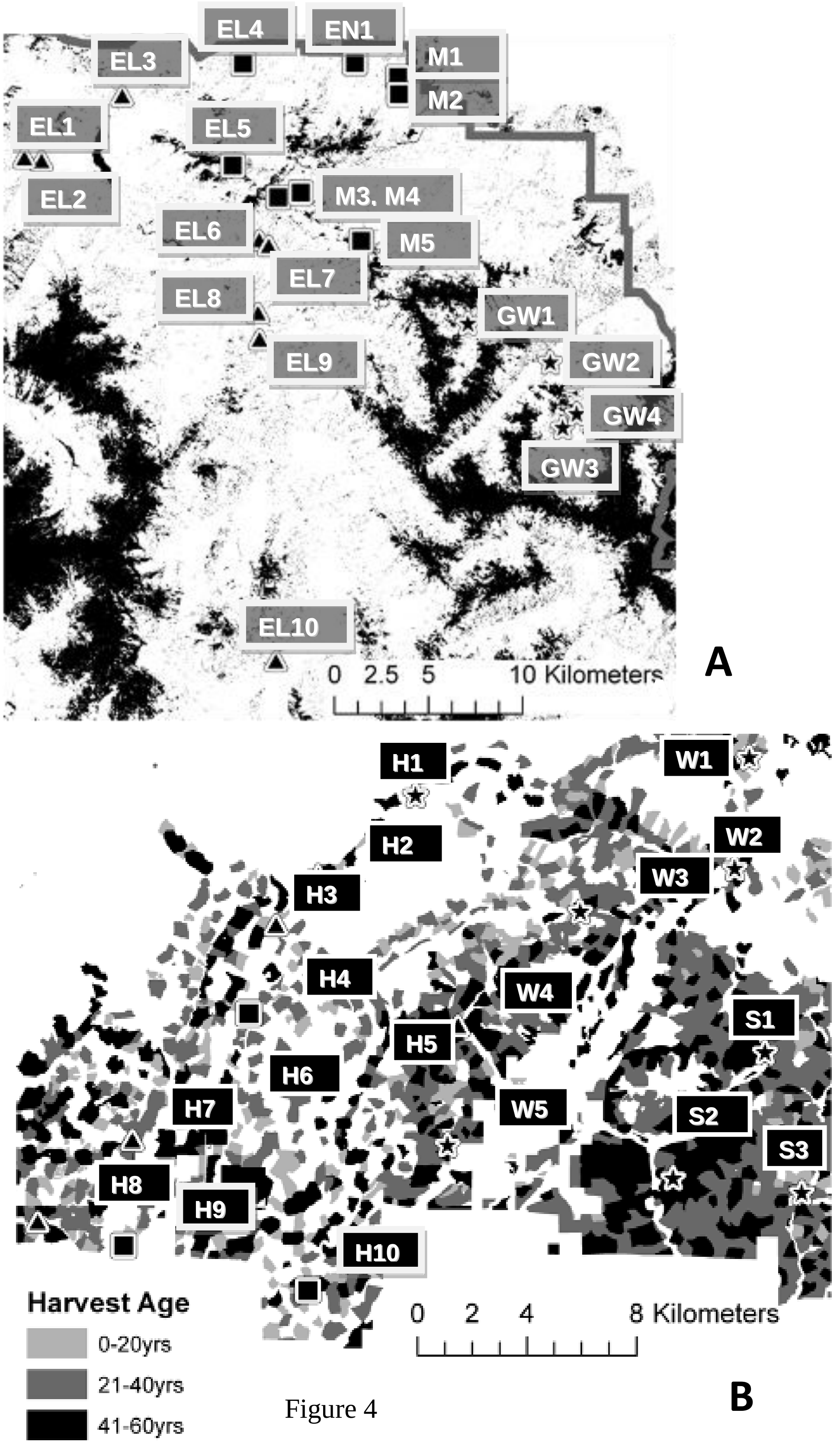


Figure 3


EL4
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H7
S2
S3
H8
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Harvest Age
0-20yrs
21-40yrs
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0 2 4 8 Kilometers
B

Figure 4

CHAPTER 2 – The influence of both anthropogenic and natural forest disturbance on extent and pattern of gene flow in a stream-associated amphibian, the Rocky Mountain tailed frog (Ascaphus montanus)

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ABSTRACT

Understanding the influence of habitat alteration on population structure and persistence is critical for effective conservation strategies. Timber harvest and wildfire are two of the most prevalent disturbances across temperate forests, yet the long-term effects of these two forces on population connectivity have rarely been studied. We studied populations of the Rocky Mountain tailed frog (*Ascaphus montanus*) across landscapes that have experienced both timber harvest and broad scale fires. Rocky Mountain tailed frogs generally require forested habitat, and are a species of concern in managed forests. We used landscape genetic techniques to test the explanatory power of alternative paths of connectivity across both burned and harvested forests and identified topographical and climatic variables that significantly influenced gene flow. We found that timber harvest and fire led to differential patterns of genetic connectivity. Across forests managed for timber harvest, gene flow among populations was facilitated by precipitation and followed riparian corridors, presumably to avoid the loss of cover due to timber harvest. In contrast, widespread terrestrial gene flow was maintained across previously burned areas and connectivity was primarily limited by solar radiation. Furthermore, the genetic response to timber harvest in Rocky Mountain tailed frogs differs from the closely related coastal tailed frog, which does not follow riparian corridors in managed forests. That is, coastal tailed frog dispersal and gene flow appears to occur primarily over land so that conservation corridors may be necessary. In contrast, Rocky Mountain tailed frog dispersal shifts from overland to riparian routes, perhaps because its inland habitat is drier. These results suggest management of Rocky Mountain tailed frogs in managed forests should differ, instead focusing on maintaining riparian buffer zones and headwater forest connectivity between basins. Thus, consideration of landscape differences is essential for conservation of closely related, morphologically similar

species. Further, this study suggests that large fires do not significantly alter population structure, perhaps due to retention of biological legacies. As such, natural succession after fire may be the best management strategy for tailed frogs and other forest dwelling amphibians.

INTRODUCTION

Understanding population responses to anthropogenically driven habitat alteration and disturbance is perhaps the single most important issue in conservation (Sanderson et al., 2002; Foley et al. 2005). While the adverse effects of complete habitat destruction may be relatively straightforward, it is less clear how recent anthropogenic disturbance may affect populations such that the majority of the world's ecosystems can now be characterized as a matrix of natural and anthropogenic disturbance. Thus, there is a need for comparative studies of population effects of natural disturbances (i.e., those historically influencing species evolution) and evolutionary challenges posed by current, anthropogenic disturbance (Lindenmayer et al., 2008).

Temperate forests provide an excellent example of an ecosystem type subject to multiple disturbances of varying source and intensity. In a majority of temperate forests, fires and timber harvest are the most widespread and frequent perturbations (Healy et al., 2008). Both fire and harvest result in spatial heterogeneity of habitat types (Thiollay and Meyburg, 1988, Turner et al., 2003) and can remove large expanses of standing cover. However, a major difference is that timber harvest usually removes vegetative cover from the site, whereas large fires leave much of the vegetation onsite in the form of biological legacies such as coarse woody debris (Lindenmayer and McCarthy, 2002, Turner et al., 2003).

Populations living in forests fragmented by different types of disturbances have been well-studied, but most such studies have assumed an island model with a forest/non-forest dichotomy (Laurance, 2008). Instead, for many landscapes (including forests subject to harvest and wildfire), the intervening habitat matrix is more heterogeneous and thus population structure and connectivity are likely better explained by considering a habitat mosaic as opposed to a dichotomy (Ricketts, 2001). In fact, a recent metanalysis of a wide variety of animal groups in fragmented landscapes demonstrated that patch size and isolation, the two key factors explaining colonization and connectivity in island models, explained relatively little variation in patch occupancy (Prugh et al., 2008). Instead, the authors concluded that the habitat type among patches was a better predictor of species occurrence. Therefore, it is critical that population studies incorporate measures of forest heterogeneity into analyses. This is especially true in areas like the American west, where managed forests contain a matrix of mature forest, clearcuts, selective cutting, and patches in different stages of regeneration for later harvest.

Amphibians represent a group for which relatively little is known regarding overall population response to forest disturbance (Naughton et al., 2000, Curtis and Taylor, 2003, Pilliod et al., 2003, Bury, 2004). Nonetheless, amphibians may be especially susceptible to disturbance effects because of relatively narrow physiological tolerances and documented recent global declines due to habitat change (Gardner et al., 2007). The studies that have addressed the effect of harvest or fire on amphibian populations have generally looked at changes in abundance or density (Welsh, 1990, Corn and Bury, 1989, Naughton et al., 2000, Hossack et al., 2005, Morris and Maret, 2007, Kroll et al., 2008, Homyack and Haas, 2009). Such studies have produced variable results. This is likely due to both differences in species' life history and the fact that amphibian populations fluctuate widely in census size from year to year (Pechmann et al., 1991; Blaustein et al. 1994). Thus abundance studies likely provide low power to predict the long-term viability of a population. Population genetic analyses are not as strongly influenced by yearly fluctuations and, when combined with landscape information, can allow for the identification of environmental factors affecting population connectivity and detection of declines in population sizes.

The forests of the northern Rocky Mountains are an example of an ecosystem in which the effects of both fire and timber harvest predominate. The area has a relatively frequent fire-return interval with a history of stand-replacing fires (Agee, 1993; Turner et al., 2003) and has been logged widely since the early 1900s. A large portion of these forests within Idaho and western Montana is also habitat for the Rocky Mountain tailed frog (*Ascaphus montanus*), a stream-breeding amphibian. *Ascaphus montanus* and its disjunct sister species, the coastal tailed frog (*A. truei* - the range of which encompasses the Pacific coast and Cascade mountains from British Columbia to California), are found only in forested areas and have among the lowest thermal and desiccation tolerances documented for amphibians (Claussen 1973, Brown 1975). Thus, there has been concern regarding the influence of timber harvest on tailed frog population dynamics due to decreased canopy cover and increased surface solar radiation (Corn and Bury, 1989; Kroll 2009). A genetic study of coastal tailed frogs on the Olympic Peninsula of Washington suggested that populations in harvested areas exhibited recent declines (Spear and Storfer, 2008), supporting the hypothesis that loss of canopy cover is detrimental to coastal tailed frog populations despite a very moist climate. However, the Rocky Mountain tailed frog has been exposed to frequent instances of open canopy through the action of fire, yet has persisted through these disturbances. Therefore, Rocky Mountain tailed frogs may have evolved to tolerate less cover and drier microhabitats. Thus, a study of Rocky Mountain tailed frogs will address whether the two species require different forest management practices, and furthermore will give insight into the genetic response of frogs to both timber harvest and fire, and may highlight important ecological differences between the two disturbances.

While relatively little research has been conducted on Rocky Mountain tailed frogs, there is evidence that suggests more restricted gene flow than that in coastal tailed frogs. Daugherty &

Sheldon (1982) used mark-recapture methods to determine that individual adult frogs were highly philopatric, often moving only a few meters along a stream, whereas juvenile females moved up to 360 meters from the stream edge. In addition, Nielson *et al.* (2006) found that at a broad geographic scale (based on allozyme data) Rocky Mountain tailed frogs had lower gene flow than coastal tailed frogs. However, mark-recapture data are generally not reliable for detecting rare migrants (Smith & Green 2006) and the allozyme results were at a broad scale and did not indicate how fine scale landscape processes might influence gene flow. Thus, we chose to use microsatellite DNA loci, which are suitable for detecting changes in gene flow and genetic diversity at a much finer spatial and temporal scale than allozymes or mitochondrial DNA (Storfer et al., 2007).

Our study of Rocky Mountain tailed frogs is designed to test three main hypotheses. First, both fires and timber harvest will initially reduce population size and connectivity compared to undisturbed areas due to loss of canopy cover, and populations across both disturbances will begin to recover as vegetative cover increases in subsequent years. However, overall connectivity will be higher in burned landscapes relative to harvested area due to greater preservation of biological legacies and less frequent disturbance intervals. Second, we expect that genetic response to either disturbance will be moderated by the topographical and climatic characteristics of the landscape. Specifically, we predict that favorable ranges of heat load index, slope, frost-free period and growing season precipitation will ameliorate harvest or fire effects, and will ultimately lead to corridors of gene flow through the heterogeneous matrix. Finally, due to a more frequent exposure to disturbance over time, Rocky Mountain tailed frogs will more effectively avoid both anthropogenic and natural disturbance such that populations will not undergo declines, in contrast to results of previous work on the coastal tailed frog. Results will provide guidance for population management that can maximize connectivity and population persistence across different disturbance types.

MATERIALS AND METHODS

Study site and Field Sampling

We sampled 1009 individuals across 41 sites across a 13,000 km^2 area across north-central Idaho, USA (Fig. 5). This region is primarily characterized by forests managed for timber production, but it also includes large tracts of U.S. Forest Service land that are characterized as “roadless areas” (continuous forest without official wilderness protection). We divided the sampling area into two subregions primarily based on type of land-use. The northern subregion (about 4500 km^2; referred to further as “harvested”) consists of forests managed by several landowners, primarily Potlatch Forest Holdings, the Idaho Panhandle National Forest, and the Idaho Department of Lands, and is currently primarily used for timber harvest. This region also had a regular fire history until the early 1930’s (after this data any fires would likely be quickly suppressed) and thus this landscape represents both historic natural disturbance and current anthropogenic alteration.

The southern subregion (8500 km^2; referred to further as “roadless”) lies almost entirely within the Clearwater National Forest and primarily contains several roadless areas separated only by thin boundaries of roads. Forest Service roadless areas are lands that lack permanent official protections (such as designated wilderness), but act as defacto wilderness due to the lack of accessibility via roads. While this area has had reduced history of logging, much of the area has been influenced by fire, most broadly the Great Burn fire of 1910, which burned much of central Idaho and western Montana over the course of two days. However, there were also very large fires in 1919 and 1934, and the region has a regular history of smaller fires continuing until the present. As such, most of the disturbance in the area occurred before extensive human interference (and in fact, the early large fires launched many fire suppression efforts (Pyne, 2001)). Additionally, we sampled several sites located in the northwest portion of this roadless subregion that lack a recorded fire history.

Potential sites within the harvested subregion were chosen based on a stratification random sample based on high and low canopy cover (above and below 50%), which would be most representative of timber harvest. Stratification occurred by presence or absence of burned areas across the roadless subregion. From all potential sites, the actual sampling sites were determined by accessibility and abundance of tailed frogs. At each sampling site, we attempted to collect tissue samples from 20-30 individuals. We collected tail tips from larvae and saliva from adults via buccal swabs (Goldberg et al. 2003). Tail tips were stored in 100% ethanol and buccal swabs were stored in a lysis buffer. Additionally, we included individuals from nine sites across the harvested subregion and four sites from the roadless subregion that were collected by Drummond et al. (unpublished).

DNA extraction, PCR amplification and genotyping

We extracted DNA from all samples using the Qiagen DNEasy 96 well plate kit (Qiagen, Inc.). We used polymerase chain reaction (PCR) to amplify 13 microsatellite DNA loci previously developed for the sister species *A. truei* (Spear et al, 2008). We ran all PCR reactions divided into 3 multiplexed panels using the Qiagen Multiplex PCR kit (see Appendix 1 for multiplex panels and PCR conditions), with a negative control included in each run. We then submitted all microsatellite products to be run on an ABI 3730 automated sequencer (Applied Biosystems, Inc.) at the Washington State University LBB1 core facility. We used GeneMapper 3.7 software to genotype all samples. For genotypes that were scored by Drummond et al. (unpublished), we ran identical samples in both labs to ensure consistent scoring of genotypes. Finally, because a large number of larval individuals were sampled, we used the software Colony (Wang 2004) to identify full siblings using a maximum likelihood algorithm to ensure that all sites had an equal number of full sibling groups.

Genetic Data Analysis

We tested whether loci and sites were in Hardy-Weinberg equilibrium and linkage equilibrium using Genepop version 3.4 (Raymond and Rousset, 1995). If sites were out of Hardy-Weinberg equilibrium, we tested for null alleles using the software FreeNA (Chapuis & Estoup, 2007), which estimates proportion of null alleles using the expectation maximization (EM) algorithm developed by Dempster et al. (1977). We expect that null alleles might be a problem for this dataset because all loci were developed using *A. truei* individuals, a species from which *A. montanus* diverged from over several million years ago. Therefore, mutations in the flanking sequence are probable and would result in null alleles (Chapuis & Estoup, 2007).

We estimated among-site differentiation using G_{ST}', a correction of F_{ST} that standardizes observed values so that the maximal value equals one, as highly variable markers such as microsatellites usually produce an uncorrected maximal value far less than one (Hedrick, 2005). We calculated uncorrected F_{ST} using FreeNA. If we detected null alleles, we used the ENA (exclude null alleles) method to estimate F_{ST} (Chapuis & Estoup, 2007), with a bootstrap resampling procedure run 1000 times to estimate 95% confidence intervals. This method simply excludes putative null alleles in the estimation of F_{ST} and Chapuis & Estoup (2007) demonstrated the effectiveness of this correction using simulated data. To calculate G_{ST}', we used RecodeData v 0.1 (Meirmans, 2006) to create a file of genotypes that were maximally differentiated, and created pairwise F_{ST} values using FreeNA based on the recoded file. We then divided the actual F_{ST} values by the maximum values to obtain a standardized G_{ST}'. The pairwise comparisons were computed separately for each subregion.

We also tested whether populations had experienced population size declines through the use of three methods: tests for both heterozygosity excess (Cornuet and Luikart, 1996), and shifted allele distributions (Luikart et al., 1998) and estimation of M-ratios (Garza and Williamson, 2001). We implemented all three tests due to the different time scales associated with each method (Cornuet and Luikart, 1996, Garza and Williamson, 2001, Spear et al., 2006). An excess of heterozygotes relative to equilibrium expectations is an ephemeral signature of recent declines within the past few generations (Cornuet and Luikart, 1996). We assumed a stepwise mutation model (appropriate for these loci) and used the Wilcoxon sign rank test to determine if a population exhibited significant heterozygosity excess. The shifted allele distribution test describes the distribution of allele frequencies, with the normal expectation that most alleles will be of low frequency. A shifted distribution in which the majority of alleles are of intermediate frequency is characteristic of populations that have experienced declines due to initial loss of rare alleles. Finally, the M-ratio, or the ratio of number of alleles (k) to allelic size range (r), with a M-ratio value below 0.68 indicating a decline. The M-ratio can represent a signature of more historic decline as not every new allele in a recovering population will increase k relative to r. (Garza and Williamson, 2001).

Spatial Analysis

We used a least cost path methodology to test the hypothesized influence of landscape and climatic variables on genetic structure in the two subregions. We developed seven different potential paths of connectivity among sites, including paths based on a straight-line, fire history, canopy cover, heat load index (hli) and stream distance (see Table 7 for specific calculations and source layers). The first path was simply a straight line connecting all sites, a path that would be expected if there was no landscape influence on gene flow as assumed under a simple isolation-by-distance model. We next created two paths based on fire history across the area. Both polygon layers contained fires in the area from the year 1900 up until present. Because fire occurrence is a categorical variable, we had to assign cost values to burned areas and we did not have previous data to guide cost assignment. Therefore, we tested two alternative cost ratios (2:1 and 10:1) in which unburned areas were always given a cost of 1, and the burned areas given the higher cost.

We next calculated the least cost path maximizing movement through high canopy cover (this path serves as a proxy for timber harvest, as we did not have specific harvest data for the entire region). For the fifth path, we developed a path minimizing hli (a measure of solar radiation ranging from 0-1 as modified by McCune and Keon, 2002). In addition, because we suspected that canopy cover and solar radiation may interact, we created a least cost path based on the combination of the two variables. Our final least cost path maximized the movement along stream corridors. We did not restrict movement to streams and rivers, as this would create distances and routes that would be highly unlikely for a small frog to travel. Instead, we assigned a higher cost to terrestrial areas, so the least cost path would tend to follow stream corridors. We used a ratio of 10 (terrestrial): 1(stream) to calculate this path. We chose a 10:1 ratio because previous investigations of *A. truei* (Spear and Storfer 2008) demonstrated very little difference in model support with different cost ratios of stream layers, and we therefore selected 10:1 as an intermediate cost ratio. All least cost paths were produced using the “costdistance” and “costpath” function in ArcGIS 9.2 (ESRI, Inc.).

We calculated the values of several independent variables along each path route. We determined the distance along each route correcting for topographical changes (topographical distance) using the “surfacelength” function in ArcGIS 9.2 using a USGS digital elevation model (DEM) as the topographic surface. To incorporate fire history, we calculated the total distance that the route traveled through burned areas. In addition, to test whether any fire response was primarily due to the Great Burn Fire of 1910, we calculated distance through areas burned during the 1910 fire only.

The remaining variables were all continuous, and we therefore calculated a weighted average along each path for each variable. We produced a weighted average by first multiplying each individual value by the percent of the overall route that passed through pixels with that value, and then adding individual calculations together. Such variables included canopy cover , hli, degree of slope, number of frost-free days (ffp), and growing season precipitation (gsp) (Table 7 for sources and calculations).

We tested the correlation of the variables along each route by estimating pairwise value of G_{ST}’ using geographically weighted regression analysis (GWR; Fotheringham et al., 2002). GWR is a modification of spatial regression analyses that accounts for local differences, and is similar to global spatial autoregression analysis in that a spatial weighting matrix (a matrix of values that describe the spatial relationship among points) is used to incorporate a spatial component into the regression equation. However, GWR is performed by estimating regression parameters at each data point using only data points within a specified bandwith of the focal point and including a spatial weight in the local equation. To create a spatial weighting matrix based on distance requires that the matrix be based on point values. However, our G_{ST}’ values are based on lines which connect site pairs and we therefore designated the x and y coordinates of the midpoint of the straight-line route as the basis for the spatial weighting matrix. To produce a weight from these midpoints, we used a bi-square weighting function in which the spatial weight between two sites *I* and *j* is calculated as $W_{ij} = (1 – (d_{ij}/b)^2)$, where d represents the distance between the two midpoints and *b* is the bandwith. Instead of using a fixed bandwith across the entire area, we used an adaptive function that optimized AIC scores to determine the best bandwith. We specified that the bandwith include 10-15% of neighboring points, with the exact percentage determined by the bandwith which produced a model with the lowest AIC for each data point. The best overall GWR model across all potential paths was chosen based on AIC weights. All GWR analyses were implemented in SAM (Spatial Analysis in Macroecology) (Rangel et al., 2006).

Our final step was to examine whether there was local variation across each subregion with respect to how much variation was explained by the best overall model at each data point (Fig. 6). To do this, we calculated the Getis-Ord local statistic using the adjusted r^2 of each local regression. The Getis-Ord local statistic is a measure of whether high and low values of a variable are clustered (Getis and Ord. 1995). The Getis-Ord statistic is a Z score, and therefore a score greater than 1.96 indicates significant clustering of high values (hot spots) and a score less than -1.96 indicating significant clustering of low values (cold spot). In our case, hot spots would be identified as areas that fit the overall model especially well. We mapped all path midpoints that were either hot spots or cold spots in ArcGIS 9.2. Additionally, because cold spots could not be described well by the overall model, we ran a separate GWR analysis on any cold spot subset to identify the process influencing that cluster.

RESULTS

Population genetic structure

We obtained genotypes from individuals at 21 sites (total number of samples = 513; average sample size = 24) across the harvested subregion and at 20 sites (total number of samples = 496; average sample size = 25) across the roadless subregion. Only five sites (emer, johns, junct, merry and twin) had any full siblings detected, and therefore we removed one individual from any full sibling pair so there were zero full siblings in the analysis. There was very little evidence for linkage disequilibrium among locus pairs, as only 3 of 78 total pairs of loci were out of equilibrium. Furthermore, each of the three pairs was at disequilibrium at different sampling sites, suggesting no consistent pattern.

In general, genetic diversity was high across both subregions, based on both average alleles per locus (harvested = 34; roadless = 35) and expected heterozygosity (harvested = 0.87; roadless = 0.852). However, several loci (particularly A17, A26, A31, and A4) were out of Hardy-Weinberg equilibrium at multiple sites across both subregions. Estimates of null allele frequencies at each locus for both subregions strongly suggested that Hardy-Weinberg violations were due to null alleles (Table 8). Therefore, we estimated G_{ST}' based on the ENA algorithm to account for the prevalence of null alleles. The global G_{ST}' value for the harvested subregion was 0.054 with a 95% confidence interval of 0.022-0.099, whereas the G_{ST}' for the roadless subregion was 0.024 with a 95% confidence interval of 0.012-0.042. As a result, it appears there is greater genetic differentiation across the harvested subregion, although there is overlap in the 95% confidence intervals.

Across the harvested subregion, the majority of moderate to great differentiation (according to Wright's (1978) definition; see Table 9) are due to seven sites (mudcab, johns, emer, carpe, renfr, mtgul and merry) (Fig. 6A), with the johns site having the strongest differentiation from other sites, as 12 of its pairwise values can be considered greatly differentiated (G_{ST}' = 0.15-0.25). Additionally, we detected no evidence of very recent population size declines as there was no significant heterozygosity excess and no shifted allele frequencies. We did find a signature of more historic declines based on the M-ratio tests (Table 10). Across the harvested subregion 19 of 21 sites had an average M-ratio less than 0.68, with overall high variance among loci (0.04-0.06).

Across the roadless subregion, differentiation is due almost entirely to three sites: fox, fire and grave (Table 11; Figure 8B). As would be expected from the global value, most differentiation is only at a moderate level (G_{ST}' = 0.06-0.13), with just two pairwise comparison with a value of 0.15. As with the harvested subregion, there was no evidence for recent population declines based on heterezygosity excess or shifted allele distribution, but there were low M-ratios across most sites. Specifically, 16 of 20 sites had M-ratios less than 0.68, and variance among loci was high as well (Table 10).

Landscape analysis

Across the harvested subregion, the best supported model followed the riparian least cost path and include total topographic distance and growing season precipitation (r^2 = 0.73, AIC weight = 0.80; Table 12). Topographic distance was positively correlated with increased genetic differentiation, whereas precipitation was negatively correlated with differentiation. The only other model that had support based on AIC weight was the least cost path maximizing canopy cover, with total topographic distance, precipitation and canopy cover as variables included (r^2 = 0.74, AIC weight = 0.18; Table 12). Total topographic distance and precipitation had the same directional relationship with G_{ST}' as in the above model and canopy cover was negatively correlated with G_{ST}'. Table 12 lists the best supported models from the other connectivity paths tested, but none of these additional paths are supported, with AIC weights at 0.1 or 0.

Across the roadless subregion, the least cost path minimizing movement through areas with high solar radiation (measured with hli) and including topographic distance through burned areas and hli as independent variables had the greatest support ($r^2 = 0.75$, AIC weight = 0.92; Table 13). Fire distance is positively correlated with differentiation, whereas hli is negatively correlated with genetic differentiation. Only two other models have any support; the least-cost path based on canopy cover which includes topographic distance and frost-free period ($r^2 = 0.74$, AIC weight = 0.05; Table 13), and the least cost path combining both canopy cover and hli, which includes total topographic distance through burned areas and hli ($r^2 = 0.74$, AIC weight = 0.03; Table 13). Both total distance and distance through burned areas are positively correlated with G_{ST}', and frost-free period and hli were negatively correlated with G_{ST}'.

Plotting the results of the Getis-Ord hot spot analysis from the local regressions of the best model across the harvested subregion identified two hot spot clusters (comparisons for which the model explained a high degree of variation) and one cold spot cluster (comparisons that had weak fit to the global model) (Fig. 7). The hot spots roughly coincided with private timberlands, whereas the cold spot stretched across land that was primarily publicly-owned. When only the cold spot pairwise values (n=73) were included in an analysis, the best model was based on straight-line distances through burned areas as the only independent variable.

Across the roadless subregion, the Getis-Ord analysis for the best supported model shows a north-south divide in model support, with the northern area representing a cold spot, and the southern region representing a hot spot (Fig. 8). These two clusters correspond well to fire history, with the unburned areas representing a cold spot for the model. Analyzing these cold spot data separately (n = 77) suggest that no tested landscape variables affect gene flow, as the best model was based on topographic distance across a straight line.

DISCUSSION

Understanding the impact of broad-scale disturbances, such as fire, in addition to anthropogenic effects, such as timber harvest, is an important future direction for conserving populations and landscapes (Lindenmayer et al., 2008). While fire and timber harvest are superficially similar, the major difference is the presence of biological legacies and more heterogeneous structure in burned landscapes (Lindenmayer and McCarthy, 2002). In this study, we demonstrate that a small forest-associated amphibian does respond differently to natural and anthropogenic disturbance, in a manner that is consistent with differences between fire and timber harvest. In particular, we demonstrate a high degree of gene flow in tailed frogs across a large study area with a history of broad natural disturbance, the resilience of populations to disturbance despite a probable initial decline, and the adjustment of movement corridors in actively harvested landscapes. Based on a comparison with a sister species in a less fire-prone landscape, our results collectively suggest that a prior history of natural disturbance may help explain differential population response to recent stressors (timber harvest), which has strong implications for current conservation. Therefore, we discuss how these results may be relevant for a variety of taxa that share similar biogeographical patterns as tailed frogs.

Genetic connectivity across disturbed landscapes

Overall, there was higher connectivity across the roadless subregion as compared to the harvested subregion, although genetic differentiation was not high in either region. The degree to which the roadless subregion had higher genetic connectivity strongly supports our hypothesis that fire is less of a hindrance to gene flow than timber harvest. The difference in genetic connectivity is certainly not due to isolation by distance, as the roadless subregion encompasses roughly double the area of the harvested subregion. The extent of genetic population differentiation is even more surprising when compared to a similar study on the closely related coastal tailed frogs on the Olympic Peninsula. The former study revealed a G_{ST}' of 0.16 across the unharvested Olympic National Park (although this subdivision was primarily due to alpine meadows in the park) and 0.04 within the managed Olympic National Forest (Spear and Storfer 2008), as compared to 0.024 (roadless subregion) and 0.054 (harvested subregion). This is despite the fact that the Olympic region has much higher precipitation, cooler temperatures and more infrequent disturbance regime than in the region considered in the present study. It is clear that Rocky Mountain tailed frogs have been able to maintain a level of genetic connectivity equal to or greater than that of its sister species despite harsher climatic or disturbance conditions across the northern Rocky Mountains, perhaps due to an enhanced ability to direct movement through suitable corridors. There is some evidence that Rocky Mountain tailed frog tadpoles survive in streams with temperatures above the general thermal limit for tailed frogs (Dunham et al. 2003) and therefore Rocky Mountain tailed frogs may be less physiologically constrained than the coastal species.

Examining the individual pairwise G_{ST}' values for the harvested subregion suggested that six sites were primarily responsible for most the genetic differentiation observed. Five of the sites (johns, carpen, emer, mtgul and mudcab) are separated from the rest of the sites by two state highways that run along the two main river systems, and this may explain a large portion of the differentiation. However, the other highly differentiated site (merry) is within the main cluster of sampling sites and has no obvious barriers surrounding it. Merry had three full sibling pairs (the only site with greater than one), and although full siblings were removed from the analysis, it might be symptomatic of a process that is uniquely affecting this site. The three sites with greatest differentiation across the roadless subregion are likely an isolation by distance effect, as they were the three most distant sites form the main cluster.

Population size trends across subregions

It does not appear that either subregion has undergone a recent severe decline (based on the heterozygosity excess and shifted distribution tests), but both regions have likely suffered more historic declines, as indicated by the M-ratios. The historic declines were widespread, as the majority of sites had low M-ratios. However, Garza and Williamson (2001) suggest a sample size of 25 and several of our sites are below this threshold. As a result, we only consider further the M-ratios at sites with sufficient sample size.

Across the roadless subregion, the most obvious explanation is that the reductions are due to the very large stand-replacing fires that took place across the area in the early 20th century. Nine sites (fern, john, twin, quartz, fox, cool, hidden, goose and orofin) have no recorded fire history. Two of these sites, quart and fox, had among the lowest M-ratios (0.504-0.569), but also had the lowest samples sizes (11 and 12, respectively). If we restrict our comparison to sites of sufficient sample size, the average M-ratio in unburned areas was near the critical value (0.675), whereas sites in burned areas had a lower M-ratio of 0.655. Additionally, three of the four individual sites that were above the critical value of 0.68 were found in unburned areas (Table 10). However, the differences in M-ratio were not great and it is hard to conclude whether they are significantly different.

The fact that fire appears to create an initial reduction in population size of Rocky Mountain tailed frogs has previously been demonstrated by research on abundance in Glacier National Park (Hossack et al., 2006) and central Idaho (Pilliod et al., 2004). In both cases, the declines occurred more severely in 1st year tadpoles as compared to older tadpoles (tailed frogs generally have a larval period of 2-3 years). These data imply that either 1st year tadpoles are less likely to survive in stream conditions immediately post-fire, or that breeding adults are reduced. However, Hossack et al. (2006) speculated that populations would likely recover. Our results are consistent with this view, as M-ratios suggest an initial decline, but the current high observed heterozygosity (0.756), lack of heterozygosity excess, and average number of alleles per locus (35), taken together suggest populations have sufficiently recovered from fire. Particularly surprising is that these apparent recoveries occurred in spite of what many consider to be the most severe fire in United States recorded history (the 1910 Great Burn fire, Pyne 2001).

The pattern of low M-ratios across the harvested subregion is more difficult to interpret. The two sites with M-ratios greater than 0.68 are not geographically close to each other. It is probable that fires led to some declines, but fires have been much more sporadic and smaller over the harvested subregion, and could not explain the M-ratio results for all sites. Therefore, we suspect that reduced M-ratios in unburned sites are due to timber harvest. This particular pattern is interesting for two reasons. First, significant reductions in effective population size of coastal tailed frogs were detected across several sites in the managed Olympic National Forest, but these were detected based on heterozygosity excess, not M-ratios (Spear and Storfer, 2008). Thus, the time frame of decline differs between the two areas, despite a similar temporal extent of harvest between the two areas. Second, timber harvest is still an ongoing activity across the Idaho harvested subregion, but the lack of heterozygosity excess and shifted distributions, as well as maintenance of high genetic diversity ($H_o = 0.78$) suggest that declines are not ongoing. We must acknowledge that presence of null alleles are a source of error for the heterozygosity excess analysis (Cornuet and Luikart, 1996) and thus could mask detection of actual declines, but we have no available evidence that population size is currently being depressed.

Landscape models of gene flow

Movement along stream corridors and growing season precipitation were, by a high AIC weight, included in the best supported model accounting for differentiation across the harvested subregion. This was an unexpected result, as gene flow along streams was not supported for coastal tailed frogs on the Olympics. That is, the stream paths were the least supported model across both protected and harvested forests (Spear and Storfer, 2008). Additionally, precipitation was not included in any of the coastal tailed frog models from the previous study, but is a significant variable explaining the spatial distribution of genetic across the harvested area studied herein. Precipitation is roughly double on the Olympic Peninsula than the northern Rockies, and therefore is not likely a limiting factor to coastal tailed frogs inhabiting this region. However, in areas with less rainfall, small differences over the landscape may have a greater impact on successful dispersal and explain its importance in our models.

We believe that these results suggest that Rocky Mountain tailed frogs may have used the riparian corridors to actively avoid the disturbed areas due to harvest. This response may be also encouraged by the practice of maintaining strips of forest along streams (buffer zones) in harvested patches. Theoretical work has found that directed dispersal evolves in the presence of autocorrelated disturbance (which describes fire and timber harvest), whereas in undisturbed areas directed movement is selectively neutral (Armsworth and Roughgarden, 2005). Directed movement between favorable, moist microhabitats has been demonstrated in wood frogs (Baldwin et al., 2006), and is quite feasible for tailed frogs which have even a lower dessication tolerance. Therefore, Rocky Mountain tailed frogs may be adapted to alter movement patterns quickly to seek out favorable microhabitats (protected riparian corridors and wet areas). In contrast, the comparatively less disturbed Olympic region would not strongly select for directed dispersal in coastal tailed frogs due to much higher levels of annual precipitation and lack of a strong selection pressure to avoid disturbance. Alternatively, tailed frogs across the Olympics may avoid stream corridors because coastal stream networks are isolated from each other (i.e. drain into the ocean), and stream use as corridors could lead to inbreeding effects.

Ultimately, the most important implication for conservation and management is that it is probably inappropriate to manage the two species of tailed frogs similarly. For Rocky Mountain tailed frogs in northern Idaho, riparian stream buffers appear necessary for protection, and a larger extent of forest protection should occur around headwater areas near divides that would provide the easiest access for frogs to move between drainage basins. Coastal tailed frogs, on the other hand, might require maintenance of forested terrestrial corridors with favorable landscape characteristics between drainages (such as low solar radiation; Spear and Storfer, 2008).

Across the roadless subregion, the terrestrial landscape appears to primarily influence genetic structure. Gene flow is best correlated with a least cost path that minimizes exposure to high solar radiation and topographical distance through burned areas had higher support than total topographical distance. Despite the inclusion of fire effects into the best model, a least cost path avoiding burned areas was not supported, regardless of the cost ratio. This is best explained by both the sheer extent of fire in this region and the coarseness of the spatial data, which simply created a polygon from the fire boundary. The size of the fires in the area was so large that it was probably impossible for a frog to completely avoid the burned region and travel around it. Secondarily, studies of the 1988 massive Yellowstone fires have demonstrated the heterogeneity of stand-replacing fires (Turner et al., 2003). A study in Spain demonstrated a positive relationship between fire occurrence and solar radiation (Díaz-Delgado et al., 2004), and so it is possible that heat load index is a proxy for fire occurrence or severity at a fine scale. If so, then perhaps gene flow is responding to fire heterogeneity to avoid the most severely burned areas. Overall, it appears that there has been little hindrance to genetic connectivity in the roadless subregion since the several large fires that occurred in the early 20th century, despite large losses of canopy cover in those fires. This may underscore the importance of biological legacies in the form of downed trees for tailed frog movement. There have been multiple studies that have demonstrated the importance of coarse woody debris to amphibians (Waldick et al, 1999, Butts and McComb, 2000, Thompson et al, 2003, Alkaslassy, 2005, Rittenhouse et al, 2008). Additionally, tiger salamanders gene flow in Yellowstone National Park was highest across previously burned areas (Spear et al, 2005). If this is the main explanation for differences between fire and logging effects, harvest regulations that maintained downed woody debris in cut areas should greatly ameliorate detrimental impacts.

Local variability in landscape influences on gene flow

An advantage of the geographically weighted regression analysis is the ability to test whether the same process is occurring over a study area (known as stationarity), and if not, identify the location of any discontinuities. The local hot spot analysis clearly showed that non-stationarity occurred in both subregions, and the pattern of the clusters gave further insight into the factors driving genetic structure. Across the harvested subregion, the clusters were layered, with hot spots (strong support for the model) at the north and south ends of the region, whereas points in the interior were a cold spot. The clusters did not seem to have any topographical or hydrological association, but do roughly match up with land ownership, with the hot spots associated with private forests and cold spots with public lands(Fig. 7). Therefore, as the hot spot represents areas which have the support the riparian movement model, the pattern suggests that frogs are most likely to modify movement in private industrial forests more so than national forest or state lands. Private forests are managed primarily for timber production, whereas public lands are managed for multiple uses, of which logging is only one part. Thus, it appears that intensive, regular logging is needed for shifts in patterns of genetic connectivity to occur. Interestingly, the G_{ST}’ values included in the cold spot (i.e. public lands) were best described by distance through burned areas along a straight line path. Thus, Rocky Mountain tailed frogs dispersing across lands managed for multiple uses (and therefore have less intensive anthropogenic disturbance) do not seem to have gene flow significantly altered. This also suggests that the presence of some reserves of protected land (as occurs in parts of the national forest in the harvested region) may alleviate detrimental effects of more intensive habitat alteration.

The roadless subregion also displays local variability in model support, with the least support in the northwest corner, which largely lacks a recorded fire history. Analyzing the subset of comparisons in this cold spot did not identify any variables that significantly influenced genetic differentiation. In other words, in the absence of any disturbance, tailed frogs in forested areas may be limited in dispersal only by distance, which may be up to tens of kilometers. This conclusion is consistent with *A. truei*, which had a gene flow to an extent of approximately 30km in the absence of alpine meadows or harvested patches (Spear and Storfer 2008).

Conclusions

We find strong evidence that natural and anthropogenic disturbances do not affect populations in the same manner. While both disturbances appear to have affected genetic diversity similarly, the response of genetic connectivity has been quite different. Thus, managers need to consider the effects of fire and timber harvest separately when developing conservation plans for species. While fire does inhibit gene flow to some extent, populations in extensively burned areas maintain high connectivity and terrestrial gene flow. In contrast, timber harvest leads to a different connectivity pattern, in which gene flow follows a narrow riparian corridor and overall differentiation is increased. From our analysis, we cannot identify the exact reasons for these differences. A likely possibility discussed above is that the downed trees remaining in natural, non-salvaged burned areas present sufficient cover to facilitate tailed frog movement across the landscape. Additionally, fires can burn down to the stream so the stream lacks a forested buffer zone found in a managed landscape. As a result, there may be no benefit to a frog to move along riparian corridors after burns if the stream sides are burned as well. Given our general lack of understanding of the influence of various types of complex disturbances on population ecology of vertebrates, we hope further studies will test the generality of our results to a variety of amphibians and other forest species.

A second major conclusion is that *A. montanus* responds to environmental disturbances differently than its sister species *A. truei*. This is significant because the two species are nearly indistinguishable morphologically and the two species were only separated recently due to strong genetic differences (Nielson et al., 2001). The difference in gene flow patterns is a rare example of a potential ecological difference between the two species. Accounting for these differences is important to many more species beyond tailed frogs. The two tailed frog species are one pair of a large number of species ranging from trees to mammals that display a disjunct pattern of a Pacific Northwest coastal (sub)species and an inland northern Rocky Mountain population (Brunsfeld et al., 2001). Although the specific responses to disturbance will obviously vary by species' ecological characteristics, one should not assume that morphological similarity will necessarily lead to the same response to alterations in the landscape and studies to guide conservation and management should occur in both regions. As direct monitoring is quite difficult for many small animals, landscape genetic techniques should continue to be a useful approach to assess population responses over a broad range and variety of habitats.

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APPENDIX 1 – Multiplex PCR conditions for *Ascaphus* (locus names are as in Spear et al. 2008)

All runs have 10 µl total volumes/sample
All multiplexes have: 5ul Qiagen Master Mix/sample
0.5 µl Q solution/sample
1 µl template DNA/sample
All primer volumes are equal for both forward and reverse primers, and are at 5 µM stock concentrations.

<u>Multiplex 1-</u> [96 deg 15 min, (94 deg 30 s, 55 deg 90 s, 72 deg 60 s)29X, 60 deg 30 min]
A2 – 0.3 µl
A4 – 0.5 µl
A12 – 0.05 µl
A31 – 0.05 µl
1.7 µl water

<u>Multiplex 2</u> – [96 deg 15 min, (94 deg 30 s, 55 deg 90 s, 72 deg 60 s)28X, 60 deg 30 min]
A15 – 0.3 µl
A24 – 0.15 µl
A26 – 0.25 µl
A29 – 0.1 µl
1.9 µl water

<u>Multiplex 3</u> – [96 deg 15 min, (94 deg 30 s, 60 deg 90 s, 72 deg 60 s)26X, 60 deg 30 min]
A1 – 0.075 µl
A3 – 0.45 µl
A13 – 0.1 µl
A14 – 0.15 µl
A17 – 0.15 µl
1.65 µl water

FIGURE LEGENDS

Figure 5. Map of sampling localities. Background is a digital elevation model, with dark areas as low elevation and lighter areas represent higher elevations. Inset indicates relative location of study areas in the state of Idaho, USA. A) Sites within the harvested subregion B) sites within the roadless subregion.

Figure 6. Flow chart depicting sequence of steps for full geographically weighted regression analysis, including identification of hot spot and cold spot clusters.

Figure 7. Results of Getis-Ord hot spot analysis across the harvested subregion. Symbols represent midpoints of pairwise comparisons. Circles represent hot spots and triangles represent cold spots. A) Background of digital elevation models B) background represents public and private ownership, with black areas as public land and white areas as private land.

Figure 8. Results of Getis-Ord hot spot analysis across the roadless subregion. Symbols represent midpoints of pairwise comparisons. Circles represent hot spots and triangles represent cold spots. A) Background of digital elevation model B) background represents fire history, with black areas as burned patches.

TABLES

Table 7. Variables used in least-cost (LC) path development and/or as independent in geographically weighted regression models.

Variable	**unit(s)**	**LC path**	**Data Source(s)**	**Resolution**	**Calculation along LC paths**
Topographic distance	meters	Yes	Digital Elevation Model (DEM)	30 x 30 m	Sum
Distance through burned areas	meters	Yes (2:1; 10:1)	Clearwater National Forest (2008), Idaho Panhandle National Forest (2008)	NA	Sum
Canopy Cover	percent	Yes (1-percent)	National Land Cover Dataset (NLCD 2001)	30 x 30 m	Weighted average
Heat Load Index (hli)	unitless, McCune and Keon, 2002	Yes	DEM	30 x 30 m	Weighted average
Canopy Cover/Heat Load Index	unitless, (hli*100 + (1 – canopy cover))	Yes	DEM, NLCD 2001	30 x 30 m	not included
Streams	meters	Yes (10:1)	Clearwater National Forest (2001), USGS National Hydrography Dataset	NA	not included
Slope	degrees	No	DEM	30 x 30 m	Weighted average
Frost-free period (ffp)	days	No	spline-based model (Rehfeldt et al. 2006)	1 x 1 km	Weighted average
Growing season precipitation (gsp)	millimeters	No	spline-based model (Rehfeldt et al. 2006)	1 x 1 km	Weighted average

Table 8. Average null allele frequency averaged across all sites in each subregion. Null allele frequency was estimated based on the EM algorithm (Dempster et al. 1977)

Locus	Harvested subregion Null allele freq.	Roadless subregion Null allele freq.
A1	0.012	0.039
A12	0.032	0.090
A13	0.012	0.012
A14	0.018	0.012
A15	0.003	0.007
A17	0.212	0.183
A2	0.015	0.053
A24	0.012	0.027
A26	0.208	0.315
A29	0.050	0.007
A3	0.070	0.053
A31	0.098	0.006
A4	0.087	0.139

Table 9. Pairwise G_{ST}' values across the harvested subregion. Site names are as in Figure 1 (abbreviated in some cases). Bolded values indicate moderate differentiation (≥0.05) and bolded and italicized values represent great differentiaton (≥0.15) (Wright 1978).

	CED	MUD	PC10	PO3	PC12	CAT	PO5	PC5	PC7	STM	M1	M7	JOH	EME	CAR	REN	HOM	MAR	LNF	MTG
MUD	**0.12**																			
PC10	0.00	**0.07**																		
PO3	0.05	**0.06**	0.02																	
PC12	0.00	0.03	-0.01	0.01																
CAT	0.01	**0.11**	-0.01	0.04	-0.01															
PO5	0.03	**0.07**	-0.01	-0.01	0.00	-0.01														
PC5	0.04	**0.08**	0.01	0.01	0.00	0.03	0.00													
PC7	0.04	**0.10**	0.03	0.04	0.04	0.01	0.01	0.01												
STM	0.03	**0.08**	-0.02	0.04	0.00	-0.01	0.00	0.01	0.00											
M1	0.04	0.05	-0.02	0.02	0.00	0.00	-0.02	0.01	0.03	-0.01										
M7	0.01	0.04	0.00	-0.01	-0.03	0.01	-0.02	-0.04	0.00	0.02	0.00									
JOH	***0.25***	***0.23***	***0.15***	**0.14**	***0.19***	***0.20***	***0.19***	**0.10**	***0.17***	***0.20***	***0.18***	**0.12**								
EME	**0.14**	***0.16***	**0.11**	**0.07**	**0.09**	**0.11**	**0.12**	0.04	**0.11**	**0.12**	**0.10**	0.03	0.02							
CAR	**0.13**	**0.13**	**0.09**	**0.06**	**0.07**	**0.11**	**0.08**	0.04	**0.09**	**0.09**	**0.08**	0.04	0.04	-0.01						
REN	**0.06**	**0.09**	**0.05**	0.03	**0.05**	**0.07**	0.01	0.02	0.04	**0.06**	**0.05**	0.00	***0.16***	**0.10**	**0.09**					
HOM	0.00	**0.07**	0.00	-0.01	0.01	0.02	0.00	-0.04	0.00	0.01	0.01	-0.05	**0.11**	0.04	**0.06**	0.01				
MAR	0.01	**0.12**	0.03	0.00	-0.01	0.00	0.01	0.00	0.02	0.03	0.01	-0.04	***0.16***	**0.05**	**0.06**	0.04	-0.03			
LNF	0.03	**0.11**	0.00	**0.06**	0.02	-0.01	0.00	**0.06**	0.02	0.01	0.01	0.04	***0.25***	***0.19***	***0.18***	**0.08**	0.03	0.04		
MTG	***0.16***	***0.18***	**0.11**	**0.09**	**0.11**	**0.14**	**0.12**	0.05	**0.11**	**0.13**	**0.11**	**0.05**	0.01	-0.02	0.00	**0.14**	**0.05**	**0.09**	*0.19*	
MER	**0.09**	***0.16***	0.03	**0.05**	**0.06**	**0.07**	**0.09**	0.01	**0.07**	**0.07**	**0.05**	0.01	**0.05**	0.04	0.02	**0.11**	-0.01	**0.06**	**0.13**	0.01

Table 10. M-ratio values (averaged over all loci) for each site in both subregions. N represents sample size at site. Bolded values are those below the critical value of 0.68 indicating a decline (Garza and Willamson 2001).

Harvested subregion				Roadless subregion			
Site	N	M-ratio	Variance	Site	N	M-ratio	Variance
CEDAR	27	**0.664**	0.049	FERN	33	**0.677**	0.044
MUDCAB	19	**0.611**	0.046	JOHN	30	0.684	0.038
PC10	30	0.706	0.043	TWIN	32	0.698	0.047
PO3	30	**0.646**	0.032	QUARTZ	11	**0.504**	0.035
PC12	18	**0.566**	0.06	FOX	12	**0.569**	0.085
CAT	30	**0.655**	0.04	JUNCT	30	**0.633**	0.047
PO5	29	**0.649**	0.061	SPRUCE	27	**0.619**	0.043
PC5	28	**0.671**	0.055	COOL	35	**0.675**	0.065
PC7	30	**0.651**	0.066	CAVE	20	**0.61**	0.058
STM	30	**0.652**	0.064	BDTRIB	32	0.701	0.039
M1	22	**0.635**	0.062	ELIZB	30	**0.67**	0.042
M7	25	**0.634**	0.062	HIDDEN	31	**0.675**	0.07
JOHNS	19	**0.662**	0.076	GOOSE	30	**0.616**	0.046
EMER	19	**0.637**	0.048	4JUL	20	**0.585**	0.06
CARPEN	20	**0.664**	0.06	BEAR	12	**0.521**	0.065
RENFRO	20	**0.621**	0.069	PETOTT	20	**0.568**	0.04
HOMEST	20	**0.609**	0.058	OROFIN	32	0.7	0.055
MARBL1	20	**0.615**	0.056	FIRE	19	**0.626**	0.06
LNFCL	20	**0.661**	0.037	GRAVE	20	**0.608**	0.069
MTGUL	40	0.73	0.046	CGRAVE	20	**0.62**	0.06
MERRY	17	**0.657**	0.044				

Table 11. Pairwise G_{ST}' values across the roadless subregion. Site names are as in Figure 2 (abbreviated in some cases). Bolded values indicate moderate differentiation (≥0.05) and bolded and italicized values represent great differentiaton (≥0.15) (Wright 1978).

	FER	JOH	TWI	QUA	FOX	JUN	SPR	COO	CAV	BDT	ELI	HID	GOO	4JU	BEA	PET	ORO	FIR	GRA
JOH	0.00																		
TWI	0.01	0.03																	
QUA	-0.03	-0.03	-0.02																
FOX	**0.06**	**0.06**	**0.09**	**0.08**															
JUN	0.01	0.02	0.05	0.00	**0.07**														
SPR	0.01	0.03	0.05	-0.03	**0.11**	0.02													
COO	-0.02	0.00	0.03	-0.04	**0.06**	0.00	0.00												
CAV	-0.02	-0.02	0.01	-0.02	**0.06**	0.01	0.01	-0.01											
BDT	-0.02	0.01	0.01	-0.02	**0.07**	0.01	0.00	0.00	0.01										
ELI	0.01	0.02	0.01	-0.02	**0.10**	0.01	0.02	0.02	0.02	0.01									
HID	0.02	0.02	0.03	-0.03	**0.09**	0.01	0.03	0.01	0.02	0.04	0.00								
GOO	0.01	0.01	0.03	-0.02	**0.12**	0.01	0.04	0.01	0.00	0.02	-0.02	0.01							
4JU	0.01	0.02	0.04	-0.04	**0.10**	0.02	-0.02	0.00	0.01	0.01	0.02	0.00	0.02						
BEA	0.02	0.06	**0.07**	-0.03	**0.13**	0.02	0.02	-0.01	0.04	0.00	0.01	0.02	0.02	-0.01					
PET	0.00	0.03	-0.01	-0.01	**0.07**	-0.01	0.02	0.00	0.00	-0.01	-0.01	0.01	0.00	-0.01	0.00				
ORO	-0.01	-0.01	0.00	-0.02	**0.08**	0.00	0.00	-0.02	-0.01	-0.02	0.01	0.02	0.00	-0.01	0.01	0.00			
FIR	**0.12**	**0.13**	**0.13**	**0.13**	**0.08**	**0.10**	**0.09**	**0.09**	**0.09**	**0.06**	**0.12**	***0.15***	***0.15***	**0.13**	**0.15**	**0.09**	**0.11**		
GRA	0.06	**0.07**	**0.10**	**0.06**	**0.08**	0.04	**0.07**	0.05	**0.05**	0.04	**0.06**	**0.08**	**0.09**	**0.09**	**0.07**	0.01	**0.05**	0.02	
CGR	0.02	0.03	**0.06**	0.02	0.05	-0.01	0.00	-0.01	0.01	0.00	0.02	0.01	0.03	0.01	0.02	0.02	0.00	0.04	0.03

Table 12. Model results from geographically weighted regression analysis for the harvested subregion. Path is the least cost path upon which the model is based. Variables are the independent variables included in the model and direction represents correlation with G_{ST}'. All models with AIC weight > 0 are included as well as the best supported model for each type of path, regardless of AIC support.

Path	**Variables**	**Direction**	**r^2**	**AIC**	**AIC wt.**
LC stream	Distance	+	0.73	-775	0.8
	Gsp	-			
LC canopy	Distance	+	0.74	-772	0.18
	Gsp	-			
	Canopy	-			
LC canopy/hli	Distance	+	0.71	-767	0.01
	Gsp	-			
LC canopy/hli	Distance	+	0.74	-765	0.01
	Gsp	-			
	Canopy	-			
Straight	Distance	+	0.73	-760	0
	Gsp	-			
	Ffp	-			
LC fire (2:1)	Distance	+	0.69	-752	0
	Gsp	-			
LC hli	Distance	+	0.71	-751	0
	Canopy	-			
LC fire (10:1)	Distance	+	0.67	-736	0
	Gsp	-			

Table 13. Model results from geographically weighted regression analysis for the roadless subregion. Path is the least cost path upon which the model is based. Variables are the independent variables included in the model and direction represents correlation with G_{ST}'. All models with AIC weight > 0 are included as well as the best supported model for each type of path, regardless of AIC support.

Path	**Variables**	**Direction**	**r^2**	**AIC**	**AIC wt.**
LC hli	Fire distance	+	0.75	-854	0.92
	Hli	-			
LC canopy	Distance	+	0.74	-848	0.05
	Ffp	+			
LC canopy/hli	Fire distance	+	0.74	-847	0.03
	Hli	-			
LC stream	Fire distance	+	0.73	-839	0
	Ffp	-			
Straight	Fire distance	+	0.7	-835	0
LC fire (2:1)	Distance	+	0.68	-832	0
LC fire (10:1)	Distance	+	0.71	-797	0
	Ffp	+			
	Gsp	+			

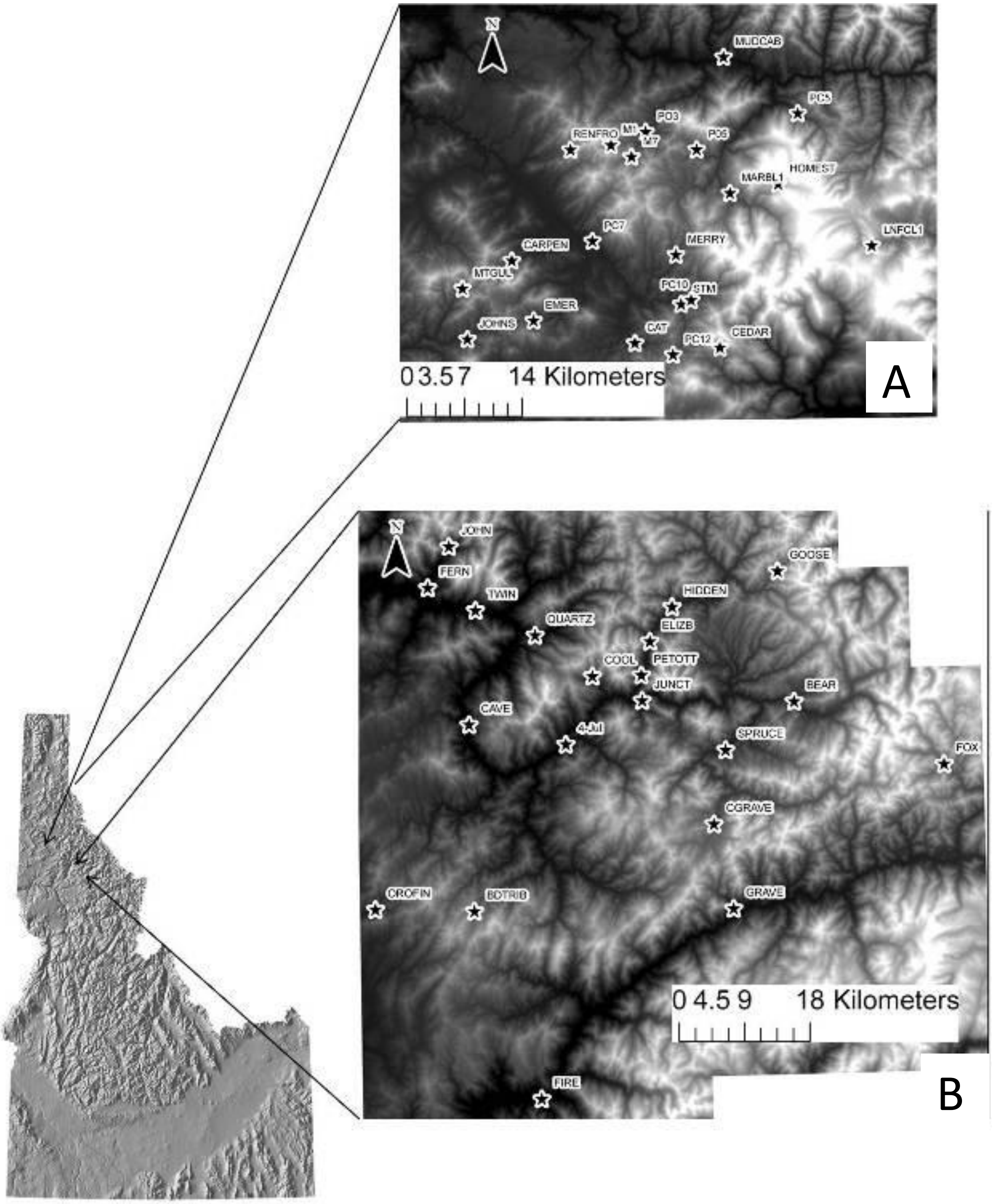

N
MUDCAB
PC5
PO3
M1
M7
RENFRO
PO6
MARBL1
HOMEST
LNFCL1
PC7
MERRY
CARPEN
MTGUL
PC10
STM
EMER
JOHNS
CAT
PC12
CEDAR
0 3.5 7 14 Kilometers
A
N
JOHN
GOOSE
FERN
TWIN
HIDDEN
QUARTZ
ELIZB
COOL
PETOTT
JUNCT
BEAR
CAVE
4-Jul
SPRUCE
FOX
CGRAVE
CROFIN
BDTRIB
GRAVE
0 4.5 9 18 Kilometers
FIRE
B

Figure 5

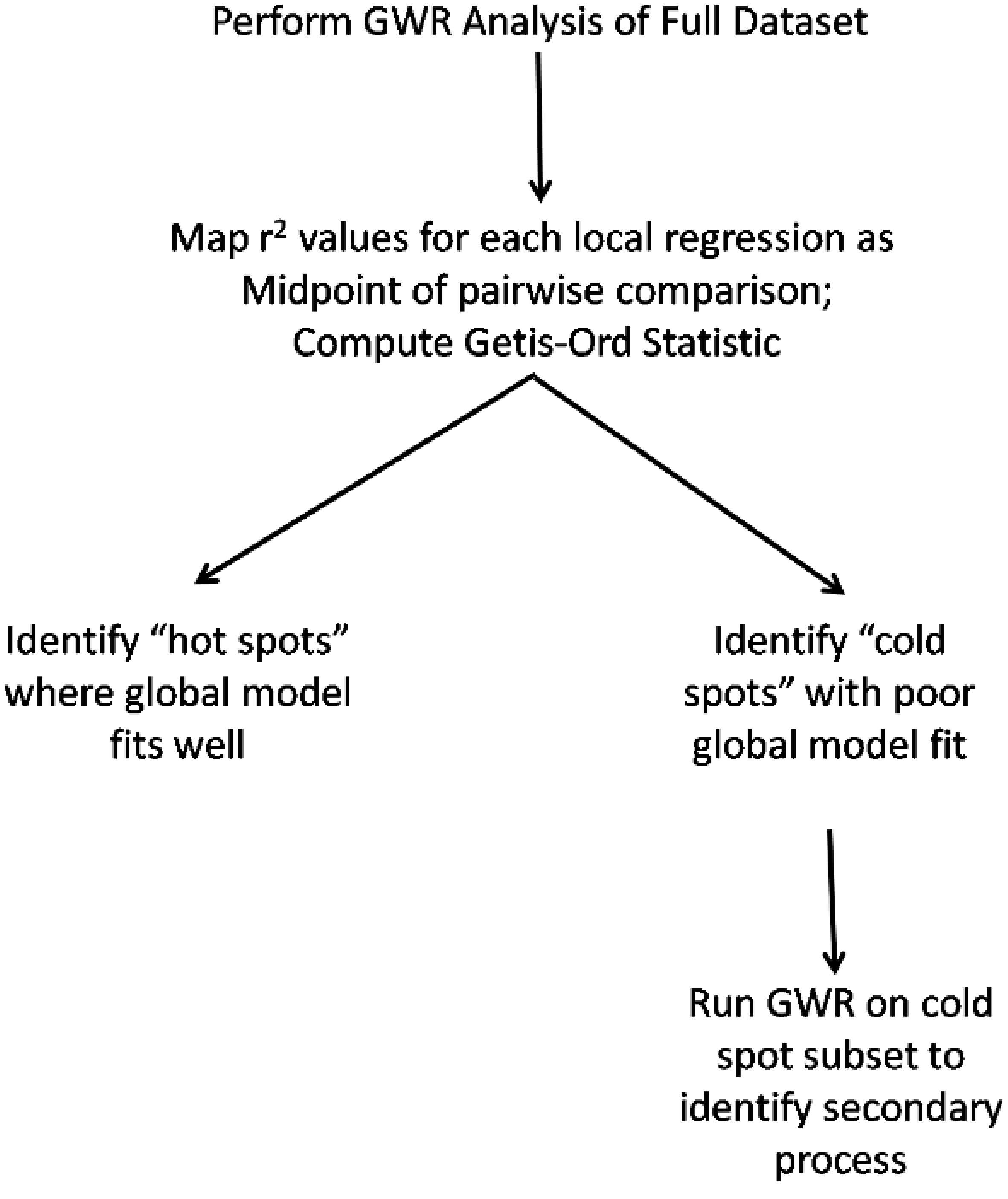
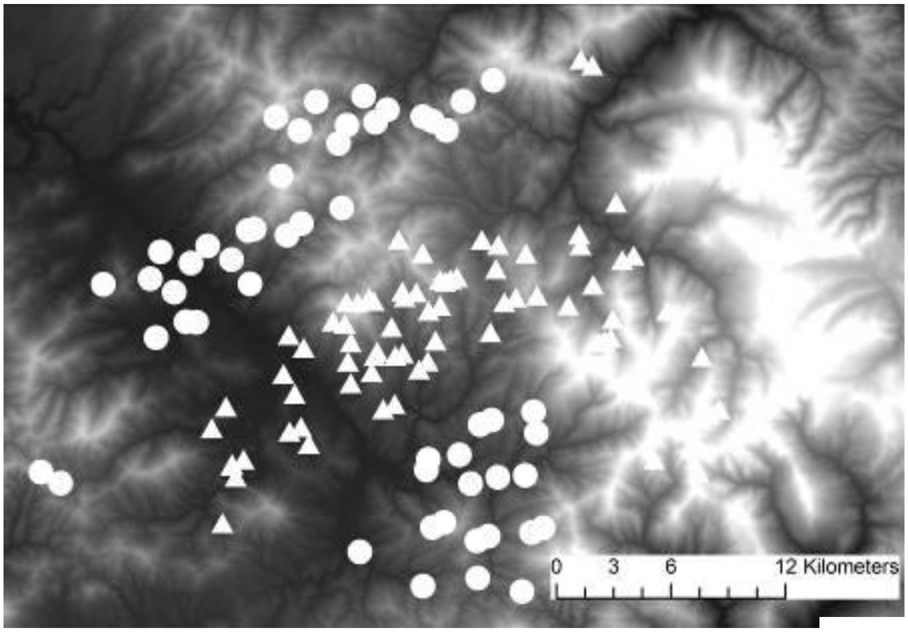
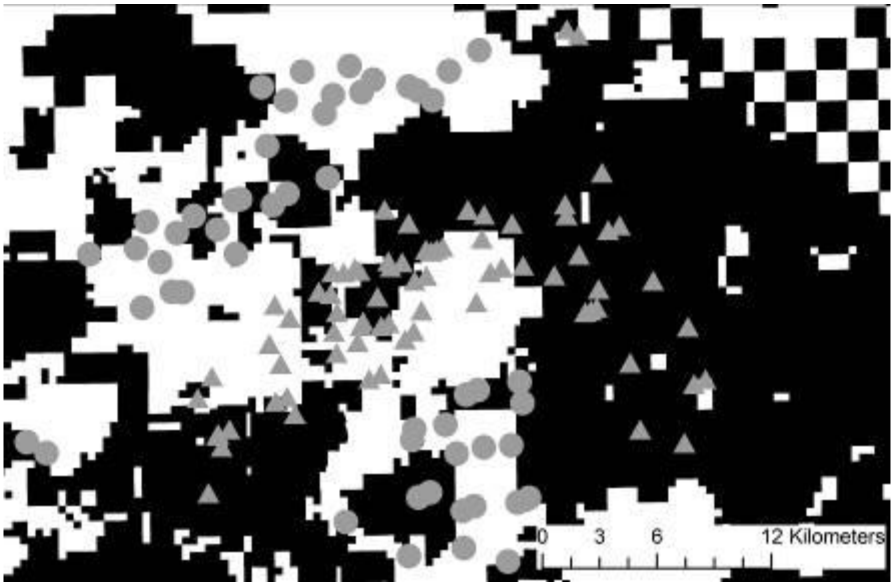

Perform GWR Analysis of Full Dataset
Map r2 values for each local regression as Midpoint of pairwise comparison; Compute Getis-Ord Statistic
Identify “hot spots” where global model fits well
Identify “cold spots” with poor global model fit
Run GWR on cold spot subset to identify secondary process

Figure 6



A



B

Figure 7

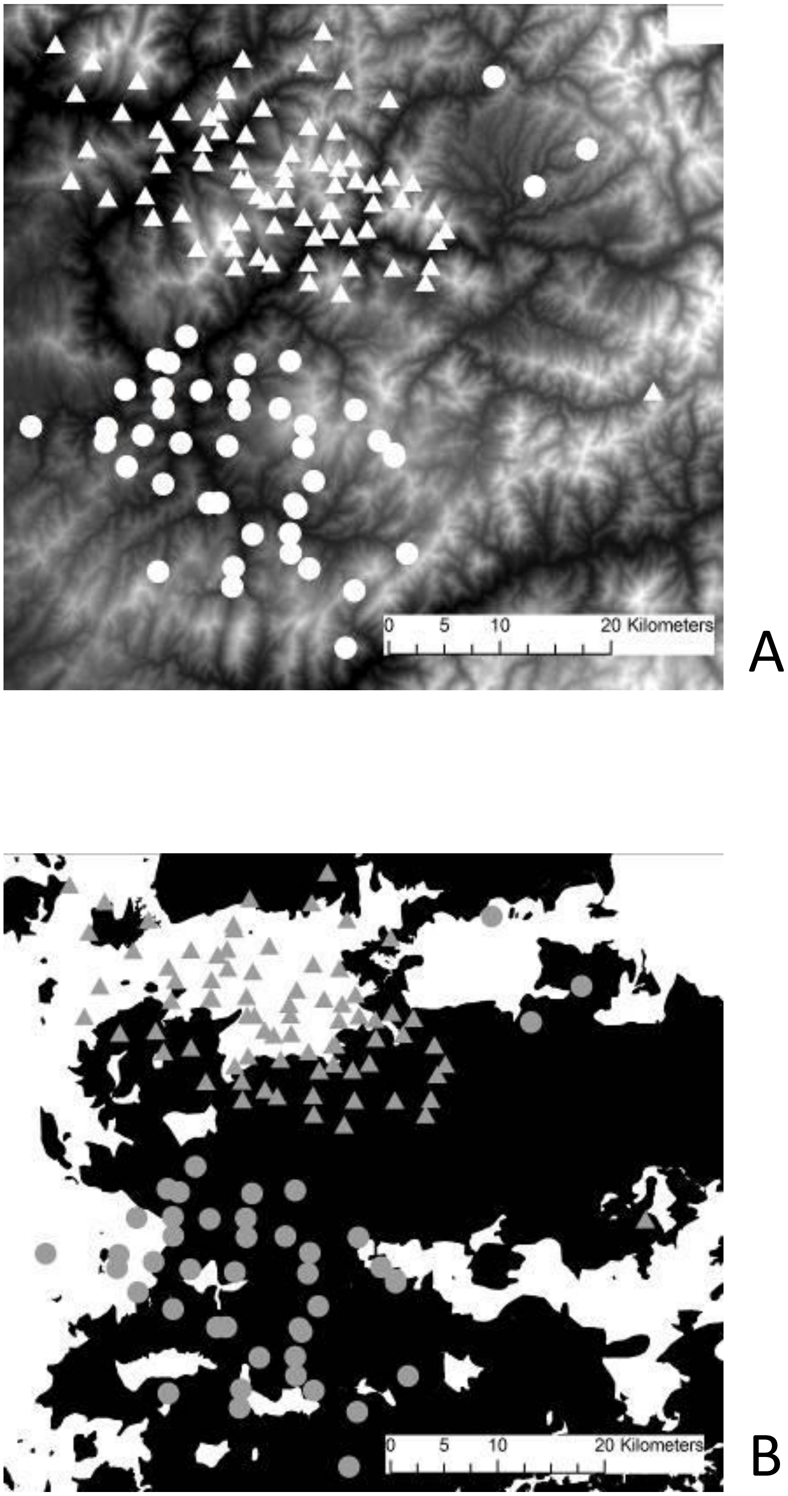


Figure 8

CHAPTER 3: Colonization and gene flow of coastal tailed frogs (Ascaphus truei) at Mount St. Helens: Population Response across Disturbance Gradients

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ABSTRACT

Large, infrequent disturbances provide “natural laboratories” to test how organisms respond to environmental change. That is, most population models consider catastrophes in long-term predictions of population viability, yet researchers are rarely afforded an opportunity to investigate species’ responses to rapid, large scale environmental change. Mount St. Helens in Washington, USA is a volcano that erupted in 1980 and immediately altered approximately 600 $km^2$of surrounding habitat. The eruption and subsequent management left four distinct disturbance zones: the national volcanic monument in which natural succession has been allowed, a managed portion of the blast zone in which dead trees were salvaged and conifers replanted, intact forest that received tephra ashfall, and forest undisturbed by the eruption. Despite massive landscape alteration, many species, including amphibians, have colonized portions of the blast zone. Coastal tailed frogs (*Ascaphus truei*) are among these species, but given the susceptibility of tailed frogs to desiccation and thermal stress, it is unlikely frogs could successfully cross the open blast zone. We had two main alternative hypotheses regarding tailed frog colonization. First, we hypothesize that populations within the blast zone were founded by a limited number of residual adults that survived the eruption and therefore blast zone sites will be genetically differentiated due to drift caused by the bottleneck, combined with no dispersal between sites. Alternatively, tailed frogs colonized the blast zone from the surrounding forest. In this case, we should be able to identify source sites and dispersal will occur through forest and stream corridors. Our results do not fully support either hypothesis. Gene flow was high among all sampled sites, regardless of disturbance history. Furthermore, genetic diversity was high across all disturbance types and there was no evidence of population bottlenecks, indicating that sites within the blast zone were colonized multiple times from across the surrounding forest.

However, hierarchical landscape models demonstrated that environmental processes affected gene flow differently dependent on disturbance history. Specifically, gene flow through the unmanaged blast zone followed a straight line route, with dispersal likely facilitated by remaining coarse woody debris. In contrast, populations within the managed blast zone followed a route restricted to forest and stream corridors, and were influenced by precipitation and heat load index, suggesting the lack of understory in salvaged areas may leave frogs more susceptible to changing environmental conditions. Overall, this research demonstrates not only the resilience of an amphibian population to large disturbances, but indicates that how landscapes are managed after the same disturbance can determine how environmental variables alter population structure, even over a relatively small area.

INTRODUCTION

Large, infrequent disturbances, such as fires, floods, hurricanes and volcanic eruptions, have a strong impact on affected biotic communities, but have received less attention as compared to smaller, more frequent disturbances (Turner and Dale 1998). However, large disturbances merit current research attention because they provide large “natural laboratories” in which to test theories of ecological response and succession. Such disturbances often lead to “ecological surprises”, or long-term changes in community state, when combined with other disturbances (Paine et al. 1998, Franklin and McMahon 2000, Turner et al. 2003). Broad disturbances create a heterogeneous landscape and involve several types of disturbance, some of which may be applicable to understanding effects of smaller, more common disturbances (Foster et al. 1998, Romme et al. 1998). Finally, severe disturbances may become much more prevalent in the future due to climate change (Dale et al. 2001), emphasizing the need to better understand the consequences of such events.

Habitat alteration and climate change are two key hypotheses for global declines of amphibians, which have been considered indicator species of environmental health due to their rate of decline exceeding that of other vertebrate classes (Stuart et al 2004). As such, amphibians are highly suitable for studies of biotic responses to broad disturbance. Such severe disturbances produce widespread habitat alterations of different intensities, and organisms will likely face increased exposure to large disturbances in the future. Thus, the population response of amphibians to broad scale disturbances should be informative for understanding population response to future pressures. Assuming high initial mortality during the disturbance event, the most important factor influencing persistence or colonization in an altered landscape is dispersal ability, and more specifically, the ability to cross through suboptimal habitat (Spiller et al. 1998), which may be difficult for many amphibians.

The eruption of Mount St. Helens, WA, in 1980 is one of the most well studied broad scale disturbances and has contributed a great deal towards ecological theory and understanding, particularly regarding patterns of succession and recovery of disturbed populations (Franklin and MacMahon 2000, Dale *et al.* 2005a). The eruption leveled approximately 600 km^2 of forest surrounding the mountain, but the severity of disturbance was not uniform, shaping the habitat within a few kilometers of the mountain into four main categories: debris avalanche/pyroclastic flow (DAPF) zone across which nearly nothing survived, the blowdown/scorch (BDSC) zone in which trees were killed but remained as biological legacies, standing forest with eruption ash-fall, and undisturbed standing forest (Swanson and Major 2005). Furthermore, the BDSC zone has been subject to two different treatments since the eruption. A portion of this area was declared Mount St. Helens National Volcanic Monument, which was left in a natural state of succession. As a result, the monument retains all downed logs and snags that were present after the eruption and has limited overstory forest cover. The other portion of the BDSC zone was designated for active management and therefore dead trees were salvaged for commercial use and trees were manually replanted (Dale et al. 2005b). As such, the managed area has a significant canopy overstory, but little coarse woody debris and understory on the ground (Titus and Householder 2007).

Highly variable genetic markers, such as microsatellites, can be used to address a number of questions that are relevant to population colonization around Mount St. Helens. Genetic methods can often be more efficient and more effective at detecting changes in population size and dispersal events than direct mark-recapture monitoring (Schwartz et al. 2007) and amphibians can exhibit fluctuations in population size from year to year (Pechmann et al. 1991) that hinder direct monitoring. Furthermore, genetic measures such as heterozygosity and inbreeding can be important components of predicting future population viability (Frankham et al. 2002). Finally, the field of landscape genetics has developed approaches to understanding how environmental features influence population connectivity (Manel et al. 2003, Storfer et al. 2007), which may particularly elucidate how different disturbance levels affect population structure.

We chose to study the population genetic response of tailed frogs (*Ascaphus truei*) across the range of disturbed habitats surrounding Mt. St. Helens to test how different severities of disturbance influences the population structure of a stream-associated amphibian. Tailed frogs are the most widespread stream-breeding amphibian in the Pacific Northwest, inhabit forested areas and have among the lowest desiccation and thermal tolerances documented for amphibians (Claussen 1973; Brown 1975). As a result, tailed frogs are likely highly susceptible to environmental disturbance, especially that which removes canopy cover and shade (Welsh 1990). The eruption likely extirpated all larval individuals in streams in both DAPF and BDSC zones, although some adults may have survived only in the BDSC zone (Crisafulli *et al.* 2005a). Tailed frogs were detected in some streams in the BDSC zone just five years post-eruption, and currently occur throughout this portion of the blast zone. Despite the fact that streams in the DAPF zone have likely been suitable habitat for breeding for at least ten years, there is currently no documented presence of any stream amphibian (Crisafulli et al. 2005a). This is in contrast to pond-breeding amphibians, which have colonized several new ponds in the DAPF zone. Bakkegard (2008) studied the genetic structure of two pond-breeding amphibians, the rough-skinned newt (*Taricha granulosa*) and the northwestern salamander (*Ambystoma gracile*).

Neither species had any degree of genetic subdivision across the blast zone, a result attributed to long-distance dispersal through open terrain. However, tailed frogs are unlikely to have long distance movements across the open blast zone, and therefore should demonstrate a more restricted genetic structure than these salamanders.

There are two main hypotheses regarding the source of the populations currently residing in the zone affected by the eruption. The first hypothesis is that a small number of blast zone residents survived the eruption, and that dispersal has not occurred into such sites since the eruption. This hypothesis is plausible for the unmanaged blast zone for two reasons. The eruption occurred in May when snow was still on the ground and amphibians were hibernating, and several amphibian species were observed soon after the eruption in the BDSC zone (Crisafulli et al. 2005a). Additionally, the unmanaged blast zone lacks a forest canopy, and open habitats have been shown to restrict gene flow in tailed frogs (Spear and Storfer 2008). Under this scenario, we predict that blast zone sites will be genetically differentiated from each other due to drift created by the eruption bottleneck, and such sites should have strong signatures of population reduction. While the colonization of sites by residual organisms may initially seem unlikely across such a disturbed area, this is indeed the pattern most often seen so far at Mount St. Helens (Franklin and McMahon 2000).

A second general hypothesis is that individuals colonized the blast zone from surrounding populations not impacted by the blast. We can further divide this pattern into two alternative explanations: colonization from one or few source sites, or gene flow from many sources across the intact forest. If colonization into the blast zone primarily occurred from one or few sites, we can use assignment tests to identify the source of migrants. Additionally, if blast zone sites were only colonized by a few individuals, measures of genetic diversity in the blast zone should be significantly lower than in the outside forest, whereas if multiple sources dispersed into the blast zone, genetic diversity would not be reduced in the blast zone (Allendorf and Lundquist 2003, Kolbe et al. 2007). We can further infer what processes facilitated or inhibited colonization through the use of landscape genetic models. Landscape genetic analyses are useful for identifying specific environmental variables that influence genetic structure, and thus can give insight into dispersal patterns through disturbed areas.

Understanding the colonization history of tailed frogs in Mt. St. Helens streams is not only an interesting ecological question, but it is also relevant to population management because aspects of the eruption are similar to more frequent forest disturbances. The monument BDSC blast zone is similar to a windstorm or stand-replacing fire because of the presence of dead blown-down or standing trees. The managed blast zone was extensively salvage logged and replanted with conifers, and as such is similar to twenty-five years after clear-cut timber harvest. Of course, there are major differences as well, such as the deposition of ash. However, the characteristics unique to a volcanic eruption occurred in both managed and unmanaged blast zone, so any differences in population genetic structure between the two areas are likely to be related to changes in forest cover and coarse woody debris.

For both of our alternative hypotheses, we expect that the open, unmanaged blast zone will be inhospitable to frog dispersal. However, we have specific predictions related to each hypothesis. First, if streams were primarily repopulated by residual adults, then we expect strong bottleneck signatures, low genetic diversity and high genetic differentiation among blast zone sites and from the surrounding intact forest. Under this hypothesis, any population connectivity detected would be restricted to stream corridors within the blast zone. Alternatively, if blast zone populations were primarily colonized from the surrounding intact forest, we expect that clustering algorithms and assignment tests will group blast zone sites with their respective source sites, and population will be differentiated from non-source areas. Furthermore, we predict that gene flow between source sites and blast zone sites will be unidirectional (i.e. from source into blast zone) and will be best explained by a combination of riparian corridors and favorable environmental conditions such as high precipitation and low solar radiation.

MATERIALS AND METHODS

Sampling design

During the summers of 2007-2008, we collected samples from 30 sites (n=844; Table 14) across four disturbance zones surrounding Mount Saint Helens. These zones included: 1) the portion of the 1980 volcano "blast zone" that was designated as unmanaged national monument and primarily consisted of blown-down trees; 2) the portion of the 1980 blast zone that was salvage logged immediately after the eruption and had trees replanted manually; 3) intact forest that was outside the 1980 blast zone, but received ashfall (known as tephra) and, 4) Intact forest not impacted by the volcano (hereafter referred to as "undisturbed", but this label refers only to 1980 volcanic activity). Sites in disturbance categories 1-2 were previously identified and surveyed sites by Crisafulli et al. (2005a) and were known to have current populations of tailed frogs (Figs. 9-10). We were able to successfully sample six sites in category 1 and seven sites in category 2, seven sites in category 3 and 10 sites in the category 4 area. We intentionally sampled from a much wider area for the undisturbed region, as previous research has documented extensive gene flow in coastal tailed frogs (Spear and Storfer 2008). All sampling sites were within either the Mount Saint Helens National Volcanic Monument or Gifford Pinchot National Forest. In all but three sampling sites, we collected tissue samples from 20-30 individuals. We sampled larval individuals by removing a small tail clip placed in 100%

ethanol, and we collected saliva samples from adults using buccal swabs, which were immediately placed in lysis buffer (Goldberg et al. 2003).

Microsatellite genotyping methods

We extracted DNA from all samples using the Qiagen DNEasy 96 well plate kit (Qiagen, Inc.). We used polymerase chain reaction (PCR) to amplify 13 microsatellite DNA loci developed for coastal tailed frogs (Spear et al. 2008). We ran all PCR reactions divided into 3 multiplexed panels using the Qiagen Multiplex PCR kit (see Appendix 1 for multiplex panels and PCR conditions), with a negative control included in each run. We submitted all microsatellite products to be run on an ABI 3730 automated sequencer (Applied Biosystems, Inc.) at the Washington State University LBB1 core facility. We used GeneMapper 3.7 software to genotype all samples. Finally, because a large number of larval individuals were sampled, we used the software Colony (Wang 2004) to identify full siblings using a maximum likelihood algorithm. This ensured that all sites had an equal number of full sibling groups and also identified sites that had a high number of related individuals.

Identification of source areas for colonization

We used Genepop version 3.4 (Raymond and Rousset, 1995) to test whether loci and sites were in Hardy-Weinberg equilibrium and linkage equilibrium. If any loci were out of Hardy-Weinberg equilibrium at multiple sites, we tested if null alleles were likely present using the software FreeNA (Chapuis & Estoup, 2007), which estimates proportion of null alleles using the expectation maximization (EM) algorithm developed by Dempster et al. (1977). We estimated among-site differentiation using G_{ST}', a correction of F_{ST} that standardizes observed values so that the maximal value equals one (Hedrick, 2005). We calculated F_{ST} using FreeNA. If we detected null alleles, we used the ENA (exclude null alleles) method to estimate F_{ST} (Chapuis &

Estoup, 2007), with a bootstrap resampling procedure run 1000 times to estimate 95% confidence intervals for global estimates. To calculate G_{ST}', we used RecodeData v 0.1 (Meirmans, 2006) to create a file of genotypes that were maximally differentiated, and then estimated F_{ST} values using FreeNA based on the recoded file. We then divided the actual F_{ST} values by the maximum values to obtain a standardized G_{ST}'. We estimated measures of observed and expected heterozygosity and inbreeding coefficient (F_{IS}) with GDA v 1.1 (Lewis and Zaykin 2001). To examine whether population genetic parameters such as F_{ST}, observed heterozygosity, allelic richness and inbreeding coefficient differed based on the four disturbance regimes, we used FSTAT 2.9.3 (Goudet et al. 2001), with significant difference determined based on 10,000 permutations.

We tested for strong clustering and/or strong divisions among sites using the genetic clustering program TESS v. 1.1 (François et al. 2006, Chen et al. 2007). This program uses a Bayesian clustering algorithm to group individuals into genetic populations, but unlike other Bayesian programs such as STRUCTURE, incorporates spatial dependency into the analysis. TESS uses hidden Markov random fields to model spatial dependency based on a specified spatial interaction parameter. After determining the most likely number of clusters (K) after a few trial runs, we used 50 different runs at the most likely K, with 50,000 simulations (10,000 burnin). We used the parameters of no F model, no admixture and a spatial interaction parameter of 0.6. Other tested interaction parameters (0.3, 0.9) had similar results but slightly lower likelihood scores. For each run, TESS estimated the probability of cluster membership (Q-value) for each individual. As we had 50 different sets of Q-values, we implemented CLUMPP v. 1.1.1 (Jakobsson and Rosenberg 2007) to produce a single matrix of Q-values that was the best representation of all replicates. We then averaged all Q-values for each cluster across all individuals within each site, and assigned the site to whichever cluster had the largest Q-value.

Clustering algorithms such as TESS should group source sites with colonized populations, but they cannot indicate the direction of gene flow. To test if gene flow occurred in a unidirectional manner, we used BayesAss v 1.3 (Wilson and Rannala 2003), which estimates migration rates using Markov chain Monte Carlo (MCMC). Due to input file constraints we were only able to analyze 20 sites at a time, so we included only the nearest potential source sites to the blast zone. We used the default parameter values suggested by the manual, which included 3,000,000 iterations, with a sampling frequency of 2000. Based on confidence intervals produced by BayesAss, we then identified sites that had a significant migration rate into another site.

Finally, we tested for signatures of hypothesized severe population size declines through the use of heterozygosity excess tests (Cornuet and Luikart, 1996), and shifted allele distributions (Luikart et al., 1998). An excess of heterozygotes relative to equilibrium expectations is an ephemeral signature of severe declines within the past few generations (Cornuet and Luikart, 1996). We assumed a stepwise mutation model and used the Wilcoxon sign rank test to determine if a population exhibited significant heterozygosity excess. The shifted allele distribution test describes the distribution of allele frequencies, with the normal expectation that most alleles will be of low frequency. A shifted distribution in which the majority of alleles are of intermediate frequency is characteristic of populations that have experienced declines due to initial loss of rare alleles.

Landscape effects on colonization

We used regression analysis and two different potential paths of gene flow to model landscape influence across all sites and then separately for each disturbance grouping. We used a hierarchical approach to detect if environmental explanatory variables differed based on spatial scale and disturbance type (Murphy et al. in review). Specifically, we divided spatial analyses into seven different sets of sites (Figure 10). First, we tested a global model (all sites). The second and third groupings separated all sites with the 1980 Mt. St. Helens blast zone (regardless of current management history) from sites in intact forest (tephra and undisturbed zones). Finally the 4th -7th groupings further divided the second and third groupings to create the following categories: sites within the national monument (unmanaged), sites within the managed blast zone, sites within the tephra ashfall zone and sites undisturbed by the 1980 eruption.

The two potential movement paths we tested were straight-line among sites and a least cost path that was based on forest and stream presence, with any open area that was not part of a stream corridor considered unsuitable (Table 15). We constructed this path because the only large expanses of open areas in the region are due to the Mt. St. Helens eruption, and thus we could test our hypothesis that tailed frogs were unable to move through the open, deforested blast zone except through following stream corridors. We assigned all forested or stream areas a cost of 1, whereas we assigned all open, non-stream areas with a cost of 1000. This cost was chosen to effectively create these open regions as impenetrable barriers. We created the cost surface using the "cost distance" function and built the least cost path with the "cost path" function, both implemented in ArcGIS 9.3 (ESRI, Inc.). Along both path types we calculated values for several independent variables, including total topographical distance, distance through non-forest, average canopy cover, average heat load index, average slope, average frost-free period and average growing season precipitation. The values of independent variables along the two potential movement paths were identified using the "intersect" function in ArcGIS 9.3. All sources and calculations for each of these variables are listed in Table 15.

We used geographically weighted regression (GWR; Fotheringham et al. 2002) or multiple linear regression to test the influence of the landscape and climatic variables along each path on G_{ST}'. GWR is a type of spatial regression analysis that accounts for local differences across the entire study area. The analysis is performed by estimating regression parameters at each data point using only data points within a specified bandwith of the focal point and including a spatial weight in the local equation. The spatial weight is defined by a spatial weighting matrix. We created a spatial weighting matrix based on the distance between the midpoints of each pairwise site comparison. Specifically, we used a bi-square weighting function in which the weight $(W_{ij}) = (1 – (d_{ij}/b)^2$, in which d represents the distance between the two midpoints *I* and *j* and *b* is the bandwith. We included 10-15% of neighboring sites as the bandwith, with the exact percentage determined by greatest AIC support (Rangel et al. 2006). For each GWR, we performed a Getis-Ord local hot spot analysis (Getis and Ord 1995) based on the r^2 of each local regression, using the midpoint as the location for each pairwise comparison. The Getis-Ord statistic is represented as a Z score, and measures whether high values are significantly clustered together (Z score > 1.96) or low values significantly clustered together (Z score < 1.96). We plotted midpoints and ran Getis-Ord analyses in ArcGIS 9.3.

However, if there is not strong spatial autocorrelation in G_{ST}', then ordinary least-squares (OLS) multiple linear regression analysis may be more appropriate. Therefore, for each model we tested whether model support (based on AIC) was greater for OLS regression compare to GWR, and used the specific regression analysis that had the highest support. We constructed

GWR or OLS regression models for each of the seven groupings. Regardless of the regression method used, the best supported model(s) was chosen based on AIC weight. All regression analyses were run in SAM (Spatial Analysis in Macroecology) (Rangel et al. 2006).

RESULTS

Genetic diversity and differentiation across disturbance types

Overall, there was some evidence of the presence of full siblings in the individuals sampled, as eleven of the thirty sites had at least one full sibling pair detected. Most of these eleven sites had only one full sibling pair, with the exception of BeanUp and Quartz (4 and 3 pairs, respectively). Sites with full siblings were evenly distributed across the three disturbed categories. Specifically, the unmanaged and managed blast zone each had three sites with full siblings, and four sites had full siblings within the tephra zone. Only one site had full siblings in the undisturbed zone. Two of the thirteen microsatellite loci were out of Hardy-Weinberg equilibrium at multiple sampling sites (A14 and A29). Both these loci had a high estimate proportion of null alleles, suggesting the reason for violations of Hardy-Weinberg assumptions. Only 7 (out of 78) loci pairs were out of linkage equilibrium at any sites. Each of these pairs was only in disequilibrium at one site each, and as these violations occurred at six different sites, there appears to be no consistent pattern of linkage disequilibrium. As the presence of null alleles would affect estimates of genetic diversity, we removed loci A14 and A29 for genetic diversity calculations. Genetic diversity was generally high and consistent among disturbance types (Table 14): average number of alleles ranged from 15.56-16.26, H_e from 0.869-0.878, H_o from 0.844-0.858 and F_{IS} from 0.023-0.039. None of the comparisons among disturbance types were significantly different.

Due to the presence of null alleles, we estimated all G_{ST}’ values using the ENA method. The global G_{ST}’ value for the unmanaged blast zone was 0.016 (95% c.i. 0 – 0.05) and for the managed, replanted blast zone the value was 0.023 (95% c.i. 0.01 – 0.04). Based on the confidence intervals, there is no difference in genetic differentiation between the two disturbance types, and the global G_{ST}’ of the unmanaged blast zone is not different from zero. Pairwise G_{ST}’ values between sites in the 1980 blast zone were quite low, with only two sites having consistently high levels of differentiation (Table 16). The two sites were BeanUp (average pairwise G_{ST}’ = 0.07) and GreenL (average pairwise G_{ST}’ = 0.09). All other sites within the blast zone had average pairwise comparisons of less than 0.05. There was no evidence of recent effective population size declines at the blast zone sites, as no sites had significant heterozygosity excess after correction for multiple comparisons and there were no shifted allele distributions.

Across the intact forest, levels of G_{ST}’ were similar to that seen within the blast zone. Sites within the tephra disturbance zone had a global G_{ST}’ of 0.024 (95% c.i. 0.01-0.05) and the global estimate across undisturbed sites was 0.038 (95% c.i. 0.01-0.07). As with the blast zone sites, there is no significant differences in genetic differentiation and both G_{ST}’ confidence intervals nearly intersect with 0. The general pattern of pairwise differentiation was higher G_{ST}’ values between sites in the northern and southern areas (Fig, 9), but comparatively little differentiation among sites that are within the northern and southern clusters (Table 17). There was no evidence for population size declines at any of the forest sites based on either heterozygosity excess or shifted allele distributions.

Bayesian clustering analyses demonstrate a very high degree of admixture among sites (Table 18). According to TESS, the most likely number of clusters was either five or six, and most individuals had some probability of being assigned to each of the clusters. For each site, the highest probability of membership to any one cluster was between 0.2-0.3. In accordance with the high degree of admixture detected by TESS, BayesAss was unable to find any information in the data to estimate pairwise migration rates between sites. However, within this high degree of gene flow, there are some geographical patterns of cluster membership, albeit very weakly supported. The undisturbed sites within the southern part of the study area had had highest membership in clusters 1 and 4, with the two clusters divided by drainage (Fig. 11). The majority of sites north and east of Mt. St. Helens belonged to Cluster 3, and this cluster included sites from all four disturbance zones. However, six of the sites within the northeast Mt. St. Helens region assign to Cluster 4 and include Greenu, Greeue, Beanup, Badger, Iron and TL1. With the exception of TL1, all these sites are found in the upper part of streams near the divide that separates the three main watersheds that drain the eastern Mt. St. Helens area (Fig. 11). Finally, HWF2 is the only site that assigns to cluster 6, although there is a similar probability of this site grouping with cluster 5, and it is also found near the main divide.

Landscape genetic patterns across disturbance types

The hierarchical regression analysis demonstrated that there were different environmental factors influencing gene flow dependent on the disturbance history. The best supported global model, which was represented by a GWR analysis, was based on the least cost forest/stream path and included distance through non-forest, length of frost-free period and slope ($r^2 = 0.392$; AIC wt. = 0.83) (Table 19). Distance through non-forest was positively correlated with G_{ST}', while both length of frost-free period and slope had a negative correlation. The only other global model with any AIC support was nearly identical, with the only difference being that total distance was included instead of distance through non-forest, and this model had an r^2 of 0.39 and an AIC wt. of 0.16 (Table 19). Getis-Ord analysis of the local regressions revealed a large hot spot (high r^2 of local regressions) among sites within and immediately surrounding the Mt.

St. Helens blast zone and a second smaller hot spot among the undisturbed sites south of the volcano (Figure 12). Significant cold spots (low r^2 of local regressions) occurred in the area between the blast zone and undisturbed sites and also within the region to the northeast of Mt. St. Helens.

The first hierarchical division, between blast zone sites and intact forest sites, produced slightly different models between the two main disturbance types. The regression analysis across the blast zone (best described by an OLS model) had the vast majority of AIC weight support (0.96) described by three models that collectively contained three variables: distance through non-forest, frost-free period and growing season precipitation. Distance through non-forest and growing season precipitation were both positively correlated with G_{ST}', while frost free period was negatively correlated. The blast zone models explained relatively little of the variation present; r^2 only ranged from 0.203-0.226. Across the intact forest, the vast majority of AIC support (combined AIC wt. = 0.87; Table 19) was for two GWR models, both of which only contained total distance as the independent variables (essentially an isolation by distance model), and the path based on the forest/stream least cost had greater support than the straight-line path (Table 19). Either of these models explained just less than 50% of the variation in gene flow. The Getis-Ord analysis identified the cluster of southern undisturbed sites as a hot spot, and the area containing the tephra sites as a model cold spot (Fig. 13).

Separate models describing the managed and unmanaged portions of the blast zone produced differing results, although the top models in both disturbance types explained a great deal of variation (r^2 = 0.612-0.638) for their respective areas. First, the sites within the monument were based on a straight line path (90% of AIC weight was attributed to such models) (Table 20). In contrast, the managed, replanted blast zone sites were best described by gene flow models following the forest/stream least cost path (combined 0.77 AIC wt.). Second, the best supported OLS models for the unmanaged blast zone included only two types of independent variables: distance (both total and non-forest were equally supported) and slope (Table 20). Gene flow across the managed blast zone in the three best supported models was explained by several independent variables that included distance through non-forest, growing season precipitation, slope, frost free period and heat load index (Table 20). Finally, slope is positively correlated with G_{ST}' in the monument, but negatively correlated across the managed blast zone, as is seen with the global model. All the remaining independent variables included in the managed blast zone model were positively correlated with G_{ST}'.

Gene flow across both the tephra and undisturbed forest areas was explained primarily by frost free period (Table 21). Differentiation across the tephra zone had the highest support along a straight line path, while the least cost path better described gene flow across the undisturbed forest. Although significant, the model for all undisturbed sites explained relatively little variation (0.146). However, if we only include the southern undisturbed sites, as indicated by clustering and hot spot analysis, the best fit model includes total distance and slope, and accounts for almost 80% of variation.

DISCUSSION

Despite a severe environmental disturbance, we saw high gene flow and genetic diversity across all sampled areas affected by the Mount St. Helens eruption. Furthermore, we detected no evidence of recent bottlenecks despite physical and survey evidence suggesting extreme population size reductions at blast zone sites. Therefore, the most likely explanation is that tailed frogs have dispersed into the blast zone from the surrounding intact forest, and probably from multiple sources, which is consistent with another colonization study across the blast zone (Yang et al. 2008). The evidence for multiple dispersals included very low degrees of substructure across all sites and high levels of genetic diversity at each site. Although there was low overall differentiation, the patterns and predictor variables of gene flow differed among disturbance categories. In particular, our results imply the importance of biological legacies for facilitating gene flow in disturbed environments. The importance of biological legacies such as downed woody debris to ecosystem recovery has been suggested by several authors (Franklin and McMahon 2000, Franklin et al. 2002, Lindenmayer and Noss 2006, Manning et al. 2006), and this study indirectly supports the importance of legacy structures to amphibians. Thus, these small stream-associated amphibians are certainly resilient to seemingly catastrophic disturbances, but environmental features such as frost free period, slope and solar radiation strongly modify this response. Specifically, we suggest that forest management that reduces understory and coarse woody debris can support high levels of connectivity, but such gene flow might be more subject to environmental changes such as climate than connectivity in sites with large coarse woody debris and vegetative understory.

Colonization and genetic diversity across disturbance zones

Based on previous research (Crisafulli et al. 2005a), we had hypothesized that residual adults would be the primary population founders, at least in the unmanaged blast zone. While residual adults may have contributed to populations in the current blast zone, gene flow has been widespread across the blast zone in addition to the surrounding intact forest. We infer this from three lines of evidence. First, there were no significant differences in genetic diversity measures in the blast zone as compared to the intact forest, and there were overall high levels of genetic diversity. Populations that have undergone severe reductions with subsequent isolation are theoretically expected to lose genetic diversity and increase inbreeding (Frankham et al. 2002), and this result has also been empirically demonstrated (Boessenkool et al. 2007, Lampert et al. 2007, Ewing et al. 2008) in bottlenecked populations on isolated islands. While we might not expect to see immediate changes in heterozygosity (Keyghobadi et al. 2006), allelic diversity and inbreeding should differ in the post-bottlenecked population. Clearly this is not the case across our study populations, as there were no significant differences between allelic diversity and inbreeding between blast zone populations and those in intact forests. Average number of alleles was similar among treatment types (mean = 16.075, SE +/- 0.52). The value of the inbreeding coefficient is significantly different from zero, but there were not significant differences among disturbance categories, including undisturbed. This indicates that any level of inbreeding is not due to the volcanic eruption.

The lack of heterozygosity excess or shifted allele distribution is a second indicator of widespread genetic exchange across the blast zone. It is possible that a number of adults survived the eruption, but the eruption eliminated all larval individuals in streams (two age classes) and any surviving adults would have had to persist in the blast zone before any vegetative succession occurred (Crisafulli et al. 2005a). Therefore, it is hard to imagine any scenario in which the blast zone populations did not have large initial reductions in effective population size. Therefore, the current genetic identity of populations must be a result of multiple dispersal events from populations outside the blast zone that would have replenished the rare alleles lost by eruption mortality.

Finally, the G_{ST}' values and the Bayesian clustering results indicate widespread gene flow and admixture across all sites, no matter the disturbance type. Across the four main disturbance types, there were no significant differences in G_{ST}' (range 0.02-0.04) and differentiation values were low. Furthermore, pairwise comparisons indicated that none of the sites in the unmanaged blast zone were highly divergent, and only two sites in the managed blast zone (BeanUp and GreenL) consistently had G_{ST}' levels greater than 0.05. Similarly, there was not high genetic differentiation among sites in the tephra forests. Finally, no sites could be assigned to a single cluster with strong confidence, and the greatest probability of membership to a single cluster for any site was 0.344. Although there were some geographical patterns of cluster probability, there is overall very weak substructuring across all sampled populations.

Surprisingly, the patterns of genetic diversity and structure are consistent with the limited studies that have investigated genetic structure at Mount St. Helens. Bakkegard (2008) observed similar high levels of gene flow in rough-skinned newts (*Taricha granulosa*) and northwestern salamanders (*Ambystoma gracile*) and Yang et al. (2008) did not detect any founder effects and found low genetic structure in the animal-dispersed black huckleberry, *Vaccinium membranaceum* (although they were able to assign half of sampled individuals to a single population cluster). Although the results of the previous two studies were unexpected by the authors, the black huckleberry is dispersed by mammals and birds that may more easily be able to cross open areas. With respect to the studied salamanders, newts are known to sometimes occur in open areas, and northwestern salamanders likely used the extensive pocket gopher tunnels across the monument. However, tailed frogs are not known to cross extensive open areas or use underground burrows, and a study on the Olympic Peninsula of Washington concluded that open alpine meadows strongly structured tailed frog populations (Spear and Storfer 2008). Thus, our results suggest that there is some aspect of the Mount St. Helens landscape that has facilitated genetic connectivity through a seemingly hostile matrix.

Landscape influences on gene flow across disturbance types

Our results demonstrated that although there were not significant differences in levels of genetic diversity and gene flow among disturbance types, gene flow was best explained by different environmental factors across the entire area. Additionally, we have evidence that route of gene flow into and through the blast zone was not random, and perhaps in some cases followed specific corridors. Our first line of evidence of more directed movement came from the Bayesian clustering analysis. There was a small cluster of sites that were primarily headwater streams near divides between drainages. This small cluster is embedded within the largest cluster that contains almost all of the remaining blast zone and tephra sites. This clustering pattern suggests that one major route of colonization followed ridges into the blast zone from a relatively narrow area in the surrounding forest and led to high connectivity between basins. Colonization to the remaining blast zone sites likely came from lower elevations in the forest and may have followed either overland or stream corridors into the blast zone sites. However, as there is very weak differentiation among clusters, we expect that there is gene flow among all sites, but that the clustering pattern perhaps reflects early colonization with subsequent admixture.

While clustering analysis reflects geographical trends in genetic connectivity, landscape genetic analyses allow us to detect and identify specific variables that facilitate or inhibit gene flow (Storfer et al. 2007). Examining a hierarchy of models demonstrated that processes affecting connectivity differed by disturbance type and management history. The global model indicated that most gene flow followed a least cost route almost exclusively through forest, and if forest cover was lacking, along stream paths. The three independent variables included in the best model were distance through non-forest, frost-free period and slope. While both topographical distance and frost free period followed an expected relationship (distance positively correlated with G_{ST}', frost free period negatively correlated), slope was surprisingly negatively correlated with G_{ST}'. In other words, as degree of slope increased, so did gene flow. Interestingly, this same relationship with slope occurred across managed forests on the Olympic Peninsula (Spear and Storfer 2008), and portions of the Gifford Pinchot National Forest are managed for timber harvest. Therefore, although Spear and Storfer (2008) were unable to satisfactorily explain the non-intuitive slope result, it may be due to the likelihood that steeper slopes are less accessible to human activities such as logging. Thus, frogs may move through these steeper areas to avoid anthropogenic disturbance.

The hot spot analysis of local regressions revealed that all four disturbance types were not subject to the same landscape process (Figure 12). Although we used a somewhat different hierarchical approach, this finding is similar to a study of boreal toad (*Bufo boreas*) gene flow that demonstrated that a different ecological model explained genetic differentiation within genetic clusters as opposed to between genetic clusters (Murphy et al. (in review)).

We grouped sites into two categories: blast zone (including managed and unmanaged) and intact forest (both tephra and undisturbed) to test if models of gene flow were best supported by this dichotomy. In fact, we found this grouping to be highly inappropriate for the blast zone sites, and less than satisfactory for intact forest. The amount of variation explained by the best model across the entire blast zone was only around 23% and was among the lowest values of r^2 for any of the hierarchical groupings. Across the intact forest, the amount of variation explained was higher (nearly 50%), but the best model was relatively simplistic and only included distance. Tailed frogs and amphibians in general, are not known to exhibit simple isolation by Euclidean distance (Funk et al. 2005, Spear et al. 2005, Giordano et al. 2007, Measey et al. 2007, Spear and

Storfer 2008), and as such suggests that there was not a single landscape process that affected the entire intact forest area. In support of this, the hot spot analysis of the intact forest GWR model suggests that the subdivision within the intact forest is between the southern sites and the tephra sites to the east of the volcano.

The final hierarchical division (managed vs unmanaged blast zone) strongly suggests that different processes are affecting gene flow in the blast zone dependent on management history after eruption. Both separate models for the managed and unmanaged area explained a much greater proportion of variation (0.612-0.638) than when the sites are combined. More striking, however, than the mere separation of the two management zone was the fact that the models describing each were quite different. We formulated the forest/stream least cost path primarily because we thought it was the only way gene flow across the unmanaged blast zone was possible, but instead the best support for gene flow within the monument was through a straight line. Moreover, in the models that comprised the top 92% of model support, only two types of variables were included: distance (either total distance or distance through non-forest; the two were basically synonymous in the unmanaged blast zone) and slope. Both were positively correlated with gene flow, and so contrary to the global model, steep slopes inhibited gene flow in this area. In contrast, across the managed blast zone, gene flow was best described by the least cost path, and several landscape variables, including distance through non-forest, growing season precipitation, slope, and heat load index. Although slope is in both managed and unmanaged blast zone models, the relationship with gene flow is in opposing directions.

Given that both managed and unmanaged blast zone were subject to approximately the same degree of disturbance from the Mount St. Helens eruption, it is curious why the landscape models are so different. We infer that the management history after eruption is the explanation.

Within the national monument, there is currently little forest cover, with primarily a shrub canopy and much coarse woody debris. Outside the national monument, in the managed blast zone, many of the large logs were salvaged and conifers planted, so that the area now resembles a tree plantation with little understory (Crisafulli et al. 2005b, Titus and Householder 2007). We suspect that the removal of coarse woody debris has somewhat restricted gene flow (as G_{ST}' values are higher in the managed blast zone) and led frogs to more closely follow forested and stream corridors. However, the most relevant effect we see is that gene flow is influenced by several more variables in the salvaged blast zone, such as heat load index and growing season precipitation. Therefore, climate changes in the future might disrupt the population processes much more severely of amphibian populations that lack the protective cover of a dense understory. The importance of coarse woody debris for amphibian populations in disturbed areas is increasingly being appreciated (Waldick et al. 1999, Butts and McComb 2000, Thompson et al. 2003, Alkaslassy 2005, Spear et al. 2005, Rittenhouse et al. 2008, but see Aubry 2000) and our results suggest that coarse woody debris might help create a stable microclimate that is more resistant to the impacts of the broader environment.

We have evidence that gene flow is extensive and does not follow a limited path in the managed blast zone. How, then do we explain the lack of tailed frogs in the DAPF zone despite the presence of pond-breeding amphibians, and seemingly suitable streams (Crisafulli et al. 2005a)? The probable answer is related to slope, as dispersal into the DAPF zone would require crossing one of the steeper slopes in the monument, and slope was negatively correlated with gene flow across the unmanaged blast zone. This does not explain why steep slopes would create a barrier for gene flow, as it seems to facilitate connectivity elsewhere in the region and in the managed blast zone. A possible hypothesis is that due to the instability of the volcanic ash deposited during the eruption and without vegetation to provide stability, large logs would be less likely to stay on steep slopes, and would instead slide down the slopes, leaving little cover at the top to allow tailed frog movement**.** If this is the case, if the upper slopes near the DAPF zone are revegetated, we should expect rapid colonization of those streams in tailed frogs.

Outside the blast zone in the intact forest, dividing the sites into our *a priori* categories of tephra and undisturbed produced similar models (length of frost free period as the primary predictor), but did not provide strong support (based on r^2) for either region. In particular, the undisturbed forest had less than 15% of variation explained. Both the Bayesian clustering analysis and Getis-Ord hot spot analysis suggested that the undisturbed forest really was two clusters: the southern sites and the eastern sites. When we reanalyzed the undisturbed forests with only the southern sites of CR, GF, PC, PL, WC and WD, we created a model that had very high support ($r^2 = 0.782$) that was similar to the monument model (distance and slope included), with the exception of the opposite relationship of slope with gene flow. This sites that formed the southern "undisturbed" cluster had actually been subjected to a very great past disturbance, the 1902 Yacolt Burn, the largest recorded fire in Washington state history. In this case, the Yacolt burned area was extensively salvaged, except in areas that were too rugged to access, and thus the relationship of gene flow with steep slopes may be associated with the maintenance of coarse woody debris and other biological legacies in this area. The similarity of the landscape model of gene flow between a volcanic landscape and a burned landscape suggests the results from ecological studies across Mount St. Helens may be applicable to burned areas further across the western U.S.

Conclusions

Our study has two major findings relevant to both amphibian conservation and disturbance ecology. First, we have demonstrated the resilience of a (seemingly) environmentally sensitive amphibian, the coastal tailed frog, to a widespread and severe disturbance. This resilience has previously been demonstrated for rough-skinned newts and northwestern salamanders across Mount St. Helens (Bakkegard 2008), but the response of the tailed frog may be even more interesting because of widespread concern about the ability of tailed frogs to withstand habitat alteration (Corn and Bury 1989, Welsh and Lind 1990, Diller and Wallace 1999, Kroll et al. 2008). However, tailed frogs have overall high gene flow, and landscape genetic models suggest that individuals might follow directed paths (Spear and Storfer 2008). Therefore, this species may be highly adapted to using favorable microhabitat conditions to allow dispersal, and thus persist among disturbances as long as some favorable conditions exist. Research such as this study and that of Bakkegard (2008) also give hope that amphibian populations initially decimated over an area may be able to recover and quickly repopulate once the habitat has stabilized.

Second, we have demonstrated that severity of disturbance and management of disturbed areas leads to different patterns of gene flow across a relatively fine scale. We have shown that tailed frogs breeding in an area that received active management display a population genetic response that is more dependent on outside environmental factors than an area without management subject to the same disturbance. In our case, we believe the most logical explanation for this difference was the practice of salvage logging removing coarse woody debris, although this hypothesis needs to be more explicitly tested. However, if our suspicions are correct, then this supports the literature that states that the use of salvage logging should be carefully considered and may have some detrimental effects (Dellasala et al. 2006, Lindenmayer and Noss 2006). Furthermore, the population response of tailed frogs on Mount St. Helens suggests that more routine disturbances such as timber harvest can be more compatible with small animal movement by leaving some remnant large woody debris in the logged area.

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FIGURE LEGENDS

Figure 9. Overview map of sites in Mount St. Helens study area. Background is a digital elevation model with lighter areas indicating higher elevations. Black rectangular box surrounds blast zone sites.

Figure 10. Site locations and names for each of the four disturbance types. Panels A and B represent the area within the black rectangular box in Figure 1. Panels C and D cover the full extent of the study areas. Background is a digital elevation model as in Figure 1. Panel A depicts the unmanaged blast zone sites within the national monument, Panel B shows the unmanaged blast zone sites, Panel C the sites within the tephra ashfall zone and Panel D the sites undisturbed by Mount St. Helens.

Figure 11. Results of the Bayesian clustering analysis implemented in TESS. Sites are assigned to the cluster in which that site has the greatest probability of membership, and clusters are indicated by different shapes. Inset is a close-up of Mount St. Helens blast zone sites.

Figure 12. Getis-Ord hot spot results for global GWR model. Circles represent hot spots that have strong support for the global model, whereas triangles represent cold spots that have low support for the model. Points represent midpoints between two sites.

Figure 13. Getis-Ord hot spot results for intact forest GWR model. Circles represent hot spots that have strong support for the global model, whereas triangles represent cold spots that have low support for the model. Points represent midpoints between two sites.

TABLES

Table 14. Sample sizes and genetic diversity measures for each sampled site. Category represents the disturbance type (1 = unmanaged blast zone, 2 = managed blast zone, 3 = tephra forest, 4 = undisturbed forest). N is the sample size (after removal of full siblings). H_e represents expected heterozygosity, H_o represents observed heterozygosity and F_{IS} is the inbreeding coefficient.

Site	Category	N	Avg # alleles	H_e	H_o	F_{IS}
GHOST	1	29	16.00	0.870	0.820	0.059
GREENU	1	30	17.09	0.893	0.863	0.034
GREEUE	1	30	18.64	0.893	0.866	0.030
SMITH3	1	31	16.36	0.855	0.818	0.044
SMITH4	1	19	13.00	0.876	0.834	0.049
SMITHE	1	30	16.45	0.876	0.862	0.016
Average		**28.17**	**16.26**	**0.877**	**0.844**	**0.039**
BEANUP	2	22	14.64	0.851	0.855	-0.005
NF3	2	31	17.64	0.872	0.870	0.003
GREENL	2	7	8.55	0.889	0.831	0.070
HWF2	2	30	17.64	0.873	0.860	0.016
HWF3	2	32	16.73	0.865	0.853	0.013
HWF4	2	32	18.00	0.871	0.836	0.040
QUARTZ	2	28	16.45	0.882	0.840	0.049
Average		**26**	**15.66**	**0.872**	**0.849**	**0.027**
BC	3	27	16.18	0.877	0.862	0.017
BADGER	3	31	16.45	0.874	0.856	0.021
F1	3	31	16.55	0.876	0.848	0.032
F2	3	32	17.09	0.867	0.840	0.032
F3	3	30	16.55	0.886	0.890	-0.005
IRON	3	32	17.64	0.885	0.845	0.046
Q2	3	28	16.64	0.880	0.862	0.021
Average		**30.14**	**16.73**	**0.878**	**0.858**	**0.023**
CR	4	31	17.64	0.884	0.857	0.031
GF	4	32	16.82	0.863	0.832	0.037
JC	4	23	15.36	0.870	0.854	0.019
L1	4	29	16.82	0.873	0.829	0.052
L3	4	20	13.36	0.860	0.852	0.010
PC	4	31	17.09	0.871	0.848	0.027
PL	4	31	16.27	0.852	0.844	0.010
TL1	4	7	8.00	0.852	0.831	0.028
WC	4	35	17.73	0.872	0.865	0.009
WD	4	29	17.36	0.889	0.864	0.028
Average		**26.8**	**15.65**	**0.869**	**0.848**	**0.025**

Table 15. Variables used in least-cost (LC) path development and/or as independent in geographically weighted regression models.

Variable	**unit(s)**	**LC path**	**Data Source(s)**	**Resolution**	**Calculation along LC paths**
Topographic distance	meters	Yes	Digital Elevation Model (DEM)	30 x 30 m	Sum
Forest/Stream	unitless	Yes (1000:1 cost)	National Land Cover Dataset (NLCD 2001) Gifford Pinchot National Forest (1998)	30 x 30 m	not included
Distance through non-forest	meters	No	NLCD 2001	30 x 30 m	Sum
Canopy Cover	percent	No	NLCD 2001	30 x 30 m	Weighted average
Heat Load Index (hli)	unitless, McCune and Keon, 2002	No	DEM	30 x 30 m	Weighted average
Slope	degrees	No	DEM	30 x 30 m	Weighted average
Frost-free period (ffp)	days	No	spline-based model (Rehfeldt et al. 2006)	1 x 1 km	Weighted average
Growing season (Apr-Sep) precipitation (gsp)	millimeters	No	spline-based model (Rehfeldt et al. 2006)	1 x 1 km	Weighted average

Table 16. Pairwise G_{ST}' values across the Mount St. Helens blast zone. Site names are as in Figure 10. Bolded values indicate moderate to great differentiation ($\geq$0.05) (Wright 1978).

	BEANUP	**NF3**	**GHOST**	**GREENL**	**GREENU**	**GREEUE**	**HWF2**	**HWF3**	**HWF4**	**QUARTZ**	**SMITH3**	**SMITH4**
NF3	**0.077**											
GHOST	**0.066**	0.033										
GREENL	**0.173**	**0.116**	0.047									
GREENU	**0.107**	**0.080**	0.007	0.029								
GREEUE	**0.082**	0.028	-0.002	0.035	-0.048							
HWF2	-0.009	0.026	0.043	**0.105**	0.042	0.015						
HWF3	0.037	0.000	0.026	**0.109**	**0.053**	0.034	-0.005					
HWF4	0.034	0.006	0.001	**0.106**	0.034	0.014	0.012	-0.003				
QUARTZ	**0.059**	0.015	0.006	**0.074**	0.015	-0.008	0.011	-0.002	0.006			
SMITH3	0.015	0.008	0.033	**0.128**	**0.087**	0.050	0.013	0.015	0.013	0.028		
SMITH4	**0.088**	0.038	0.000	**0.073**	0.031	-0.008	0.047	0.012	0.028	0.025	-0.003	
SMITHE	0.046	0.016	0.019	**0.065**	0.021	-0.003	-0.002	-0.017	0.013	0.001	0.004	0.004

Table 17. Pairwise G_{ST}' values across the intact forest. Site names are as in Figure 10. Bolded values indicate moderate to great differentiation (≥0.05) (Wright 1978).

	BC	**BADGER**	**CR**	**F1**	**F2**	**F3**	**GF**	**IRON**	**JC**	**L1**	**L3**	**PC**	**PL**	**Q2**	**TL1**	**WC**
BD	0.013															
CR	**0.062**	**0.095**														
F1	0.029	0.027	**0.072**													
F2	0.023	0.033	**0.077**	-0.001												
F3	-0.001	0.009	0.049	0.016	0.023											
GF	**0.053**	**0.082**	0.019	**0.052**	0.023	**0.056**										
IC	**0.054**	0.042	**0.132**	0.043	**0.058**	0.018	**0.136**									
JC	**0.055**	**0.085**	0.030	**0.051**	**0.064**	**0.065**	0.025	**0.120**								
L1	0.000	0.012	0.030	0.007	0.003	-0.005	0.032	0.050	0.023							
L3	0.022	0.034	**0.067**	**0.051**	0.037	0.035	**0.081**	**0.102**	0.042	-0.010						
PC	**0.055**	**0.115**	**0.054**	**0.055**	0.047	**0.073**	0.007	**0.133**	0.000	0.021	0.046					
PL	**0.082**	**0.106**	**0.051**	**0.081**	0.027	**0.088**	0.004	**0.170**	0.012	0.047	**0.058**	0.010				
Q2	-0.007	0.006	0.047	0.036	0.009	-0.003	0.046	0.051	0.025	0.006	0.031	0.046	0.042			
TL1	0.034	**0.093**	**0.071**	**0.054**	-0.009	0.042	0.002	**0.164**	0.009	0.019	0.023	0.036	0.002	0.006		
WC	**0.081**	**0.079**	0.030	0.038	0.048	**0.061**	0.001	**0.126**	0.019	0.017	**0.069**	0.018	0.024	**0.053**	**0.066**	
WD	**0.075**	**0.070**	**0.054**	**0.059**	**0.063**	0.036	**0.089**	0.033	**0.083**	0.031	0.044	**0.092**	**0.114**	0.040	**0.139**	**0.087**

Table 18. Assignment probabilities for each site to one of six clusters based on the Bayesian clustering alghorithm implemented in TESS. The highest cluster probability for each site is bolded.

Site	**Cluster 1**	**Cluster 2**	**Cluster 3**	**Cluster 4**	**Cluster 5**	**Cluster 6**
GHOST	0.129	0.081	**0.237**	0.117	0.236	0.194
GREENU	0.158	0.060	0.199	0.096	**0.248**	0.233
GREEUE	0.183	0.083	0.188	0.087	**0.243**	0.213
SMITH3	0.177	0.083	**0.281**	0.113	0.163	0.182
SMITH4	0.180	0.073	**0.262**	0.098	0.163	0.223
SMITHE	0.155	0.103	**0.252**	0.137	0.163	0.188
BEANUP	0.233	0.047	0.169	0.122	**0.267**	0.162
NF3	0.194	0.104	**0.298**	0.108	0.125	0.170
GREENL	0.141	0.099	**0.329**	0.130	0.090	0.210
HWF2	0.152	0.067	0.194	0.129	0.226	**0.229**
HWF3	0.151	0.088	**0.344**	0.082	0.160	0.174
HWF4	0.208	0.088	**0.239**	0.108	0.172	0.182
QUARTZ	0.175	0.096	**0.262**	0.157	0.126	0.180
BC	0.140	0.069	**0.253**	0.077	0.249	0.211
BADGER	0.135	0.088	0.189	0.100	**0.266**	0.221
F1	0.152	0.135	**0.216**	0.158	0.158	0.179
F2	0.193	0.092	**0.224**	0.106	0.223	0.161
F3	0.134	0.099	**0.276**	0.149	0.122	0.220
IRON	0.100	0.098	0.202	0.118	**0.293**	0.188
Q2	0.155	0.060	**0.271**	0.108	0.218	0.187
CR	0.213	0.074	0.156	**0.318**	0.040	0.180
GF	**0.278**	0.078	0.183	0.219	0.082	0.139
JC	0.217	0.075	**0.262**	0.183	0.149	0.112
L1	0.189	0.102	**0.300**	0.131	0.134	0.143
L3	0.119	0.057	**0.274**	0.098	0.196	0.254
PC	**0.334**	0.058	0.231	0.139	0.089	0.125
PL	**0.267**	0.057	0.199	0.133	0.208	0.136
TL1	0.116	0.076	0.179	0.161	**0.285**	0.182
WC	**0.244**	0.066	0.158	0.241	0.151	0.138
WD	0.183	0.072	0.172	**0.200**	0.188	0.181

Table 19. Model results from geographically weighted regression (GWR) or ordinary-least squares (OLS) analysis for the global, blast zone and intact forest models. Path is the least cost path upon which the model is based. Variables are the independent variables included in the model and direction represents correlation with G_{ST}'.

	Path	Variables	Direction	r^2	AIC	AIC wt.
Global (GWR)	LC forest/stream	non-forest dist ffp slope	+ - -	0.392	-1771	0.83
	LC forest/stream	total dist ffp slope	+ - -	0.390	-1769	0.16
Blast Zone (OLS)	LC forest/stream	non-forest dist ffp	+ -	0.226	-298	0.41
	LC forest/stream	non-forest dist gsp	+ +	0.220	-298	0.31
	LC forest/stream	non-forest dist	+	0.203	-297	0.24
	LC forest/stream	total dist	+	0.139	-291	0.01
	Straight line	non-forest dist gsp	+ +	0.143	-290	0.01
	Straight line	non-forest dist ffp	+ -	0.137	-290	0.01
	Straight line	non-forest dist	+	0.122	-290	0.01
Intact Forest (GWR)	LC forest/stream	total dist	+	0.482	-552	0.51
	Straight line	total dist	+	0.480	-551	0.36
	LC forest/stream	ffp	-	0.466	-548	0.08
	Straight line	ffp	-	0.454	-547	0.05

Table 20. Model results from ordinary-least squares regression (OLS) analysis for the unmanaged and managed blast zone. Path is the least cost path upon which the model is based. Variables are the independent variables included in the model and direction represents correlation with G_{ST}’.

	Path	Variables	Direction	r^2	AIC	AIC wt.
Natl Vol. Monument (OLS)	Straight line	non-forest dist	+	0.638	-67	0.31
		slope	+			
	Straight line	total dist	+	0.635	-66	0.30
		slope	+			
	Straight line	total dist	+	0.495	-64	0.11
	Straight line	non-forest dist	+	0.492	-64	0.10
	Straight line	slope	+	0.443	-63	0.05
	LC forest/stream	non-forest dist	+	0.438	-63	0.05
	Straight line	gsp	+	0.400	-62	0.03
	LC forest/stream	total dist	+	0.398	-62	0.03
	LC forest/stream	% canopy	+	0.364	-61	0.02
Managed blast zone (OLS)	LC forest/stream	non-forest dist	+	0.612	-70	0.23
		gsp	+			
		slope	-			
		hli	+			
	LC forest/stream	non-forest dist	+	0.555	-70	0.23
		gsp	+			
		slope	-			
	LC forest/stream	non-forest dist	+	0.597	-69	0.15
		hli	+			
		gsp	+			
		ffp	+			
	Straight line	non-forest dist	+	0.425	-69	0.14
	LC forest/stream	non-forest dist	+	0.517	-68	0.10
		hli	+			
		gsp	+			
	Straight line	% canopy	-	0.388	-68	0.07
	LC forest/stream	total dist	+	0.313	-65	0.02
	LC forest/stream	non-forest dist	+	0.376	-65	0.02
		gsp	+			
	LC forest/stream	non-forest dist	+	0.309	-65	0.02

Table 21. Model results from ordinary-least squares regression (OLS) analysis for the tephra and undisturbed forest. Path is the least cost path upon which the model is based. Variables are the independent variables included in the model and direction represents correlation with G_{ST}'. Asterisk indicates model including only southern sites.

	Path	**Variables**	**Direction**	**r^2**	**AIC**	**AIC wt.**
Tephra (OLS)	Straight line	ffp	-	0.328	-107	0.57
	LC forest/stream	ffp	-	0.232	-105	0.24
	Straight line	gsp	+	0.255	-104	0.19
Undisturbed forest (OLS)	LC forest/stream	ffp	-	0.146	-180	0.44
	Straight line	ffp	-	0.138	-179	0.36
	Straight line	slope	-	0.115	-178	0.20
Undisturbed forest* (OLS)	Straight line	total dist slope	+ -	0.782	-104	0.74
	LC forest/stream	total dist slope	+ -	0.740	-100	0.12
	LC forest/stream	total dist	+	0.704	-99	0.09
	Straight line	total dist	+	0.691	-99	0.05

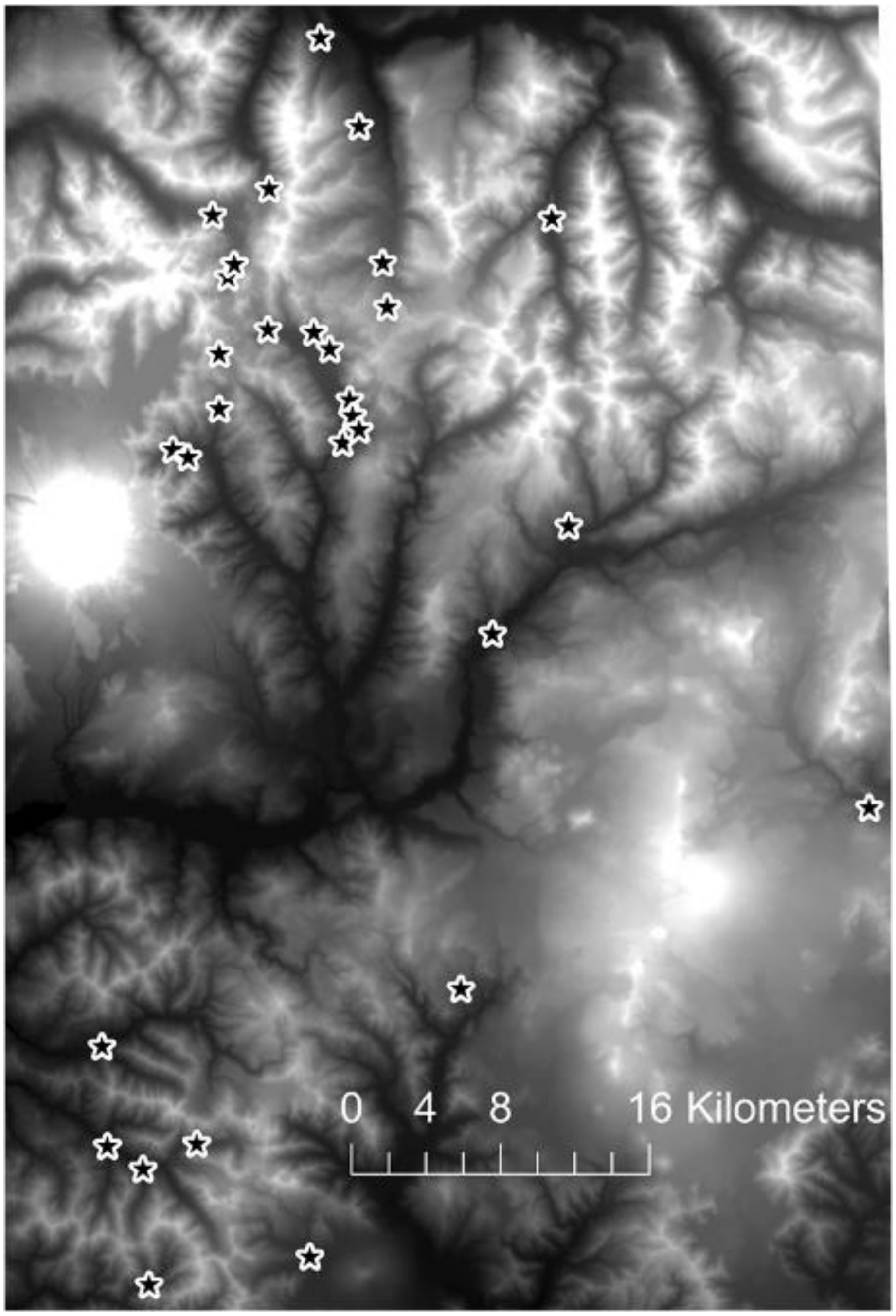

Figure 9

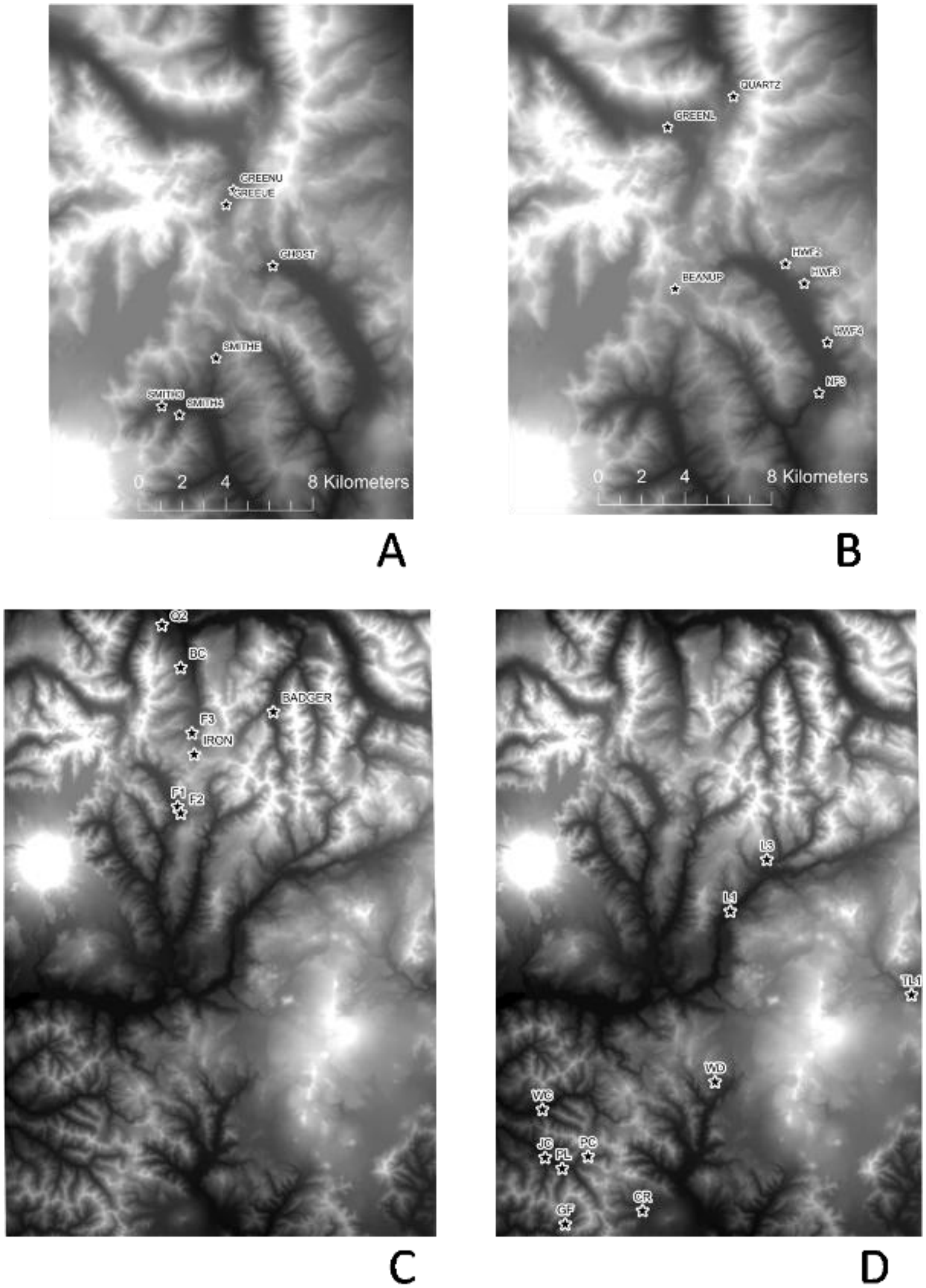
GREENU
GREEUE
GHOST
SMITHE
SMITH3
SMITH4
0
2
4
8 Kilometers
A
QUARTZ
GREENL
HWF2
HWF3
BEANUP
HWF4
NF3
0
2
4
8 Kilometers
B
O2
BC
BADGER
F3
IRON
F1
F2
C
L3
L1
TL1
WD
WC
JC
PC
PL
CR
GF
D

Figure 10

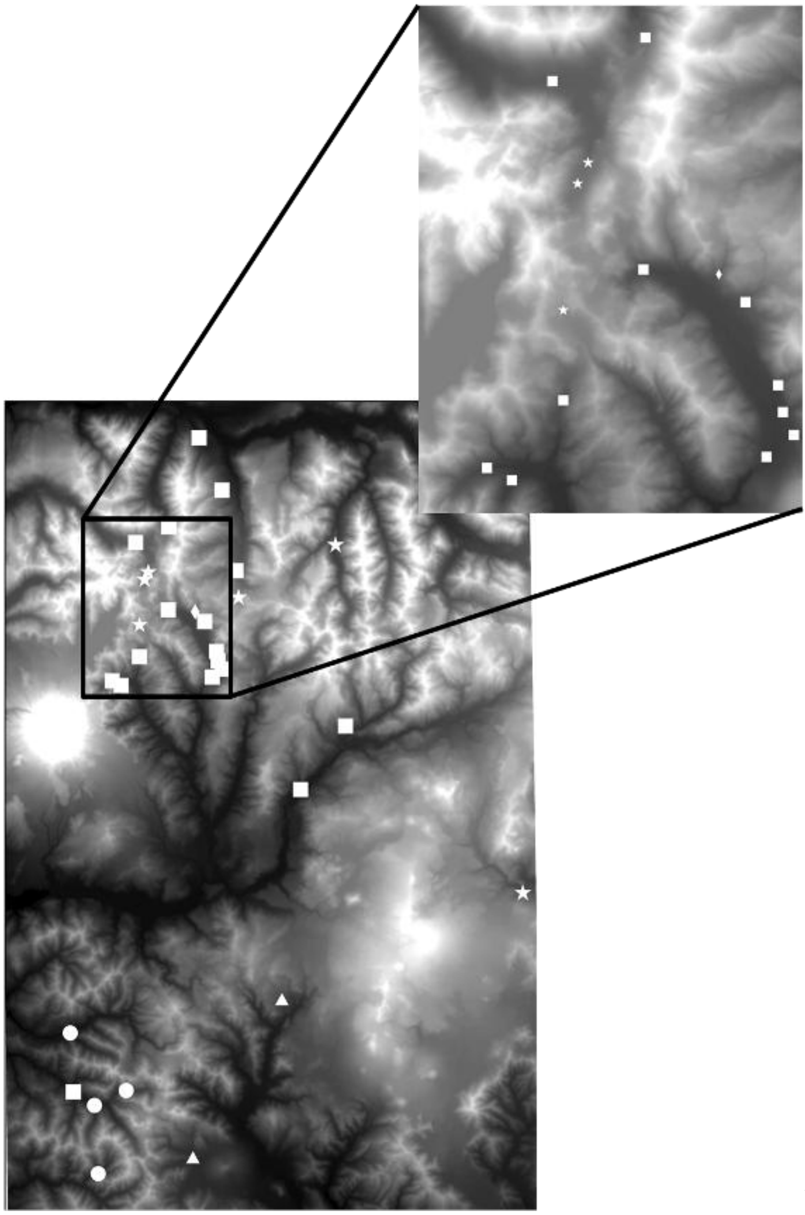

Figure 11

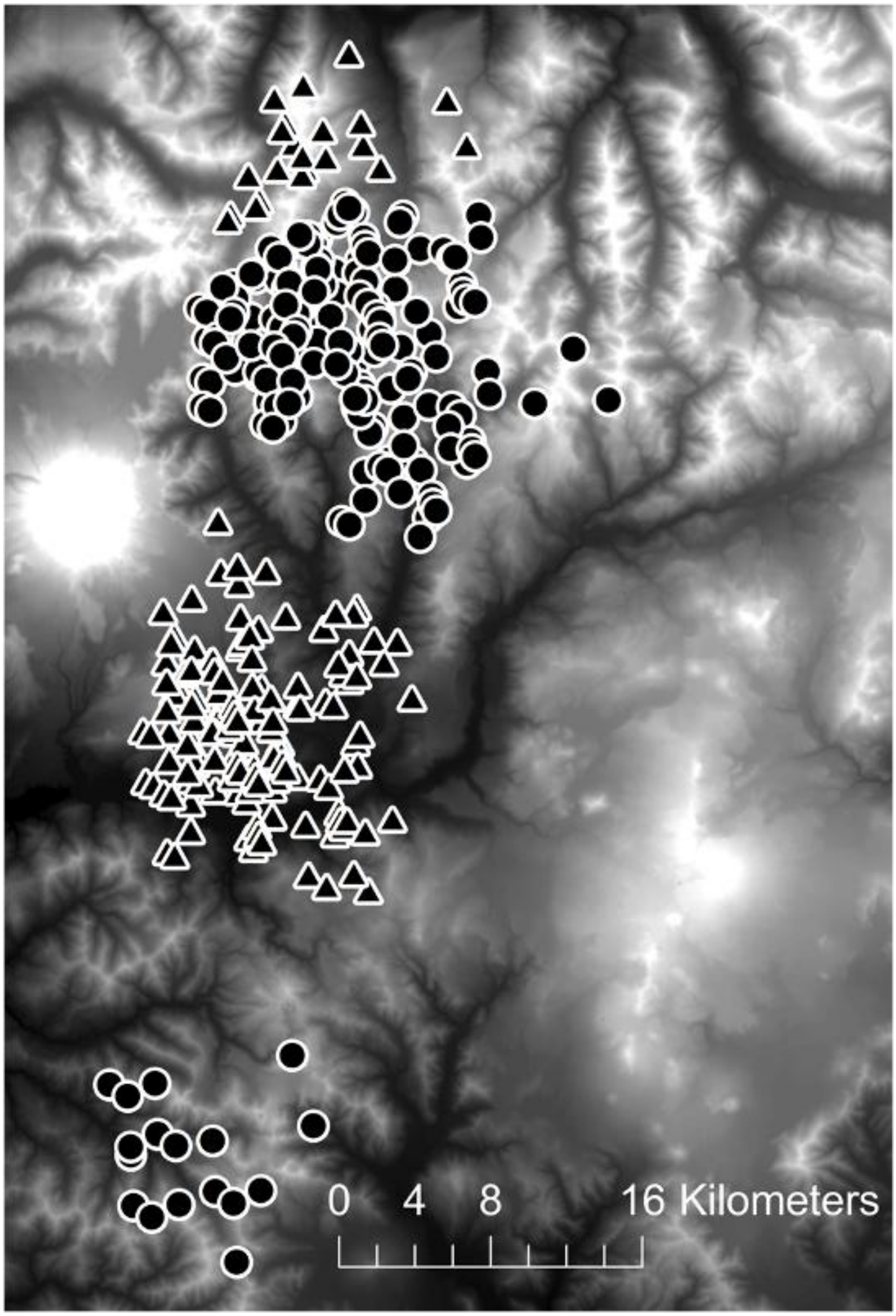

Figure 12

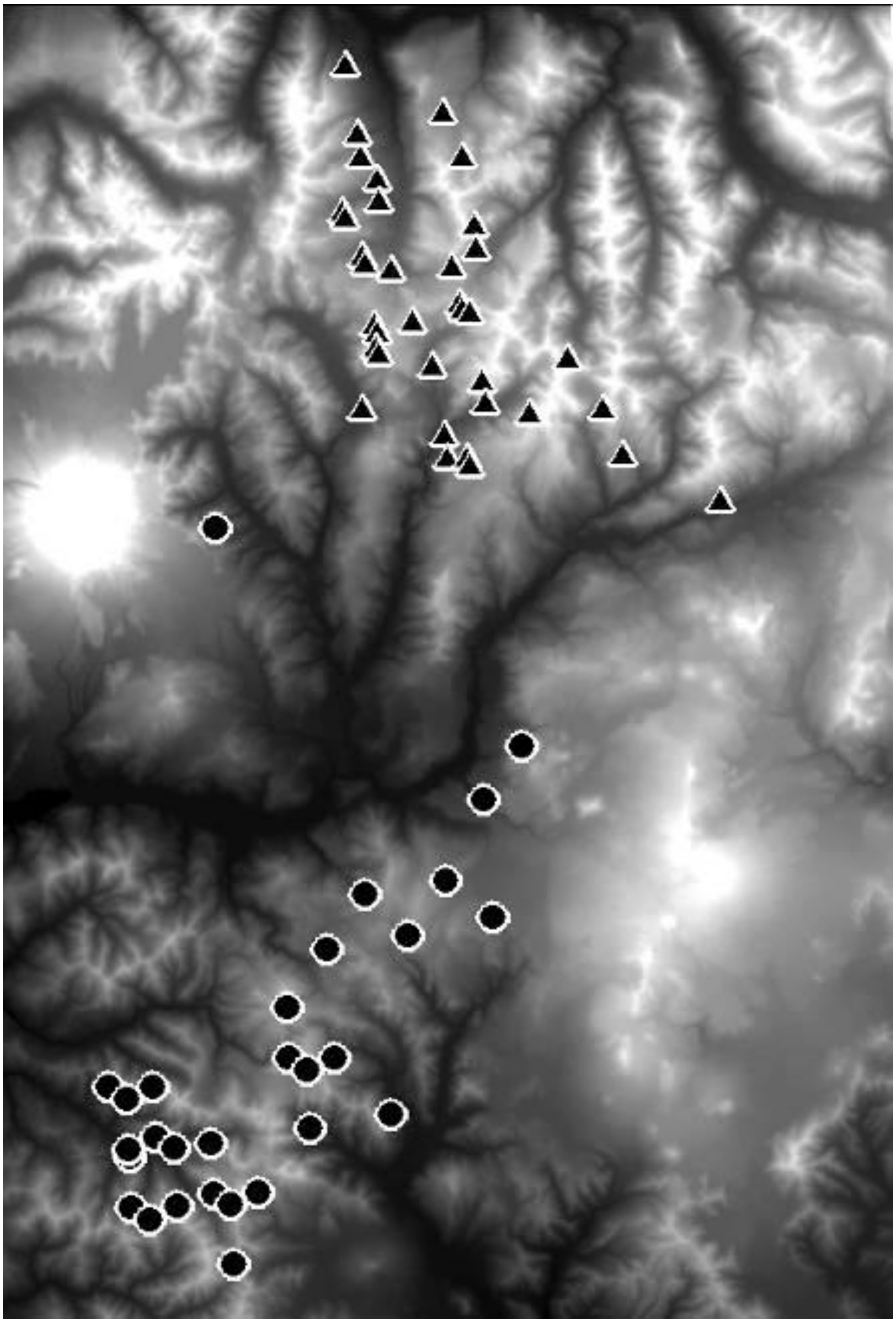

Figure 13