Utah State University
DigitalCommons@USU

All Graduate Theses and Dissertations | Graduate Studies, School of

12-1-2008

The Genetics of Colonization in Two Amphibian Species After the 1980 Eruption of Mount St. Helens

Kristin Ann Bakkegard
Utah State University

Recommended Citation
Bakkegard, Kristin Ann, "The Genetics of Colonization in Two Amphibian Species After the 1980 Eruption of Mount St. Helens" (2008). *All Graduate Theses and Dissertations.* Paper 77.
http://digitalcommons.usu.edu/etd/77

This Dissertation is brought to you for free and open access by the Graduate Studies, School of at DigitalCommons@USU. It has been accepted for inclusion in All Graduate Theses and Dissertations by an authorized administrator of DigitalCommons@USU. For more information, please contact becky.thoms@usu.edu.

THE GENETICS OF COLONIZATION IN TWO AMPHIBIAN SPECIES AFTER THE 1980 ERUPTION OF MOUNT ST. HELENS

by

Kristin A. Bakkegard

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Biology

Approved:

E. D. Brodie, Jr. Major Professor	M. E. Pfrender Committee Member
P. G. Wolf. Committee Member	K. H. Beard Committee Member
T. A. Crowl Committee Member	B. R. Burnham Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, UT

2008

Copyright © Kristin A. Bakkegard, 2008

All Rights Reserved

ABSTRACT

The Genetic Response of Two Amphibian Species After the

1980 Eruption of Mount St. Helens

by

Kristin A. Bakkegard, Doctor of Philosophy

Utah State University, 2008

Major Professor: Dr. Edmund D. Brodie, Jr.
Department: Biology

The genetics of colonization is understudied in salamanders but has large conservation implications as new habitats are formed or restored to their previous condition. The 1980 eruption of Mount St. Helens provided a natural experiment to study the genetic effects of a large infrequent environmental disturbance on two species of salamander, *Taricha granulosa* (Rough-skinned newt) and *Ambystoma gracile* (Northwestern salamander). Both these species breed in ponds, and are thought to exhibit high breeding site fidelity and low vagility. I designated three treatments based on the effects of the eruption: new ponds (created by the eruption, immigrants only), recovery lakes (in blast zone, survivors plus immigrants), and reference lakes (unaffected by eruption, assumed to represent pre-eruption genetic diversity measures). Salamanders took at least nine years to colonize the new ponds. I studied the population genetics of colonization and recovery using microsatellites and AFLPs (amplified fragment length polymorphisms) to measure genetic diversity, gene flow, and population substructure at

Mount St. Helens National Volcanic Monument. Based on population genetics theory and the life history characteristics of these pond-breeding amphibians, I predicted that genetic diversity would be lower in newly colonized ponds compared to recovery or reference sites. I also expected significant levels of population substructuring. Finally, I predicted that because of their lower vagility and large number of neotenes, that *A. gracile* would have less gene flow and a greater degree of population substructuring than *T. granulosa*. My predictions were not supported by my data. There was no loss of genetic diversity in new or recovery populations in either species. There was no strong evidence for population structure by either AMOVA, isolation by distance or principal components analysis. Gene flow (F_{ST}) was high in both species. *Taricha granulosa* and *A. gracile* were found to be resistant to a large infrequent environmental disturbance. Loss of genetic variability in new populations cannot automatically be assumed. Predicting dispersal and colonization ability based on the broad category of pond-breeding amphibian is not always reliable.

(137 pages)

ACKNOWLEDGMENTS

I would like to thank Dr. Edmund D. Brodie, Jr., an advisor and mentor without equal. He and his spouse, Judy, always went above and beyond the call of duty. They attended the change of command ceremony in Fort Worth, TX for my Navy reserve unit, Mobile Inshore Undersea Warfare Unit 109, and visited me in the hospital after my appendectomy. I was discharged from the hospital the same day of the lab's big fall party. Butch picked me up at the hospital and took me to their house where I got to sit in "his" chair during the festivities. Judy Brodie sent me care packages containing the best pralines in the world during my mobilization and deployment to Kuwait in 2004.

Dr. Michael E. Pfrender served as a co-advisor on all genetic matters. Without his advise, technical expertise, and use of his lab, the genetics work would have been impossible to complete. He also suggested Mount St. Helens as a study site. My interest in population genetics was spurred by discussions in Dr. Paul Wolf's population genetics class. Lab mates Chris Feldman and Eric O'Neill taught me how to extract DNA and perform PCRs, and answered a myriad of questions about these techniques. The AFLP work would not have been possible without the assistance of Dr. Mark P. Miller and his technician, Mr. Tom Orwin. Dr. Miller provided advice and lab supplies. Tom taught me the lab techniques for AFLPs and assisted in performing many of the DNA extractions and PCR reactions. Committee members Drs. Paul Wolf, Todd Crowl and Karen Beard provided helpful comments on this manuscript and asked all the questions I had not considered.

At Mount St. Helens, Charlie M. Crisafulli provided helpful information on where to find animals and a map of the Hummocks and Maratta ponds. He provided traps for my

first field season and provided access to a prime camping spot for the Hummocks and Maratta ponds. Dr. Adam G. Jones provided aliquots of primers for *Taricha granulosa*. Rod Williams (*Ambystoma texanum*), Tim L. King and Colleen Callahan (*A. maculatum* and *A. jeffersonianum*), and Angie Stevenson and Kelly Zamudio (*A. maculatum*) provided aliquots of primer for testing in *Ambystoma gracile*. Stephen Spear provided advice on optimizing *A. tigrinum* primers and provided a tissue sample of *A. tigrinum*, Quinn Shurtliff provided advise on using Abgene's Reddy-Mix, and Dr. Peter Ducey provided a tissue sample of *A. maculatum* that was used as a control. Mr. Ken Jones of Genetic Identification Services tested *A. gracile* DNA in PCR with *A. macrodactylum* primers for free. Their assistance saved me considerable amounts of time and money.

The USU herpetology group, including Kevin Young, Megan Lahti, Chris Feldman, Eric O'Neill, Dan Mulcahy, Becky Williams, Amber Brouillette, Leigh Latta, Ryan O'Donnell, Charles Hanifin, Shana Geffney, Ali Scoville, Michelle Cummer, and Mike Edgehouse, provided good friendship, feedback on work, and encouragement throughout the entire process. Best wishes to them on their future endeavors.

Funding sources included Sigma Xi and the American Museum of Natural History through a Theodore Roosevelt Memorial Grant. Research was approved by the Utah State University IACUC, State of Washington scientific collection permits, and administrative closure access permits at Mount St. Helens National Volcanic Monument.

Finally, I am grateful to Mom, Dad, my brother, John, and sister, Koren. They knew that this was important to me. My cats, Shadow, Merlin, and Lena, listened, snuggled, and always knew when to purr.

Kristin Bakkegard

CONTENTS

Page

ABSTRACT……...……...iii

ACKNOWLEDGMENTS v

LIST OF TABLES ix

LIST OF FIGURES xi

CHAPTER

1. INTRODUCTION 1

2. RAPID GENETIC RECOVERY BY *TARICHA GRANULOSA* (CAUDATA: SALAMANDRIDAE) AFTER THE 1980 ERUPTION OF MOUNT ST. HELENS 14

Introduction.....14
Materials and Methods.....20

Study site 20
Population sampling 21
DNA extraction and microsatellite amplification.....22
Data analysis 23

Results.....24
Discussion.....26
Literature Cited 33

3. GENETICS OF COLONIZATION IN *AMBYSTOMA GRACILE* (CAUDATA: AMBYSTOMATIDAE) AFTER THE 1980 ERUPTION OF MOUNT ST. HELENS 53

Introduction.....53
Materials and Methods.....57

Study site 57
Study organism 58
Population sampling 59
DNA extraction and amplification.....60

Microsatellites 60

AFLP 61

Within-population patterns of genetic diversity 63
Among-population patterns of genetic diversity 63
Assignment tests 64

Results 65

Sampling 65
Within-population measures of genetic diversity 66
Among-populations patterns of genetic diversity 67
Assignment tests 68

Discussion 69
Literature Cited 75

4. CONCLUSIONS 94

APPENDICIES 101

Appendix A. Collections by locality and year 102
Appendix B. Allele frequency tables 104
Appendix C. Distance Matrix 113
Appendix D. All *Ambystoma* primers 115

CURRICULUM VITAE 122

LIST OF TABLES

Table Page

2.1 Effects of the 1980 eruption of Mount St. Helens on the local landscape 43

2.2 Total collections of *Taricha granulosa* by zone and treatment 44

2.3 PCR conditions and basic data on six microsatellite loci for *T. granulosa* from MSHNVM 45

2.4 Genetic measures for 6 *T. granulosa* microsatellite loci (L) by collection site 46

2.5 Genetic measures for 5 *T. granulosa* microsatellite loci by treatment..... 47

2.6 Comparisons between treatment groups over all loci in *T. granulosa* at MSHNVM 48

2.7 Pairwise F_{ST} values and P-values by collection site 49

3.1 Effects of the 1980 eruption of Mount St. Helens on the local landscape 83

3.2 Total collection of *A. gracile* by zone and treatment 84

3.3 PCR conditions and basic data on four microsatellite loci used in *A. gracile* From MSHNVM 85

3.4 Measures of genetic diversity for all collection sites at MSHNVM by Microsatellite locus 86

3.5 Measures of genetic diversity by treatment at MSHNVM by microsatellite and AFLP loci 87

3.6 Measures of genetic diversity for all collection sites at MSHNVM based on 81 AFLP loci 88

3.7 Pairwise population Φ_{PT} values (below diagonal) for microsatellites (top number of pair) and AFLPs (bottom number of pair) 89

3.8 Results of hierarchical AMOVA comparing genetic variation among the three treatments (new, recovery, reference), among collection sites within treatments and among collection sites for microsatellites and AFLPs..... 90

A.1 Collections by location and year of *Taricha granulosa* and *Ambystoma gracile* at Mount St. Helens National Volcanic Monument 103

B.1 Allele frequencies for *Taricha granulosa* and *Ambystoma gracile* from Mount St. Helens National Volcanic Monument 105

C.1 Matrix of Nei's unbiased genetic distances and topographic distances between collection sites at Mount St. Helens National Volcanic Monument 114

D.1 *Ambystoma* microsatellite primers in *A. gracile* 119

LIST OF FIGURES

Figure Page

2.1 Collection sites at MSHNVM 50

2.2 Isolation by distance in *T. granulosa* from six collection sites in MSHNVM 51

2.3 Principal components analysis for *T. granulosa* at MSHNVM by treatment 52

3.1 Collection sites at MSHNVM 91

3.2 Geographic distance versus Nei's unbiased genetic distance in *A. gracile* at MSHNVM 92

3.3 Principal component analysis for microsatellite and AFLPs for *A. gracile* at MSHNVM 93

CHAPTER 1

INTRODUCTION

The genetics of colonization is an important topic in evolutionary biology. Colonization can occur with relatively few individuals, leading to founder effects or a bottleneck, resulting in the loss of alleles and a decrease in genetic diversity (Nei *et al.* 1975). Loss of genetic variability may adversely affect a population, leading to decreased fitness or a decrease in the probability of persistence, especially in small populations (Frankham 1995; Lesbarréres *et al.* 2005). Conversely, new habitats can be colonized by a large number of individuals or by multiple waves of immigrants from different source populations, increasing the amount of genetic variation in the newer populations (Kolbe *et al.* 2007). Colonization can, over many generations, act as an engine for evolution, leading to speciation events as evident in the Galapagos and Hawaiian Islands with well known examples such as spiders (*Tetragnatha*) and birds (*Geospiza*; Grant *et al.* 2001; Gillespie 2004). On an ecological time scale, the success of a colonization event is dependent on the life-history of the colonizer, the selective pressures, reproductive output and survival. Therefore, each colonization event is unique.

Where does life-history and population genetic structure intersect? Wright's (1931) effective population size (N_e) is strongly influenced by age at first reproduction, life span, fecundity, and mating system. Population genetics theory predicts a positive correlation between effective population size and genetic diversity (Kimura 1983) and smaller populations are considered to be at higher risk of suffering the deleterious effects of low genetic diversity (Rowe & Beebee 2003; 2005). One way to contextualize the role of life history in the genetic structure of multiple populations is to compare similar

species, preferably from the same collection sites. With that approach, genetic differences among populations has been attributed to differences in dispersal capabilities, extinction-recolonization events, habitat fragmentation, and natural or anthropogenic disasters (Gallardo *et al.* 1995; Matocq *et al.* 2000; Newman & Squire 2001; Miller *et al.* 2002; Whiteley *et al.* 2004). Thus, a full understanding of the colonization process can provide guidance for research and conservation priorities, provide data for understanding demographic and dispersal patterns as related to life-history traits, and show if, or how much, genetic diversity is lost when a local population goes extinct and the habitat is later recolonized.

Since erupting on May 18, 1980, Mount St. Helens, located in the southwestern corner of Washington State, has become a classic study site for testing ecological theories of colonization (Dale *et al.* 2005). Researchers have studied how plants and animals have responded to and recovered from this major environmental disturbance, which has been allowed to proceed with minimal anthropogenic interference. Surprisingly, in the 25 plus years since the eruption, there are only two published studies on the genetic impacts of the eruption of Mount St. Helens for any taxa, both plants. Bishop (1996) compared survivor populations with newly colonized populations in *Lupinus lepidus* (prairie lupine) and found strong founder effects as expected from theory. Yang *et al.* (2008) found no evidence of founder effects or reduction in genetic diversity in new populations colonizing primary succession areas as compared to secondary succession or survivor plants in *Vaccinium membranaceum* (black huckleberry); evidence of high gene flow and long distance dispersal of seeds of this plant by animals. Worldwide, genetic studies of recolonization, after a recent volcanic eruption are few. The fossorial rodent *Ctenomys*

maulinus brunnenus on Lonquimay volcano in Chile showed a drastic reduction in genetic diversity of populations resulting in bottleneck effects when compared to levels of pre-eruption genetic diversity (Gallardo *et al.* 1995). The recolonization of *Hypochaeris tenuifolia*, a flowering herb on the same volcano, had no loss of genetic diversity within five founder populations (less than 10 yrs old) as compared to survivor or isolated populations from other parts of the range (Tremsetsberger *et al.* 2003). The tree *Antirhea borbonica* that colonized young lava flows on La Réunion island showed no evidence of founder effects (Litrico *et al.* 2005). Other studies on the impact of volcanoes on population genetics were from eruptive events greater than 50 yrs ago (e.g. Vandergast *et al.* 2004).

The impact of the 18 May 1980 eruption on the surrounding landscape was swift, violent and dramatic. All areas on the northern side and to the northeastern side of the volcano were severely affected by the debris-avalanche, blast and ashfall (Peterson 1986). The collapse of the volcano's north slope caused a debris-avalanche, leaving a deposit of rock, tephra and ash up to 195 m deep where nothing is believed to have survived (Swanson & Major 2005). The blast created a blowdown zone (approx. 570 km^2 of forest affected). Some plants and animals survived the blast, including some fish in 67% of the lakes within the blowdown zone, and small burrowing animals (and their commensals), such as the northern pocket gopher, *Thomonys talpoides*, which were still in their winter retreats (Andersen 1982; Andersen & MacMahon 1985; Crawford 1986). In contrast, areas to the south of the volcano were relatively unaffected except to receive approximately 5 to 25 cm of tephra fall (Dahm *et al.* 2005). Several lakes on the south side have been used in other studies as reference sites, representing pre-eruption

conditions (Wissmar *et al.* 1982; Dahm *et al.* 2005). Out of this mosaic of effects upon the landscape I delineated three treatments: new ponds, generated by the debris-avalanche when depressions in the large hummocky deposits filled with water; recovery lakes, those within the blowdown zone; and reference lakes, those unaffected by the eruption. The biota of the new ponds consist entirely of colonists. Recovery lakes contain colonists and either survivors or their descendents. Animals collected in the reference lakes should be representative of the genetic diversity present in the Mount St. Helens area prior to the eruption.

The purpose of this research was to measure the amount of genetic diversity, gene flow, and amount of population subdivision in common two amphibian species found in and around Mount St. Helens. This study is the first to examine the population genetics of any animal species in response to the 1980 eruption. Based on population genetics theory, the amount of genetic diversity in ponds colonized since the eruption should be less than that of reference areas, if low gene flow and few colonists is assumed. These assumptions are developed by making a thorough consideration of the life-history characteristics of the colonizing species. However, predictions based on life-history characters may not always hold true (Colson & Hughes 2004). Therefore, each species must be considered on its own merits.

The genetics of colonization is understudied in salamanders, especially in those species found in the western United States. Understanding the outcomes of the colonization process is important in elucidating how well amphibians can recover from a major disturbance. In this study, the disturbance is natural. However, most amphibian conservation efforts include restoring degraded or severely damaged habitats back to a

more natural state. Restoration of genetic diversity is as important as restoring numbers of animals (Mace *et al.* 1996; Reed & Frankham 2003). A colonization event could result in a large number of individuals but the population as a whole may suffer from the deleterious effects of low genetic diversity, reducing the probability that a newly restored population will persist (Reed *et al.* 2007).

I studied two species of locally common pond-breeding amphibian, *Taricha granulosa*, the rough-skinned newt, and *Ambystoma gracile*, the northwestern salamander. *Taricha granulosa* has a typical biphasic life-cycle; aquatic eggs and larvae, with terrestrial adults. Over the course of several months, adults migrate to a breeding pond, court, females lay eggs, and then leave. Upon transformation (4-5 months, depending upon site conditions), larvae depart the breeding pond, returning when sexually mature at approximately 4-5 years old (Petranka 1998). Females mate with multiple males then deposit single eggs (112-226, mean = 172) over the course of several weeks (Jones *et al.* 2002). *Taricha granulosa* is assumed to be philopatric to breeding sites because they do not appear to move far from it when not breeding (Pimentel 1960). However, a population genetics study at a geographic scale comparable to mine measured an average F_{ST} value of 0.005, indicating high gene flow (Jones *et al.* 2001). The life span of *T. granulosa* is estimated to be about 12 years and they are capable of homing and returning to their capture site when displaced (Efford & Mathias 1969). *Taricha granulosa* colonized the new ponds created by the debris-avalanche 10 years post eruption and the closest source population was estimated to be 5.7 km away (Crisafulli *et al.* 2005).

Ambystoma gracile also has a biphasic life-style though different from *T. granulosa*. In some populations, adults do not metamorphose into a lunged, terrestrial

form but instead remain in the natal pond and upon reaching maturity, breed as gilled adults called neotenes (Sprules 1974). Current Mount St. Helens populations appear to be highly neotenic, especially in the debris-avalanche zone (Crisafulli *et al.* 2005). Females attach gelatinous egg masses, containing 40–270 eggs, to vegetation or sticks in a pond (Nussbaum *et al.* 1983; MacCracken 2007). Larvae take longer to mature (over a year) which is an extended aquatic phase as compared to most other *Ambystoma* (Eagleson 1976; Petranka 1998). The life-span of neotenic *A. gracile* is estimated to be 5 years (Efford & Mathias 1969). The lifespan of a terrestrial adult is unknown. Ambystomatid salamanders are considered highly site-fidelic, usually moving less than 1 km from their breeding ponds, exhibit low migration rates between subpopulations, and have low vagility (Spear *et al.* 2005; Gamble *et al.* 2007; Kinkead *et al.* 2007; Zamudio & Wieczorek 2007). It took 9 years for *A. gracile* to colonize the new ponds and their closest source population was 3.7 km away (Crisafulli *et al.* 2005).

Based on the life-history of these pond-breeding amphibians and population genetics theory, I predict that populations from recently colonized habitat (immigrants only) will have less genetic diversity than populations from recovering (survivors plus immigrants) or unaffected areas (source populations). New ponds, created in 1980, were not colonized by salamanders until 9 years post eruption. Since colonization, I estimate there have been only 3–5 generations of *T. granulosa* and 5–10 generations of *A. gracile*. The debris-avalanche zone appears to be a moonscape (bare rock, little vegetation) with little cover or shelter to protect migrating salamanders. Therefore, it is reasonable to expect that new ponds have been colonized by only a few individuals because of the harsh environment and putative philopatry of breeding adults. Coupled with low gene

flow from other populations, new ponds should have low genetic diversity as compared to recovery lakes or source populations. Due to the rugged terrain, sampled populations across the area should also exhibit high levels of population substructure.

Another goal of this study was to compare the population genetics of two species collected from the same localities, so that they would be subjected to the same landscape and environmental conditions, to observe how differences in life-history affect the genetic diversity of colonized populations. There are few studies comparing the population genetics of two sympatric species. Miller *et al.* (2002) compared direct observation of dispersal with patterns of genetic diversity in four aerial dispersing aquatic insects and found that all four species showed different patterns of genetic diversity due to differences in dispersal ability. Monaghan *et al.* (2002) found differences in genetic patterns between three species of aquatic insects (2 mayflies, 1 caddisfly) due to differences in dispersal ability but overall, small-scale patterns were affected by demographic processes (recent habitat fragmentation within 100 yrs) and large-scale patterns were due to historical processes (habitat fragmentation on geologic scale). A forest specialist species of carabid beetle showed higher amounts of population substructuring and a lower dispersal capability than a generalist carabid beetle that could live in forest and open areas (Brouat *et al.* 2003). Matocq *et al.* (2000) used ecological and life- history differences to predict then measure the population genetic structure and amounts of gene flow in two species of sympatric spiny rats. Whiteley *et al.* (2004) found spawning location, population size and mating system were good predictors of gene flow patterns and genetic variation in sympatric populations of bull trout and mountain whitefish. They also provided a general framework for discussing the interactions among

ecological and life-history factors and neutral and adaptive divergence. Amphibian studies include the population genetics of three species of sympatric *Ambystoma* (Kinkead *et al.* 2007). Brede & Beebee (2004) found that populations of the toad *Bufo bufo*, had lower genetic variability than the frog *Rana temporaria*, even though the toad had larger breeding assemblages than the frog. They attributed this result to high levels of gene flow in the frog. Therefore, comparison of genetic diversity in sympatric species is applicable to a wide variety of ecological and evolutionary scenarios because with population genetics tools, specific hypotheses centered upon species-specific life-history differences can be addressed.

Taricha granulosa and *A. gracile* vary in critical life-history characteristics such as fecundity, age at first reproduction, life-span, and vagility and should respond differently to the eruption. *Taricha granulosa* is more vagile than *A. gracile* because they are less susceptible to desiccation. They also have a longer generation time resulting in fewer generations post-eruption. Comparing the two caudates, fecundity is about equal. Obviously, the first colonizers of *A. gracile* were terrestrial adults. However, current populations appear to be highly neotenic. Therefore, I predict that there have been fewer migrating *A. gracile* than *T. granulosa* and genetic diversity, as measured by heterozygosity, and gene flow, as measured by F_{ST} and population substructuring, will be lower in Northwestern salamanders.

In Chapter 2, I report on the genetics of colonization in *Taricha granulosa*, the Rough-skinned newt, in lakes and ponds at Mount St. Helens using microsatellites as the molecular marker. In Chapter 3, I study the colonization genetics of *Ambystoma gracile*, the Northwestern salamander. It differs from *T. granulosa* in some key life-history traits,

most notably, reproduction in the neotenic form. At many of the same ponds where I also collected *T. granulosa*, I measured genetic diversity, gene flow and population substructure using two classes of genetic markers to determine the effects of the eruption on a natural population of this salamander over an ecological timescale. In Chapter 4, I compare the results of the two species. Because few microsatellite primers worked in *A. gracile* and because the results for both species were similar, I was unable to speculate as to which life-history variable would have attributed to any species-specific differences.

Literature Cited

Andersen DC (1982) Observations on *Thomomys talpoides* in the region affected by the eruption of Mount St. Helens. *Journal of Mammalogy* **63**, 652-655.

Andersen DC, MacMahon JA (1985) The effects of catastrophic ecosystem disturbance: the residual mammals at Mount St. Helens. *Journal of Mammalogy* **66**, 581-589.

Bishop JG (1996) *Demographic and genetic variation during colonization by the herb Lupinus lepidus on Mount St. Helens.* PhD. Dissertation, University of Washington.

Brede EG, Beebee TJC (2004) Contrasting population structures in two sympatric anurans: implications for species conservation. *Heredity* **92**, 110-117.

Brouat C, Sennedot F, Audiot P, Leblois R, Rasplus J-Y (2003) Fine-scale genetic structure of two carabid species with contrasted levels of habitat specialization. *Molecular Ecology* **12**, 1731-1745.

Colson I, Hughes RN (2004) Rapid recovery of genetic diversity of dogwhelk (*Nucella lapillus* L.) populations after local extinction and recolonization contradicts predictions from life-history characteristics. *Molecular Ecology* **13**, 2223-2233.

Crawford B (1986) The recovery of surviving fish populations within the Mount St. Helens National Volcanic Monument and adjacent areas. In: *Mount St. Helens: Five Years Later* (ed Keller SAC), pp. 293-296. Eastern Washington University Press, Cheney.

Crisafulli CM, Trippe LS, Hawkins CP, MacMahon JA (2005) Amphibian responses to the 1980 eruption of Mount St. Helens. In: *Ecological Responses to the 1980 Eruption of Mount St. Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 183-197. Springer, New York.

Dahm CN, Larson DW, Petersen RR, Wissmar RC (2005) Response and recovery of lakes. In: *Ecological Responses to the 1980 Eruption of Mount St. Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 255-274. Springer, New York.

Dale V, Swanson FJ, Crisafulli CM (2005) *Ecological Responses to the 1980 Eruption of Mount St. Helens.* Springer, New York.

Eagleson GW (1976) A comparison of the life histories and growth patterns of *Ambystoma gracile* (Baird) from permanent low-altitude and montane lakes. *Canadian Journal of Zoology* **54**, 2098-2111.

Efford IE, Mathias JA (1969) A comparison of two salamander populations in Marion Lake, British Columbia. *Copeia* **1969**, 723-736.

Frankham R (1995) Inbreeding and extinction: a threshold effect. *Conservation Biology* **9**, 792-799.

Gallardo MH, Kohler N, Araneda C (1995) Bottleneck effects in local populations of fossorial *Ctenomys* (Rodentia, Ctenomyidae) affected by vulcanism. *Heredity* **74**, 638-646.

Gamble LR, McGarigal K, Compton BW (2007) Fidelity and dispersal in the pond-breeding amphibian, *Ambystoma opacum*: implications for spatio-temporal population dynamics and conservation. *Biological Conservation* **139**, 247-257.

Gillespie R (2004) Community assembly through adaptive radiation in Hawaiian spiders. *Science* **303**, 356-359.

Grant PR, Grant BR, Petren K (2001) A population founded by a single pair of individuals: establishment, expansion, and evolution. *Genetica* **112-113**.

Jones AG, Adams EM, Arnold SJ (2002) Topping off: a mechanism of first-male sperm precedence in a vertebrate. *Proceedings of the National Academy of Sciences* **99**, 2078-2081.

Jones AG, Blouin MS, Arnold SJ (2001) Genetic variation in two populations of the rough-skinned newt (*Taricha granulosa*) assessed using novel tetranucleotide microsatellite loci. *Molecular Ecology Notes* **1**, 293-296.

Kimura M (1983) *The Neutral Theory of Molecular Evolution.* Cambridge University Press, Cambridge.

Kinkead KE, Abbott AG, Otis DL (2007) Genetic variation among *Ambystoma* breeding populations on the Savannah River site. *Conservation Genetics* **8**, 281-292.

Kolbe JJ, Glor RE, Schettino LR, Lara AC, Larson A, Losos JB (2007) Multiple sources, admixture, and genetic variation in introduced *Anolis* lizard populations. *Conservation Biology* **21**, 1612-1625.

Lesbarréres D, Primmer CR, Laurila A, Merila J (2005) Environmental and population dependency of genetic variability-fitness correlations in *Rana temporaria*. *Molecular Ecology* **14**, 311-323.

Litrico I, Ronfort J, Verlaque R, Thompson JD (2005) Spatial structure of genetic variation and primary succession in the pioneer tree species *Antirhea borbonica* on La Réunion. *Molecular Ecology* **14**, 1575-1584.

MacCracken JG (2007) Northwestern salamander (*Ambystoma gracile*) oviposition sites and hatching success in the Cascade mountains of southern Washington. *Herpetological Conservation and Biology* **2**, 127-134.

Mace GM, Smith TB, Bruford MW, Wayne RK (1996) An overview of the issues. In: *Molecular Genetic Approaches in Conservation* (eds Smith TB, Wayne RK), pp. 3-21. Oxford University Press, New York.

Matocq MD, Patton JL, da Silva MNF (2000) Population genetic structure of two ecologically distinct Amazonian spiny rats: separating history and current ecology. *Evolution* **54**, 1423-1432.

Miller MP, Blinn DW, Keim P (2002) Correlations between observed dispersal capabilities and patterns of genetic differentiation in populations of four aquatic insect species from the Arizona White Mountains, U.S.A. *Freshwater Biology* **47**, 1660-1673.

Monaghan MT, Spaak P, Robinson CT, Ward JV (2002) Population genetic structure of 3 alpine stream insects: influences of gene flow, demographics, and habitat fragmentation. *Journal of North American Benthological Society* **21**, 114-131.

Nei M, Maruyama T, Chakroborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution* **29**, 1-10.

Newman RA, Squire T (2001) Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). *Molecular Ecology* **10**, 1087-1100.

Nussbaum RA, Brodie Jr ED, Storm RM (1983) *Amphibians and Reptiles of the Pacific Northwest.* University Press of Idaho, Moscow.

Peterson DW (1986) Mount St. Helens and the science of volcanology: a five-year perspective. In: *Mount St. Helens: Five Years Later* (ed Keller SAC), pp. 3-19. Eastern Washington University Press, Cheney.

Petranka JW (1998) *Salamanders of the United States and Canada.* Smithsonian Institution Press, Washington, DC.

Pimentel RA (1960) Inter- and intrahabitat movements of the rough-skinned newt, *Taricha torosa granulosa* (Skilton). *American Midland Naturalist* **63**, 470-496.

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Conservation Biology* **17**, 230-237.

Reed DH, Nicholas AC, Stratton GE (2007) Genetic quality of individuals impacts population dynamics. *Animal Conservation* **10**, 275-283.

Rowe G, Beebee TJC (2003) Population on the verge of a mutational meltdown? Fitness costs of genetic load for an amphibian in the wild. *Evolution* **57**, 177-181.

Rowe G, Beebee TJC (2005) Intraspecific competition disadvantages inbred natterjack toad (*Bufo calamita*) genotypes over outbred ones in a shared pond environment. *Journal of Animal Ecology* **74**, 71-76.

Spear SF, Peterson CR, Matocq MD, Storfer A (2005) Landscape genetics of the blotched tiger salamander (*Ambystoma tigrinum melanostictum*). *Molecular Ecology* **14**, 2553-2564.

Sprules WG (1974) Environmental factors and the incidence of neoteny in *Ambystoma gracile* (Baird) (Amphibia: Caudata). *Canadian Journal of Zoology* **52**, 1545-1552.

Swanson FJ, Major JJ (2005) Physical events, environments, and geological-ecological interactions at Mount St. Helens: March 1980-2004. In: *Ecological Responses to the 1980 Eruption of Mount St. Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 27-44. Springer, New York.

Tremsetsberger K, Stuessy TF, M SR, Baeza CM, Fay MF (2003) Genetics of colonization in *Hypochaeris tenuifolia* (Asteraceae, Lactuceae) on Volcán Lonaquimay, Chile. *Molecular Ecology* **12**, 2649-2659.

Vandergast AG, Gillespie RG, Roderick GK (2004) Influence of volcanic activity on the population genetic structure of Hawaiian *Tetragnatha* spiders: fragmentation, rapid population growth and the potential for accelerated evolution. *Molecular Ecology* **13**, 1729-1743.

Whiteley AR, Spruell P, Allendorf FW (2004) Ecological and life history characteristics predict population genetic divergence of two salmonids in the same landscape. *Molecular Ecology* **13**, 3675-3688.

Wissmar RC, Devol AH, Staley JT, Sedell JR (1982) Biological responses of lakes in the Mount St. Helens blast zone. *Science* **216**, 178-181.

Wright S (1931) Evolution in Mendelian populations. *Genetics* **16**, 97-159.

Yang S, Bishop JG, Webster MS (2008) Colonization genetics of an animal-dispersed plant (*Vaccinium membranaceum*) at Mount St Helens, Washington. *Molecular Ecology* **17**, 731-740.

Zamudio KR, Wieczorek AM (2007) Fine-scale spatial genetic structure and dispersal among spotted salamander (*Ambystoma maculatum*) breeding populations. *Molecular Ecology* **16**, 257-274.

CHAPTER 2

RAPID GENETIC RECOVERY BY *TARICHA GRANULOSA* (CAUDATA: SALAMANDRIDAE) AFTER THE 1980 ERUPTION OF MOUNT ST. HELENS

Introduction

Colonization is the establishment of a population in a new area due to one or several dispersal events. The success of a colonization event is dependent on the life-history of the colonizer, the number of immigrants later adding to the new population, selective pressures during colonization, and survivorship and reproductive success after establishment (Slatkin 1977; Grant *et al.* 2001). An *a priori* expectation of colonization is a strong founder effect in the newly established population. Founder effects are characterized by an immediate loss of alleles followed by a decrease in genetic diversity and are most apparent in a population established by only a few individuals (Nei *et al.* 1975; Maruyama & Fuerst 1985; Allendorf 1986; Dlugosch & Parker 2008). Loss of genetic variability may adversely affect a population, leading to decreased fitness or an increase in the probability of extinction, especially in small populations (Hitchings & Beebee 1997; Frankham 2005; Johansson *et al.* 2007; Reed *et al.* 2007). Additionally, smaller populations are considered to be at higher risk of suffering the deleterious effects of low genetic diversity than larger ones because of drift and inbreeding (Rowe & Beebee 2003). Conservation biologists have learned that it is important to conserve both the number of individuals and the genetic diversity within a population in order to maximize fitness and avoid the loss of adaptive variation due to drift (Mace *et al.* 1996; Reed &

Frankham 2003). Therefore, newly colonized populations may be at greater risk of extinction because they are usually established by few individuals, have lower genetic diversity, and thus may suffer from the deleterious effects of inbreeding and drift (Lesbarréres *et al.* 2005; Rowe & Beebee 2005). Understanding colonization can guide research and conservation priorities, provide data for understanding demographic and dispersal patterns as related to life-history traits, and show if or how much genetic diversity is lost when a local population is extirpated and the habitat is later recolonized.

Pond-breeding amphibians (anurans and caudates) are considered to share some general life-history characteristics: low vagility, high site fidelity with respect to breeding site, slow spread across a landscape because they are prone to desiccation, low effective population size, high levels of population subdivision, and spatial dynamics comparable to metapopulations (Semlitsch & Pechmann 1985; Blaustein *et al.* 1994; Funk *et al.* 1999; Marsh & Trenham 2001; Palo *et al.* 2004). Here, the phrase "pond-breeding amphibian" denotes those species with a terrestrial adult, aquatic eggs and larvae phases, that migrate to/from a perennial or permanent breeding pond. In North America, this definition includes most salamanders in the families Ambystomatidae and Salamandridae and anurans in the traditional families Bufonidae, Hylidae, and Ranidae (Salthe 1969; Duellman & Trueb 1986). This also appears to be how some others use the term pond-breeding amphibian, especially (but not always) in reference to metapopulations (Semlitsch 1998; Petranka *et al.* 2004; Jehle *et al.* 2005; Gamble *et al.* 2007). There are other amphibians that breed in ponds (e.g. Sirenids, *Amphiuma*, Hemidactyliini, many anurans) but researchers do not seem to apply the label "pond-breeding amphibian" to them. The traditional herpetological literature recognizes three forms of salamander

larvae based on their morphology: stream, mountain-brook, and pond (Valentine & Dennis 1964) but I was unable to find any other formal definition of "pond-breeding" amphibian.

Population genetics and life history traits such as age at first reproduction, life span, fecundity, and mating system are linked through the concept of effective population size which is positively correlated with genetic diversity (Wright 1931; Kimura 1983). Newly colonized populations of a pond-breeding amphibian are expected to have low effective population size, low genetic diversity, be negatively impacted by founder effects or a bottleneck, and be subject to low gene flow. For example, genetic bottlenecks have been found in newly established (less than 20 years) and severely declining populations in *Bufo calamita* (natterjack toads) in Great Britain (Beebee & Rowe 2001). An established population of the threatened Mallorcan midwife toad, *Alytes muletensis,* showed evidence of a bottleneck in a low gene flow system (Kraaijeveld-Smit *et al.* 2005). An island population of tungara frog, *Physalaemus pustulosus,* established 12 years earlier, showed evidence of a severe bottleneck (Lampert *et al.* 2007) and recently introduced (6-12 generations) populations of bullfrogs, *Rana catesbeiana*, in Europe showed a stong genetic bottleneck (Ficetola *et al.* 2008). However, not all colonization events by amphibians may lead to a bottleneck or a reduction in genetic diversity. The crested newt, *Triturus cristatus,* showed no significant genetic bottleneck in ponds it had colonized only decades earlier (Jehle *et al.* 2001) and a few individuals of the frog *Rana ridibunda* effectively colonized Great Britain in 1935 without a strong bottleneck effect, a result attributed to their rapid expansion (Zeisset & Beebee 2003). Similarly, the cane toad, *Bufo marinus*, showed no evidence of isolation by distance after an estimated 25–35

generations in Australia (Leblois *et al.* 2000). Apparently, each species must be evaluated individually.

The 1980 eruption of Mount St. Helens provides a natural experiment to study colonization at the ecological time scale and a unique opportunity to assess how species recover from the effects of a major environmental disturbance (Dale *et al.* 2005). There are only two published studies on genetic impacts of the eruption, both were on plants (Bishop 1996; Yang *et al.* 2008). The genetics of colonization after a recent volcanic eruption show a variety of results. At Mount St. Helens, Bishop (1996) found evidence of founder effects in new populations of the herb, *Lupinus lepidus*, while Yang *et al.* (2008) did not find evidence for founder effects in the animal dispersed *Vaccinium membranaceum*. The fossorial rodent *Ctenomys maulinus brunnenus* experienced a drastic reduction in genetic diversity and showed evidence of a bottleneck effect three years after the 1988 eruption of the Lonquimay volcano in Chile (Gallardo *et al.* 1995) and the recolonization of *Hypochaeris tenuifolia*, a flowering herb on the same volcano, showed no loss of genetic diversity within the five founder populations (less than 10 yrs old) compared to survivor or isolated populations from other parts of the range (Tremsetsberger *et al.* 2003). There was no evidence of founder effects in a tree species that colonized young lava flows on La Réunion island (Litrico *et al.* 2005). Most studies on the impact of volcanoes on population genetics were from eruptive events greater than 50 yrs ago and emphasized evolutionary or phylogeographical events (e.g. Gillespie 2004; Vandergast *et al.* 2004; Emerson *et al.* 2006) and thus had different goals than this one.

The impact of the 18 May 1980 eruption of Mount St. Helens on the surrounding landscape was swift, violent, and dramatic. All areas north and north east of the volcano were severely affected by the debris-avalanche, blast and ashfall (Peterson 1986). The eruption created a mosaic of conditions across the landscape. The debris-avalanche zone, created by the collapse of the northern flank of the volcano, was the largest landslide in recorded history, affecting 60 km^2 and covering the landscape in some areas with up to 195 m of rock and tephra (Swanson & Major 2005). All plant and animal life was extirpated, buried under the rubble. However, when the ash settled, new habitat was available for colonization. The blowdown zone (approx. 570 km^2 of forest affected) was created by the lateral blast where trees toppled, ponds and lakes heated to near-lethal temperatures. Ashfall and organic debris dramatically altered the landscape and lakes (Dahm *et al.* 2005; Swanson & Major 2005). However, snow-covered ground and ice-covered lakes provided some protection to animals in their winter retreats. Although there were survivors, subsequent mortality was high (Crisafulli *et al.* 2005b). For example, some fish survived in 67% of the lakes within the blowdown zone (Crawford 1986) and northern pocket gophers, *Thomomys talpoides*, were found less than a year after the eruption in the blowdown zone (Andersen 1982). Areas on the volcano's south side were relatively unaffected except for receiving about 5 to 25 cm of tephra fall (Dahm *et al.* 2005). Other post-eruption studies have used lakes on the south side as a reference (Wissmar *et al.* 1982; Dahm *et al.* 2005). In this study, reference populations are assumed to represent the levels of genetic diversity that would have been present across the entire area if the volcano had not erupted. Thus, the volcano created conditions by which both colonization and recovery can be studied in concert.

Taricha granulosa, the rough-skinned newt is a common pond-breeding amphibian in the Pacific Northwest with a typical amphibian biphasic life-cycle: pond-breeding, aquatic eggs and larvae, and terrestrial adults (Nussbaum *et al.* 1983). Adults migrate to a breeding pond (males arrive first), court, females lay individual eggs (122–226) over the course of several weeks, then leave (Jones *et al.* 2002). Upon transformation (4–5 months, depending upon temperature), larvae depart the breeding pond for several years, returning when sexually mature (Petranka 1998). The life span of *T. granulosa* is estimated to be about 12 years and age to first reproduction is approximately 4–5 years (Chandler 1918; Efford & Mathias 1969). They are capable of homing, returning to their capture site when displaced (Efford & Mathias 1969). *Taricha granulosa* may be philopatric with respect to breeding site, similar to *T. rivularis* (Pimentel 1960; Packer 1963; Twitty 1966; Oliver & McCurdy 1974). However, genetic studies suggest high gene flow (Jones *et al.* 2001; Kuchta & Tan 2005; Ridenhour *et al.* 2007). At Mount St. Helens, it took 10 years for *T. granulosa* to reach new ponds created by the debris-avalanche zone and the closest known source population was 5.7 km away, in the blast zone (Crisafulli *et al.* 2005b). Based on generation time, I estimate there have been at most 3–5 generations of *T. granulosa* in the new ponds since colonization in 1990.

This study examines the genetic response of *T. granulosa* to the eruption of Mount St. Helens by comparing genetic diversity in three treatments: new ponds (debris-avalanche), recovery areas (blowdown zone), and reference (unaffected areas). I predicted that *T. granulosa* in the new ponds (founding populations) would have less genetic variability than those from recovery populations or reference populations.

Because the terrain is rugged and likely to limit movement through the landscape, I expected to find genetic substructuring by collection sites and treatment. If *T. granulosa* are philopatric, new populations would show a strong founder effect, as evidenced by a bottleneck, and gene flow will be low, as indicated by high F_{ST} values and extensive population substructuring. If *T. granulosa* moves freely across the landscape, gene flow would be high, and genetic diversity in founding populations may or may not be different than reference populations. However, since only 3 to 5 generations are estimated to have passed since the eruption, I expected to see lower genetic diversity in the new populations. This study is unique in that it is the first to examine the genetic response of any animal to the eruption at Mount St. Helens and one of a handful measuring the genetic consequences at the ecological scale of organisms in response to a contemporary volcanic eruption. Little to no loss of genetic diversity would be a positive indicator that at least one species of amphibian can quickly recover genetic diversity after major habitat loss.

Materials and Methods

Study site

This study was conducted in the Mount St. Helens National Volcanic Monument, Washington State, USA (Figure 1). Collection sites were assigned to one of three treatments based on the effects of the eruption (Tables 2.1 and 2.2). The first treatment, new ponds, consisted of the Maratta and Hummocks sites, approximately 11 and 12 km northwest from the volcano's crater. Neither are discrete lakes but a collection of ponds created when the hummocky deposits from the initial landslide filled with water. Maratta is a complex of approximately 35 ponds and Hummocks is a complex of approximately

15 ponds within the boundary delineated by the Hummocks (Forest Service Trail 229) trail. These ponds are now surrounded by red alder (*Alnus rubra*) and small willows (*Salix* spp.). The area between ponds is mainly loose piles of rock and tephra, covered with small herbs and grasses. The second treatment, recovery lakes, were pre-existing that occur in the blowdown zone. St. Charles (13km NE of the crater), Strawberry (17.8 km NE) and Ghost (16.9km NE) Lakes all have open canopies but some trees and shrubs are growing up around them. Tephra is the predominate substrate for these lakes and surrounding area. Treatment three, references lakes, were Goat Marsh and McBride, 8.4 and 8.2 km southwest of the crater. Goat Marsh Lake is surrounded by forest (primarily *Abies*, *Pseudotsuga* and *Tsuga*) and is in a research natural area (Franklin & Wiberg 1979) and the southern slope of Mount St. Helens is clearly visible from Goat Marsh Lake. McBride Lake is also surrounded by coniferous forest characteristic of the area (Franklin & Dyrness 1973). Using Goat Marsh and McBride lakes as reference is not unusual. Others have used lakes on the southern side of Mount St. Helens as reference sites for their post-eruption studies (Wissmar *et al.* 1982; Dahm *et al.* 2005).

Population sampling

I captured *T. granulosa* in May-June 2003 and in July 2005 using minnow traps (set and left overnight) and dipnet (Table 2.2). Collection effort by site and year is in Appendix A. Collection sites were chosen based on the probability that they would contain *T. granulosa* (Crisafulli, pers comm.) and were accessible within a 3 hr hike. I captured adults except for these number of small larvae (young of 2005); 1 in St. Charles Lake, 21 in Goat Marsh, and 7 in McBride Lake. *Taricha granulosa* was collected from all localities both years except for Strawberry Lake, which was sampled only in 2005.

They were immediately released at their capture site after taking a tissue sample by removing approximately 1 cm of tail tip from each individual.

DNA extraction and microsatellite amplification

Each tissue was stored individually in 95% ethanol until return to the lab when samples were stored at -80º C. Total genomic DNA was isolated by digesting approximately 0.5mm of tissue sample in 550µl of lysis buffer (50mM Tris HCl, pH 8.0, 10mM EDTA, pH 8.0, 200mM NaCl) with 11 µl of Proteinase K (20 µg/ml) at 55º C followed by either a chloroform purification and isopropanol precipitation (Mullenbach *et al.* 1989) or Qiagen DNeasy kits (Qiagen, Inc, Valencia, CA). I used a Nanodrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) to measure the concentration of the isolated DNA and used ddH_2O to standardize each sample to 25ng/µL.

I used six *T. granulosa* microsatellite loci: Tgr 01, Tgr 02, Tgr 04, Tgr 06, Tgr 10, and Tgr 14 (Jones *et al.* 2001). Each PCR was carried out in a 20µL total volume with 50ng of DNA. Reagents were as in Jones *et al.* (2001) with modifications to annealing temperatures, primer concentrations, and $MgCl_2$ concentrations (Table 2.3). Thermal cycling was preceded by a 2 min denaturation at 92º C and followed by a 5 min extension period at 72º C. Each PCR consisted of 35 cycles. A cycle consisted of 1 min at 92º C, 1 min at the optimal annealing temperature and 2 min at 72º C for extension. Fragments were separated and visualized on an ABI PRISM® 3730 DNA Analyzer by the Nevada Genomics Center, Reno, NV for all loci except Tgr 02 which was run on an ABI PRISM® 3100 Genetic Analyzer by the Center for Integrated BioSystems at Utah State

University, Logan, UT. I used GENEMAPPER 3.0 (Applied Biosystems Inc.) to score each sample and rounded allele sizes to the appropriate whole number.

Data analysis

Data were analyzed by collection site and by treatment. I used GENEPOP web version 3.4 (Raymond & Rousset 1995) and FSTAT (Goudet 2001) to determine if the results varied by year. I found no significant differences so I pooled all years. I used ARLEQUIN version 3.1 (Excoffier *et al.* 2005) to estimate observed (H_O) and expected (H_E) heterozygosities, calculate pairwise F_{ST} values and their significant values (10100 permutations), and AMOVA (Analysis of molecular variation; distance method: number of alleles;16000 permutations) to look for evidence of genetic substructure by collection site and treatment group. The AMOVA tested each treatment with two populations (collection sites) per group. I used GENEPOP to test for departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD). FSTAT was used to calculate genetic diversity indices such as number of alleles, allelic richness (corrected for minimum sample size), Nei's gene diversity, F_{IS} and F_{ST} values, and average relatedness of individuals within samples when compared to the whole, corrected for population substructure. I tested for null alleles and scoring errors using MICROCHECKER (Van Oosterhout *et al.* 2004) at three different levels: all samples pooled, samples by treatment, and samples by collection site. I set the confidence interval for Bonferroni with 1000 Monte-Carlo simulations. I tested for isolation by distance with a Mantel test (9999 permutations) conducted in TFPGA (Miller 1997) with Nei's unbiased genetic distances from GENALEX 6.1 (Peakall & Smouse 2006) and geographic distance between

collection sites (accounting for topography) measured with TOPO 6.0 (DeLorme, Yarmouth, ME).

When a population loses a large number of individuals (bottleneck) or is descended from a few number of individuals (founder effects), alleles are lost more rapidly than heterozygosity (Maruyama & Fuerst 1985; Allendorf 1986). To test for a bottleneck, I used the program BOTTLENECK (Cornuet & Luikart 1997) using the stepwise mutational model (SMM) and two-phased model of mutation (TPM). This program develops a distribution of expected heterozygosity values under mutation drift equilibrium and compares that with observed heterozygosity to determine if there is a heterozygote excess or deficiency at each locus. Statistical tests determine whether the allele frequency distribution is approximately L-shaped, as would be expected if the population had not undergone a bottleneck. I used the sign test only when collection sites were grouped by treatment to meet the minimal requirements of the test (Luikart & Cornuet 1998) and the Wilcoxon test for individual collection sites and treatments. Based on the authors' recommendations, I set TPM with probability equal to 95%, variance set at 10% and 10,000 replications. I also used GENALEX 6.1 to visualize population structuring by treatment performing a principal component analysis (PCA) of genotypic distances using a standardized covariance matrix with 9999 permutations. PCA makes no prior assumptions about population structure.

Results

All loci were highly polymorphic (Table 2.4). Tgr 04 had the lowest observed heterozygosity of any loci at all collection sites and showed evidence of null alleles with all animals pooled (102 homozygotes observed, 48.3 expected, $P < 0.001$) or by treatment

($P < 0.001$). Therefore, it was eliminated from further analysis. There was no evidence of significant linkage disequilibrium or departures from HWE either by collection site or by treatment for all remaining loci. By treatment, levels of genetic diversity were high and had small F_{IS} values (Table 2.5).

There were no significant differences in allelic richness, H_E, H_S, F_{IS}, F_{ST}, or relatedness when individual collection sites were grouped by treatment (Table 2.6). Pairwise F_{ST} values by collection site and treatment were low. By collection site, only two F_{ST} values were significantly different from each other, Goat Marsh Lake with Strawberry and Goat Marsh with St. Charles Lake (Table 2.7). There was no evidence of population substructure indicated by AMOVA. By treatment, 98.92% ($P< 0.001$) of the variation was within populations (collection sites). Less than 1% of the variation was among treatments (0.67%, $P = 0.0650$) or among collection sites within treatments (0.41%, $P = 0.0809$). There was no evidence for a relationship between genetic distance and geographic distance (Figure 2.2; Mantel test, $r = 0.3544$, $Z = 15.51$, $P = 0.0539$). Principal components analysis showed extensive overlap between treatments (Figure 3) indicating low levels of genetic structuring. PC axis 1 explains 22.9% of the variation, axis 2 explains 18.0% and axis 3 explains 16.5% for a cumulative total of 57.4%.

BOTTLENECK tests showed no evidence of recent bottlenecks either by treatment or by collection site under SMM or TPM by either the sign or Wilcoxon test. The smallest P value testing by treatment was 0.3125 (Wilcoxon, TPM, blowdown). The smallest P value for the testing by collection site was 0.0941 (Sign test, SMM, Goat Marsh Lake). Allele frequency distribution did not deviate significantly from the L-

shaped distribution expected under mutation-drift equilibrium by treatment or collection site.

Discussion

Taricha granulosa collected around Mount St. Helens did not show population structure. Rather, they appeared to be part of a panmictic population. There was no evidence of isolation by distance (IBD) and no measurable loss of genetic variability in the new or recovery populations. New ponds, resulting from the eruption, appear to have been colonized by a large number of individuals. Recovery ponds either had a large number of survivors, large migration pulse(s) or both. Pre-eruption Mount St. Helens was mostly surrounded by National Forest and managed forest lands, both of which most likely harbored large source populations (Franklin *et al.* 1995). Slatkin's (1993) examination of isolation by distance models found that failure to find evidence of IBD for species with low F_{ST} values may indicate recent colonization, a result supported by these data. The results also suggest that *T. granulosa* are not site-fidelic with regards to breeding and that they move extensively and perhaps randomly (at least to us) across the landscape.

Other genetic studies support this result. *Taricha granulosa* from two Oregon populations separated by 16 km (a distance within the geographic scale of this study) showed significant differences in distributions of allele frequencies with five microsatellite loci but also displayed high levels of gene flow (F_{ST} = 0.005; Jones *et al.* 2001). At larger scales, high gene flow, represented by low levels of population substructure (θ_{ST} = 0.031), characterized populations from 20 sites across three transects in the Pacific Northwest. Isolation by distance was detected at the 200-km scale

(Ridenhour *et al.* 2007). Finally, in an indirect measure of gene flow covering the entire range of *T. granulosa*, the largest Nei's genetic distance (D_N; between a CA and a WA population) was 0.247 but the average among a cluster of WA to AK populations was low, D_N=0.013 (Kuchta & Tan 2005). While not a direct measure of gene flow at an ecological scale, these populations of *T. granulosa* were not completely isolated from one another as some population groupings did not show isolation by distance (Kuchta & Tan 2005). Therefore, high gene flow may be normal for *T. granulosa*.

Alternatively, short dispersal distances of *T. granulosa* observed in ecological studies and high gene flow measured in molecular studies can be reconciled through their mating system and large clutch size if populations had been founded by a few individuals. If you compared a polyandrous population with a monogamous one, heterozygosity could theoretically remain equal but the number of different allele combinations and allelic richness would be higher in the polyandrous population. *Taricha granulosa* are polyandrous and females store sperm from multiple males (2–5, mean 2.1) via a "topping off" mechanism where sperm from the first male has precedence (Jones *et al*. 2002). Mating continues until a female's spermathecae are full (Jones *et al.* 2002). This could result in an effective population size greater than the census population size (Baer & Schmid-Hempel 1999; Trontti *et al.* 2007). Polyandry in caudates is not unique to *T. granulosa* but is also found in *Ambystoma, Plethodon, Desmognathus, Salamandra* and *Lissotriton* (ex *Triturus*; Myers & Zamudio 2004; Adams *et al.* 2005; Gopurenko *et al.* 2006; Liebgold *et al.* 2006; Steinfartz *et al.* 2006; Jehle *et al.* 2007). With a clutch size in *T. granulosa* ranging from 122–226 eggs (Jones *et al.* 2002), even a few individuals could dramatically influence the allelic richness in subsequent generations, a process

similar to some *Ambystoma* (Kinkead *et al.* 2007) but not others (Zamudio & Wieczorek 2007). Multiple paternity has resulted in increased genetic diversity in other organisms including *Anolis* lizards, loggerhead sea turtles, and blue tits (Foerster *et al.* 2003; Calsbeek *et al.* 2007; Zbinden *et al.* 2007). However, in *Ambystoma maculatum*, individual larvae sired by stored sperm had lower genetic diversity than expected by chance (Chandler & Zamudio 2008) and in lemon sharks, *Negaprion brevirostris*, polyandrous litters did not have greater genetic diversity than monoandrous litters (DiBattista *et al.* 2008). To determine whether polyandry in *T. granulosa* increases genetic variability in the next generation of founder populations requires further testing but it could contribute to no loss of alleles in the new ponds of this study.

Remarkably, new populations showed no significant loss of genetic diversity, by collection site or treatment, even after only 3 to 5 generations (at most) passed since the eruption. It is well known that only one migrant is needed to introduce new alleles and prevent drift. In other taxa, known demographic declines have not always shown a detectable genetic bottleneck effect. For example, a population of kangaroo rats, *Dipodomys spectabilis*, although having suffered a 50% reduction in the number of adults, did not show any genetic evidence of a bottleneck when tested 5–6 generations after the major demographic decline (Busch *et al.* 2007). This result was attributed to immigration despite extensive mark-recapture and trapping studies showing low dispersal rates in Kangaroo rats. In ornate box turtles, *Terrapene ornata*, the failure to detect a bottleneck approximately six generations post disturbance was attributed to their long lifespan (Kuo & Janzen 2004). Other examples due to long lifespan or generation time include the copper redhorse, *Moxostoma hubbsi*, which takes at least 10 years to reach

maturity (Lippe *et al.* 2006) and the white-tailed eagle, *Haliaeetus albicilla* (Hailer *et al.* 2006). In the case of *T. granulosa*, high migration rates and a polyandrous mating system are the processes most likely erasing any bottleneck signature. In those taxa where genetic methods may be the only means available for determining demographic trends (Spear *et al.* 2006), any evidence of a bottleneck should be viewed with alarm, especially in sensitive or imperiled species. As in fish species with high gene flow (Waples 1998), the lack of a genetic signal indicating population differentiation should be viewed cautiously so that populations that really are discrete are not inappropriately grouped with others.

There may be few constraints on *T. granulosa* dispersal, especially across open areas, as would be encountered at Mount St. Helens. Across their entire range, *T. granulosa* may prefer to migrate across open areas and prefer (when not breeding) drier microclimates within their normal range. Landscape disturbances, such as clear cuts, do not appear to negatively impact presence in the Oregon Coast Range (Cole *et al.* 1997). Additionally, *T. granulosa* had the highest mean relative abundance (compared to 8 other salamanders) in clearcuts. Abundance in clearcuts was higher than in old growth stands and they were found in 100% of the dry stands (Corn & Bury 1991). However, presence of *T. granulosa* may just represent movement of animals through the forest to breeding sites (Bury *et al.* 1991). In a private industrial forest approximately 9 km north of Mount St. Helens, *T. granulosa* were most closely associated with open wetlands and early successional habitats (clear cut and sapling conifer) (Bosakowski 1999). *Taricha granulosa* were present (although not in large numbers) in both clearcut and forested sites in a managed forest in Southwest Washington State (Grialou *et al.* 2000) and although

few animals were found (N= 34 over 2 yrs), 80% of these were captured in a clearcut or in pre-canopy forest in another managed, second-growth forest in western Washington State (Aubry 2000). Bury and Corn (1988) found that *T. granulosa* prefer drier old growth forest and are also found in young forest and clearcuts. In the Mattolle River watershed (coastal Northern California), *T. granulosa* were most closely associated with grasslands and second growth forest rather than late serial forest (Welsh *et al*. 2005). Additionally, *T. granulosa* appear to choose clear migration routes and were positively associated with bare ground in managed forest land during a spring trapping season, potentially due to migration to breeding ponds and selection for clear pathways (Butts & McComb 2000). When not using streams as migratory corridors, *T. granulosa* used portions of a dirt road at two localities (Pimentel 1960). Thus, the terrain surrounding the new ponds may have been attractive to *T. granulosa* as an obstacle-free environment, promoting instead of deterring migration.

For juveniles, how far they migrate into the landscape to mature sexually or how they subsequently "choose" a breeding pond is unknown. Because smaller amphibians are less resistant to desiccation than larger ones (Spight 1968), it cannot be assumed that the habitat preferences of adults are the same as for juveniles (Rothermel & Semlitsch 2002). Burrows created by fossorial northern pocket gophers, *Thomomys talpoides*, which were found throughout the affected areas at Mount St. Helens (Andersen 1982; Andersen & MacMahon 1985), could certainly act as refuges for dispersing *T. granulosa*. Additionally, *T. granulosa* are hardy creatures and capable of surviving challenging environmental conditions. Pimentel (1960) buried 10 *T. granulosa* for six months and stored them at 15.6ºC. Although desiccated, seven survived and appeared normal after

rehydration. He also placed 10 *T. granulosa* in an earth-filled outdoor enclosure where they lived without food or water for four months. Nine survived and moved to water when soil moisture increased. Therefore, what may appear to be an inhospitable landscape may in fact be a survivable habitat.

This study also highlights how different types of studies (e.g. demographic, ecological or molecular) may lead to different conclusions (e.g. Busch *et al.* 2007). If only non-genetic studies were considered, one would conclude that *T. granulosa* behave similarly to other typical pond-breeding amphibians: poor dispersal, site-fidelic, low gene flow, and metapopulation structure. For example, *Taricha rivularis*, a closely related species, traveled at least 8.0 km over rough terrain and across areas of suitable breeding habitat to return to within approximately 15 m of their original capture site (Packer 1963; Grant *et al.* 1968). *Taricha granulosa* are also capable of homing, similar to, although not as dramatic as *T. rivularis*. In a British Columbia population, over 96% of the *T. granulosa* captured in inlet or outlet streams then released in the middle of a lake, returned to their capture location. The furthest distance moved was 550 m and one salamander moved at an average of 56 m/hr, returning to its capture location in 9 hrs (Efford & Mathias 1969). A later study at the same locality found similar results (Neish 1971).

Two additional studies also support the conclusion that *T. granulosa* remain in or near their breeding ponds. In Oregon, most were believed to have moved to underground retreats within 6.2–23 m of their breeding pond with an estimate of 90% moving to underground retreats within 61.5 m of their breeding pond (Pimentel 1960). On southern Vancouver Island, females overwintered on land, migrating into the pond to breed in the

spring and departing in the fall. Newly metamorphosed juveniles also left their natal pond in the fall while males appeared to be permanently aquatic (Oliver & McCurdy 1974). However, males in an Oregon population were not permanently aquatic and left breeding ponds at the same rates as females (Pimentel 1960). Pimentel (1960) also described a new movement activity, wandering, consisting of short departures (1 to 45 days) from a breeding pond, generally limited to short distances. While highly valuable, life-history and ecological studies may not match genetic data due to the differences in underlying assumptions (Schmeller & Merila 2007). Additionally, different molecular markers (mitochondrial versus nuclear) may produce different conclusions (Monsen & Blouin 2003; Canestrelli *et al.* 2007). Dependence on one type of data could lead to poor conservation recommendations. If an endangered species is involved, we cannot afford to choose poorly.

The rapid recovery and colonization of new ponds by *T. granulosa* is good news. Given large source populations and time, this species is resistant to large environmental disturbances. Loss of genetic variability in new populations cannot automatically be assumed. The results from demographic, ecological and behavior studies, even within the same species do not always lead to the same conclusion (e.g. Perret *et al.* 2003; Kinkead *et al.* 2007) and amphibian spatial dynamics are more complex than the metapopulation model (Marsh & Trenham 2001; Smith & Green 2005). In plants, life-history traits were poor predictors of population genetic structure (Duminil *et al.* 2007). There are over 60 species of anuran and caudates that could be classified as a pond-breeding amphibian, by my definition, in the United States (Lannoo 2005). *Taricha granulosa* is a classic pond-breeding amphibian. However, they are not site-fidelic, have high gene flow, are good

dispersers and ready colonizers. Thus, I caution against using labels such as "pond-breeding amphibian" as a general predictor variable when making conservation recommendations, especially in poorly-known species. I recommend increased use of the complex life cycle concept (Istock 1967; Wilbur 1980) in its place. I agree with Cushman (2006) that more species specific knowledge in a landscape setting is needed to improve and refine amphibian conservation strategies.

Literature Cited

Adams EM, Jones AG, Arnold SJ (2005) Multiple paternity in a natural population of a salamander with long-term sperm storage. *Molecular Ecology* **14**, 1803-1810.

Allendorf FW (1986) Genetic drift and the loss of alleles versus heterozygosity. *Zoo Biology* **5**, 181-190.

Andersen DC (1982) Observations on *Thomomys talpoides* in the region affected by the eruption of Mount St. Helens. *Journal of Mammalogy* **63**, 652-655.

Andersen DC, MacMahon JA (1985) The effects of catastrophic ecosystem disturbance: the residual mammals at Mount St. Helens. *Journal of Mammalogy* **66**, 581-589.

Aubry KB (2000) Amphibians in managed, second-growth Douglas-fir forests. *The Journal of Wildlife Management* **64**, 1041-1052.

Baer B, Schmid-Hempel P (1999) Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. *Nature* **397**, 151-154.

Beebee T, Rowe G (2001) Application of genetic bottleneck testing to the investigation of amphibian declines: a case study with Natterjack toads. *Conservation Biology* **15**, 266-270.

Bishop JG (1996) *Demographic and genetic variation during colonization by the herb Lupinus lepidus on Mount St. Helens.* PhD. Dissertation, University of Washington.

Blaustein AR, Wake DB, Sousa WP (1994) Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. *Conservation Biology* **8**, 60-71.

Bosakowski T (1999) Amphibian microhabitat associations on a private industrial forest in western Washington. *Northwestern Naturalist* **20**, 61-69.

Bury RB, Corn PS (1988) Douglas-fir forests in the Oregon and Washington Cascades: abundance of terrestrial herpetofauna related to stand age and moisture. In: *Management of Amphibians, Reptiles, and Small Mammals in North America* (eds Szaro RC, Severson KE, Patton DR), pp. 11-22. USDA Forest Service General Technical Report, RM-166, Fort Collins.

Bury RB, Corn PS, Aubry KB (1991) Regional patterns of terrestrial amphibian communities in Oregon and Washington In: *Wildlife and Vegetation of Unmanaged Douglas-fir Forests* (eds Ruggiero LF, Aubry KB, Carey AB, Huff MH), pp. 341-350. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, PNW-GTR-285, Portland.

Busch JD, Waser PM, DeWoody JA (2007) Recent demographic bottlenecks are not accompanied by a genetic signature in banner-tailed kangaroo rats (*Dipodomys spectabilis*). *Molecular Ecology* **16**, 2450-2462.

Butts SR, McComb WC (2000) Associations of forest-floor vertebrates with coarse woody debris in managed forests of western Oregon. *The Journal of Wildlife Management* **64**, 95-104.

Calsbeek R, Bonneaud C, Prabhu S, Manoukis NC, Smith TB (2007) Multiple paternity and sperm storage lead to increased genetic diversity in *Anolis* lizards. *Evolutionary Ecology Research* **9**, 495-503.

Canestrelli D, Verardi A, Nascetti G (2007) Genetic differentiation and history of populations of the Italian treefrog *Hyla intermedia*: lack of concordance between mitochondrial and nuclear markers. *Genetica* **130**, 241-255.

Chandler AC (1918) The Western newt or Water-dog (*Notophthalmus torosus*). *Oregon Agricultural College Experiment Station Bulletin* **152**, 1-24.

Chandler CH, Zamudio KR (2008) Reproductive success by large, closely related males facilitated by sperm storage in an aggregate breeding amphibian. *Molecular Ecology* **17**, 1564-1576.

Cole EC, McComb WC, Newton M, Chambers CL, Leeming JP (1997) Response of amphibians to clearcutting, burning, and glyphosate application in the Oregon Coast Range. *Journal of Wildlife Management* **61**, 656-664.

Corn PS, Bury RB (1991) Terrestrial amphibian communities in the Oregon Coast Range. In: *Wildlife and Vegetation of Unmanaged Douglas-fir Forests* (eds Ruggiero LF, Aubry KB, Carey AB, Huff MH), pp. 305-317. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, PNW-GTR-285, Portland.

Cornuet J-M, Luikart G (1997) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**, 2001-2014.

Crawford B (1986) The recovery of surviving fish populations within the Mount St. Helens National Volcanic Monument and adjacent areas. In: *Mount St. Helens: Five Years Later* (ed Keller SAC), pp. 293-296. Eastern Washington University Press, Cheney.

Crisafulli CM, Swanson FJ, Dale VH (2005a) Overview of the ecological responses to the eruption of Mount St. Helens: 1980-2005. In: *Ecological Responses to the 1980 Eruption of Mount St. Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 287-299. Springer, New York.

Crisafulli CM, Trippe LS, Hawkins CP, MacMahon JA (2005b) Amphibian responses to the 1980 eruption of Mount St. Helens. In: *Ecological Responses to the 1980 Eruption of Mount St. Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 183-197. Springer, New York.

Cushman SA (2006) Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation* **128**, 231-240.

Dahm CN, Larson DW, Petersen RR, Wissmar RC (2005) Response and recovery of lakes. In: *Ecological Responses to the 1980 Eruption of Mount St. Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 255-274. Springer, New York.

Dale V, Swanson FJ, Crisafulli CM (2005) *Ecological Responses to the 1980 Eruption of Mount St. Helens.* Springer, New York.

DiBattista JD, Feldheim KA, Gruber SH, Hendry AP (2008) Are indirect genetic benefits associated with polyandry? Testing predictions in a natural population of lemon sharks. *Molecular Ecology* **17**, 783-795.

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* **17**, 431-449.

Duellman WE, Trueb L (1986) *Biology of Amphibians.* McGraw-Hill, New York.

Duminil J, Fineschi S, Hampe A, Jordano P, Salvini D, Vendramin GG, *et al.* (2007) Can population genetic structure be predicted from life-history traits? *American Naturalist* **169**, 662-672.

Efford IE, Mathias JA (1969) A comparison of two salamander populations in Marion Lake, British Columbia. *Copeia* **1969**, 723-736.

Emerson BC, Forgie S, Goodacre S, Oromi P (2006) Testing phylogeographic predictions on an active volcanic island: *Brachyderes rugatus* (Coleoptera: Curculionidae) on La Palma (Canary Islands). *Molecular Ecology* **15**, 449-458.

Excoffier L, Laval G, Schneider S (2005) Arlequin version 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**, 47-50.

Ficetola GF, Bonin A, Miaud C (2008) Population genetics reveals origin and number of founders in a biological invasion. *Molecular Ecology* **17**, 773-782.

Foerster K, Delhey K, Johnsen A, Lifjeld JT, Kempenaers B (2003) Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature* **425**, 714-717.

Frankham R (2005) Genetics and extinction. *Biological Conservation* **126**, 131-140.

Franklin JF, Dyrness CT (1973) Natural vegetation of Oregon and Washington. PNW-8, US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland.

Franklin JF, Frenzen PM, Swanson FJ (1995) Re-creation of ecosystems at Mount St. Helens: contrasts in artificial and natural approaches. In: *Rehabilitating Damaged Ecosystems, 2nd Ed* (ed Cairns Jr. J), pp. 287-333. CRC Press, Boca Raton.

Franklin JF, Wiberg C (1979) Goat Marsh Research Natural Area Supplement No. 10. In: *Federal Research Natural Areas in Oregon and Washington: A Guidebook for Scientists and Educators* (eds Franklin JF, Hall FC, Dyrness CT, Maser C), pp. GH1-GH19. US Government Printing Office, Washington DC.

Funk WC, Tallmon DA, Allendorf FW (1999) Small effective population sizes in the long-toed salamander. *Molecular Ecology* **8**, 1633-1640.

Gallardo MH, Kohler N, Araneda C (1995) Bottleneck effects in local populations of fossorial *Ctenomys* (Rodentia, Ctenomyidae) affected by vulcanism. *Heredity* **74**, 638-646.

Gamble LR, McGarigal K, Compton BW (2007) Fidelity and dispersal in the pond-breeding amphibian, *Ambystoma opacum*: implications for spatio-temporal population dynamics and conservation. *Biological Conservation* **139**, 247-257.

Gillespie R (2004) Community assembly through adaptive radiation in Hawaiian spiders. *Science* **303**, 356-359.

Gopurenko D, Williams RN, McCormick CR, DeWoody JA (2006) Insights into the mating habits of the tiger salamander (*Ambystoma tigrinum tigrinum*) as revealed by genetic parentage analyses. *Molecular Ecology* **15**, 1917-1928.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995).

Grant D, Anderson O, Twitty V (1968) Homing orientation by olfaction in newts (*Taricha rivularis*). *Science* **160**, 1354-1356.

Grant PR, Grant BR, Petren K (2001) A population founded by a single pair of individuals: establishment, expansion, and evolution. *Genetica* **112-113**.

Grialou JA, West SD, Wilkins RN (2000) The effects of forest clearcut harvesting and thinning on terrestrial salamanders. *Journal of Wildlife Management* **64**, 105-113.

Hailer F, Helander B, Folkestad AO, Ganusevich SA, Garstad S, Hauff P, *et al.* (2006) Bottlenecked but long-lived: high genetic diversity retained in white-tailed eagles upon recovery from population decline. *Biology Letters* **2**, 316-319.

Hitchings SP, Beebee TJC (1997) Genetic substructuring as a result of barriers to gene flow in urban *Rana temporaria* (common frog) populations: implications for biodiversity conservation. *Heredity* **79**, 117-127.

Istock CA (1967) The evolution of complex life cycle phenomenon: an ecological perspective. *Evolution* **21**, 592-605.

Jehle R, Arntzen JW, Burke T, Krupa AP, Hödl W (2001) The annual number of breeding adults and the effective population size of syntopic newts (*Triturus cristatus, T. marmoratus*). *Molecular Ecology* **10**, 839-850.

Jehle R, Burke T, Arntzen JW (2005) Delineating fine-scale genetic unit in amphibians: probing the primacy of ponds. *Conservation Genetics* **6**, 227-234.

Jehle R, Sztatecsny M, Wolf JBW, Whitlock A, Hodl W, Burke T (2007) Genetic dissimilarity predicts paternity in the smooth newt (*Lissotriton vulgaris*). *Biology Letters* **3**, 526-528.

Johansson M, Primmer CR, Merila J (2007) Does habitat fragmentation reduce fitness and adaptability? A case study of the common frog (*Rana temporaria*). *Molecular Ecology* **16**, 2693-2700.

Jones AG, Adams EM, Arnold SJ (2002) Topping off: a mechanism of first-male sperm precedence in a vertebrate. *Proceedings of the National Academy of Sciences* **99**, 2078-2081.

Jones AG, Blouin MS, Arnold SJ (2001) Genetic variation in two populations of the rough-skinned newt (*Taricha granulosa*) assessed using novel tetranucleotide microsatellite loci. *Molecular Ecology Notes* **1**, 293-296.

Kimura M (1983) *The Neutral Theory of Molecular Evolution.* Cambridge University Press, Cambridge.

Kinkead KE, Abbott AG, Otis DL (2007) Genetic variation among *Ambystoma* breeding populations on the Savannah River site. *Conservation Genetics* **8**, 281-292.

Kraaijeveld-Smit FJL, Beebee TJC, Griffiths RA, Moore RD, Schley L (2005) Low gene flow but high genetic diversity in the threatened Mallorcan midwife toad *Alytes muletensis*. *Molecular Ecology* **14**, 3307-3315.

Kuchta SR, Tan A-M (2005) Isolation by distance and post-glacial range expansion in the rough-skinned newt, *Taricha granulosa*. *Molecular Ecology* **14**, 225-244.

Kuo CH, Janzen FJ (2004) Genetic effects of a persistent bottleneck on a natural population of ornate box turtles (*Terrapene ornata*). *Conservation Genetics* **5**, 425-437.

Lampert KP, Bernal XE, Rand SA, Mueller UG, Ryan MJ (2007) Island populations of *Physalaemus pustulosus*: history influences genetic diversity and morphology *Herpetologica* **63**, 311-319.

Lannoo MJ (2005) *Amphibian Declines: The Conservation Status of United States Species.* University of California Press, Berkeley.

Leberg PL (2002) Estimating allelic richness: Effects of sample size and bottlenecks. *Molecular Ecology* **11**, 2445-2449.

Leblois R, Rousset F, Tikel D, Moritz C, Estoup A (2000) Absence of evidence for isolation by distance in an expanding cane toad (*Bufo marinus*) population: an individual-based analysis of microsatellite genotypes. *Molecular Ecology* **9**, 1905-1909.

Lesbarréres D, Primmer CR, Laurila A, Merila J (2005) Environmental and population dependency of genetic variability-fitness correlations in *Rana temporaria*. *Molecular Ecology* **14**, 311-323.

Liebgold EB, Cabe PR, Jaeger RG, Leberg PL (2006) Multiple paternity in a salamander with socially monogamous behaviour. *Molecular Ecology* **15**, 4153-4160.

Lippe C, Dumont P, Bernatchez L (2006) High genetic diversity and no inbreeding in the endangered copper redhorse, *Moxostoma hubbsi* (Catostomidae, Pisces): the positive sides of a long generation time. *Molecular Ecology* **15**, 1769-1780.

Litrico I, Ronfort J, Verlaque R, Thompson JD (2005) Spatial structure of genetic variation and primary succession in the pioneer tree species *Antirhea borbonica* on La Réunion. *Molecular Ecology* **14**, 1575-1584.

Luikart G, Cornuet J-M (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequencies. *Conservation Biology* **12**, 228-237.

Mace GM, Smith TB, Bruford MW, Wayne RK (1996) An overview of the issues. In: *Molecular Genetic Approaches in Conservation* (eds Smith TB, Wayne RK), pp. 3-21. Oxford University Press, New York.

Marsh DM, Trenham PC (2001) Metapopulation dynamics and amphibian conservation. *Conservation Biology* **15**, 40-49.

Maruyama T, Fuerst PA (1985) Population bottlenecks and nonequilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. *Genetics* **111**, 675-689.

Miller MP (1997) Tools for population genetic analysis (TFPGA) 1.3: a Windows program for the analysis of allozyme and molecular population genetic data, Computer software distributed by author (http://bioweb.usu.edu/mpmbio).

Monsen KJ, Blouin MS (2003) Genetic structure in a montane ranid frog: restricted gene flow and nuclear-mitochondrial discordance. *Molecular Ecology* **12**, 3275-3286.

Mullenbach R, Lagoda P, Welter C (1989) An efficient salt-chloroform extraction of DNA from blood and tissues. *Trends in Genetics* **5**, 391.

Myers EM, Zamudio KR (2004) Multiple paternity in an aggregate breeding amphibian: the effect of reproductive skew on estimates of male reproductive success. *Molecular Ecology* **13**, 1951-1963.

Nei M (1987) *Molecular Evolutionary Genetics.* Columbia University Press, New York.

Nei M, Maruyama T, Chakroborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution* **29**, 1-10.

Neish IC (1971) Comparison of size, structure and distributional patterns of two salamander populations in Marion Lake, British Columbia. *Journal of Fishery Research Board of Canada* **28**, 49-58.

Nussbaum RA, Brodie Jr ED, Storm RM (1983) *Amphibians and Reptiles of the Pacific Northwest.* University Press of Idaho, Moscow.

Oliver MG, McCurdy HM (1974) Migration, overwintering, and reproductive patterns of *Taricha granulosa* on southern Vancouver Island. *Canadian Journal of Zoology* **52**, 541-545.

Packer WC (1963) Observations on the breeding migration of *Taricha rivularis*. *Copeia* **1963**, 378-382.

Palo JU, Schmeller DS, Laurila A, Primmer CR, Kuzmin SL, Merila J (2004) High degree of population subdivision in a widespread amphibian. *Molecular Ecology* **13**, 2631-2644.

Peakall ROD, Smouse PE (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288-295.

Perret N, Pradel R, Miaud C, Grolet O, Joly P (2003) Transience, dispersal and survival rates in newt patchy populations. *Journal of Animal Ecology* **72**, 567-575.

Peterson DW (1986) Mount St. Helens and the science of volcanology: a five-year perspective. In: *Mount St. Helens: Five Years Later* (ed Keller SAC), pp. 3-19. Eastern Washington University Press, Cheney.

Petranka JW (1998) *Salamanders of the United States and Canada.* Smithsonian Institution Press, Washington, DC.

Petranka JW, Smith CK, Scott AF (2004) Identifying the minimal demographic unit for monitoring pond-breeding amphibians *Ecological Applications* **14**, 1065-1078.

Pimentel RA (1960) Inter- and intrahabitat movements of the rough-skinned newt, *Taricha torosa granulosa* (Skilton). *American Midland Naturalist* **63**, 470-496.

Raymond M, Rousset F (1995) Population genetics software for exact test and exumenicism. *Journal of Heredity* **86**, 248-249.

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Conservation Biology* **17**, 230-237.

Reed DH, Nicholas AC, Stratton GE (2007) Genetic quality of individuals impacts population dynamics. *Animal Conservation* **10**, 275-283.

Ridenhour BJ, Brodie Jr ED, Brodie III ED (2007) Patterns of genetic differentiation in *Thamnophis* and *Taricha* from the Pacific Northwest. *Journal of Biogeography* **34**, 724-735.

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

Rowe G, Beebee TJC (2003) Population on the verge of a mutational meltdown? Fitness costs of genetic load for an amphibian in the wild. *Evolution* **57**, 177-181.

Rowe G, Beebee TJC (2005) Intraspecific competition disadvantages inbred natterjack toad (*Bufo calamita*) genotypes over outbred ones in a shared pond environment. *Journal of Animal Ecology* **74**, 71-76.

Salthe SN (1969) Reproductive modes and the numbers and sizes of ova in the urodeles. *American Midland Naturalist* **81**, 467-490.

Schmeller DS, Merila J (2007) Demographic and genetic estimates of effective population and breeding size in the amphibian *Rana temporaria*. *Conservation Biology* **21**, 142-151.

Semlitsch RD (1998) Biological delineation of terrestrial buffer zones for pond-breeding salamanders. *Conservation Biology* **12**, 1113-1119.

Semlitsch RD, Pechmann JHK (1985) Diel pattern of migratory activity for several species of pond-breeding salamanders. *Copeia* **1985**, 86-91.

Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoretical Population Biology* **12**, 253-262.

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47**, 264-279.

Smith MA, Green DM (2005) Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography* **28**, 110-128.

Spear SF, Peterson CR, Matocq MD, Storfer A (2006) Molecular evidence for historical and recent population size reductions of tiger salamanders (*Ambystoma tigrinum*) in Yellowstone National Park. *Conservation Genetics* **7**, 605-611.

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

Steinfartz S, Stemshorn K, Kuesters D, Tautz D (2006) Patterns of multiple paternity within and between annual reproduction cycles of the fire salamander (*Salamandra salamandra*) under natural conditions. *Journal of Zoology* **268**, 1-8.

Swanson FJ, Major JJ (2005) Physical events, environments, and geological-ecological interactions at Mount St. Helens: March 1980-2004. In: *Ecological Responses to the 1980 Eruption of Mount St. Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 27-44. Springer, New York.

Tremsetsberger K, Stuessy TF, M SR, Baeza CM, Fay MF (2003) Genetics of colonization in *Hypochaeris tenuifolia* (Asteraceae, Lactuceae) on Volcán Lonaquimay, Chile. *Molecular Ecology* **12**, 2649-2659.

Trontti K, Thurin N, Sundstrom L, Aron S (2007) Mating for convenience or genetic diversity? Mating patterns in the polygynous ant *Plagiolepis pygmaea*. *Behavoral Ecology* **18**, 298-303.

Twitty VC (1966) *Of Scientists and Salamanders.* W.H. Freeman and Company, San Francisco.

Valentine BD, Dennis DM (1964) A comparision of the gill-arch system and fins of three general of larval salamanders, *Rhyacotriton*, *Gyrinophilus*, and *Ambystoma*. *Copeia* **1964**, 196-201.

Van Oosterhout C, Hutchinson WF, Wills D, P. M. (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535-538.

Vandergast AG, Gillespie RG, Roderick GK (2004) Influence of volcanic activity on the population genetic structure of Hawaiian *Tetragnatha* spiders: fragmentation, rapid population growth and the potential for accelerated evolution. *Molecular Ecology* **13**, 1729-1743.

Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity* **89**, 438-450.

Welsh Jr HH, Hodgson GR, Lind AJ (2005) Ecogeography of the herpetofauna of a northern California watershed: linking species patterns to landscape processes. *Ecography* **28**, 521-536.

Wilbur HM (1980) Complex life cycles. *Annual Review of Ecology and Systematics* **11**, 67-93.

Wissmar RC, Devol AH, Staley JT, Sedell JR (1982) Biological responses of lakes in the Mount St. Helens blast zone. *Science* **216**, 178-181.

Wright S (1931) Evolution in Mendelian populations. *Genetics* **16**, 97-159.

Yang S, Bishop JG, Webster MS (2008) Colonization genetics of an animal-dispersed plant (*Vaccinium membranaceum*) at Mount St Helens, Washington. *Molecular Ecology* **17**, 731-740.

Zamudio KR, Wieczorek AM (2007) Fine-scale spatial genetic structure and dispersal among spotted salamander (*Ambystoma maculatum*) breeding populations. *Molecular Ecology* **16**, 257-274.

Zbinden JA, Largiader CR, Leippert F, Margaritoulis D, Arlettaz R (2007) High frequency of multiple paternity in the largest rookery of Mediterranean loggerhead sea turtles. *Molecular Ecology* **16**, 3703-3711.

Zeisset I, Beebee TJC (2003) Population genetics of a successful invader: the marsh frog *Rana ridibunda* in Britain. *Molecular Ecology* **12**, 639-646.

Table 2.1. Effects of 1980 eruption of Mount St. Helens on the local landscape (Crisafulli *et al.* 2005a; Swanson & Major 2005). The eruption also generated mudflows but these were localized to drainages and are not under consideration.

Zone	Caused by	Characterized by	Created	Survivors	Treatment
Debris-Avalanche	Collapse and landslide of volcano's north slope.	Hummocks/hills of rock. Covering of landscape by rock to 195m.	A clean slate with new lakes and ponds nonexistent prior to the eruption.	None	New
Blow down	High pressure blast, Air temps to 300ºC**.** High velocity rock and tephra moving across landscape.	Blowdown of trees, heating of lakes/ponds, heavy levels of ash and organic material into lakes/ponds.	Complex mosaic of disturbance dependent upon topography and distance from volcano.	Some (fossorial animals, fish, *T. granulosa*, *A. gracile*, underground plant parts)	Recovery
Reference	South of volcano. Not in direct path of eruptive forces.	Life as normal. Some ash/tephra fall.	No major effects.	Probably 100%	Reference

Table 2.2. Total collections of *Taricha granulosa* by zone and treatment (TRMT) in and around the Mount St. Helens National Volcanic Monument. DA is Debris-Avalanche, BLOW is Blowdown, REF is Reference, REC is Recovery, and N is the total number of animals collected. Latitude, longitude, and elevation were measured with a handheld GPS. Latitude and longitude are in degrees and minutes.

	Zone	TRMT	N	Lat (N)	Long (W)	Elevation (m)
Maratta complex	DA	NEW				
M5			1	46 17.48	122 16.98	730.8
M6			10	46 17.47	122 16.97	749.8
M26			1	Not recorded		
M27			3	46 17.54	122 17.05	725.2
M28			1	Not recorded		
M30			8	46 17.52	122 16.98	744.3
Hummocks complex	DA	NEW				
H6			4	46 16.77	122 16.15	763.7
H23			2	46 16.81	122 16.22	765.2
St Charles Lake	BLOW	REC	11	46 19.34	122 05.23	1223.4
Strawberry Lake	BLOW	REC	46*	46 19.64	122 03.21	1456.0
Ghost Lake**	BLOW	REC	2	46 19.17	122 03.68	1154.2
Goat Marsh Lake	REF	REF	106	46 08.42	122 16.72	889.8
McBride Lake	REF	REF	52	46 08.44	122 15.11	830.7

*Many more were observed visually. **Ghost Lake not included in any analysis due to small sample size.

Table 2.3. PCR Conditions and basic data on six microsatellite loci for *T. granulosa* from MSHNVM. Some reaction conditions differ from Jones *et al*. (2001).

Locus	Anneal temp ºC	$MgCl_2$ mM	Primer mM	Dye	Size range (base pairs)	Total alleles all pops
Tgr01	56	1.50	2.0	6 FAM	215–271 (tetra)	14
Tgr02	56	1.50	1.5	HEX	179–255 (bi & tetra)	16
Tgr04	56	1.50	2.0	NED	207–235 (bi & tetra)	9
Tgr06	59	1.50	1.5	VIC	145–173 (bi & tetra)	12
Tgr10	56	1.75	1.5	NED	174–214 (tetra)	11
Tgr14	57	1.50	2.0	6-FAM	218–328 (tetra)	31

Table 2.4. Genetic measures for 6 *T. granulosa* microsatellite loci (L) by collection site. N is the number of animals genotyped for that locus, and H_O, H_E are observed and expected heterozygosities. F_{IS} is a within population estimation of inbreeding. A is the number of alleles and R is the allelic richness (corrected for minimum sample size (6) using rarefaction (Leberg 2002). An allele frequency table is in Appendix B.

	Maratta						Hummocks					
L	N	H_O	H_E	F_{IS}	A	R	N	H_O	H_E	F_{IS}	A	R
Tgr 01	26	0.8077	0.8778	0.081	12	6.89	6	1.0000	0.8788	-0.154	7	7.00
Tgr 02	26	0.8462	0.8477	0.002	9	5.94	6	0.8333	0.9091	0.091	7	7.00
Tgr 06	25	0.8000	0.7894	-0.014	8	5.31	6	0.6667	0.7576	0.130	5	5.00
Tgr 10	26	0.8077	0.8559	0.057	10	6.26	6	1.0000	0.8939	-0.132	7	7.00
Tgr 14	26	0.9615	0.9479	-0.015	20	9.11	6	1.0000	0.9545	-0.053	9	9.00
ALL		0.8446	0.8638	0.034				0.9000	0.8788	-0.003		

	St. Charles						Strawberry					
L	N	H_O	H_E	F_{IS}	A	R	N	H_O	H_E	F_{IS}	A	R
Tgr 01	11	0.9091	0.8788	-0.036	8	6.66	41	0.8780	0.8681	-0.012	11	6.62
Tgr 02	11	0.5454	0.8311	0.355	6	5.25	37	0.7568	0.8012	0.056	10	5.73
Tgr 06	11	0.8181	0.7879	-0.040	7	5.38	41	0.8293	0.8244	-0.006	10	5.75
Tgr 10	11	0.9091	0.8874	-0.026	8	6.62	41	0.7317	0.8341	0.124	8	5.71
Tgr 14	11	0.8182	0.8788	0.072	11	7.47	41	0.9024	0.9407	0.041	20	8.82
ALL		0.8000	0.8528	0.047				0.8196	0.8537	0.078		

	Goat Marsh						McBride					
L	N	H_O	H_E	F_{IS}	A	R	N	H_O	H_E	F_{IS}	A	R
Tgr 01	75	0.8400	0.8197	-0.025	10	5.54	43	0.8607	0.8159	-0.055	12	5.81
Tgr 02	73	0.8630	0.8428	-0.024	13	6.08	40	0.9000	0.8674	-0.038	14	6.52
Tgr 06	76	0.7895	0.8024	0.016	9	5.30	41	0.8049	0.8329	0.034	9	5.75
Tgr 10	75	0.8800	0.8484	-0.037	11	6.05	43	0.8605	0.8509	-0.011	11	6.27
Tgr 14	74	0.9459	0.9500	0.004	25	9.26	41	0.9512	0.9446	-0.007	22	9.06
ALL		0.8637	0.8527	0.054				0.8754	0.8623	0.063		

Table 2.5. Genetic measures for 5 *T. granulosa* microsatellite loci by treatment. Tgr 04 is not included because it contains a null allele in several populations. A is the number of alleles, R is allelic richness (corrected for minimum sample size (31) using rarefaction (Leberg 2002), N is the number of animals genotyped for that locus, H_O and H_E are observed and expected heterozygosities. F_{IS} is a within population estimation of inbreeding.

	total		NEW						RECOVERY						REFERENCE					
Locus	A	R	N	H_O	H_E	F_{IS}	A	R	N	H_O	H_E	F_{IS}	A	R	N	H_O	H_E	F_{IS}	A	R
Tgr 01	14	10.58	32	0.8438	0.8834	0.046	13	12.90	54	0.8846	0.8799	-0.005	11	10.43	118	0.8475	0.8191	-0.035	12	9.58
Tgr 02	16	10.52	32	0.8438	0.8492	0.007	9	8.97	50	0.7083	0.8092	0.126	10	9.36	113	0.8761	0.8557	-0.024	15	11.12
Tgr 06	12	9.01	31	0.7742	0.7784	0.006	8	8.00	54	0.8269	0.8219	-0.006	10	9.44	117	0.7949	0.8122	0.021	10	8.72
Tgr 10	11	9.50	32	0.8438	0.8517	0.009	10	9.94	54	0.7692	0.8536	0.100	8	7.96	118	0.8729	0.8482	-0.029	11	9.85
Tgr 14	31	21.59	32	0.9688	0.9464	-0.024	21	20.78	54	0.8846	0.9350	0.054	21	18.34	115	0.9478	0.9492	0.001	28	22.01
means				0.8548	0.8618	0.008				0.8147	0.9599	0.053				0.8678	0.8569	-0.013		

Table 2.6. Comparisons between treatment groups over all loci in *T. granulosa* at MSHNVM. R is allelic richness, H_O is observed heterozygosity, H_S is Nei's (1987) gene diversity, F_{IS} is a within population estimate of inbreeding, F_{ST} is a measure of among population genetic diversity and Relc is the average relatedness of individuals within samples when compared to the whole, corrected for population structure.

	New	Recovery	Reference	P value
R	6.86	6.40	6.56	0.1980
H_O	0.855	0.815	0.868	0.2001
H_S	0.867	0.854	0.856	0.2034
F_{IS}	0.014	0.046	-0.014	0.2001
F_{ST}	-0.018	0.020	0.002	0.1870
Relc	-0.028	-0.097	0.027	0.2001

Table 2.7. Pairwise F_{ST} values and P-values by collection site. Number above the diagonal is the F_{ST} value, number below is the exact P-value obtained after 10100 permutations. Alpha, signifying a difference, adjusted for multiple comparisons is 0.00333. Significant values are bolded.

	Maratta	Hummocks	St Charles	Strawberry	Goat Marsh	McBride
Maratta		-0.0167	0.0227	0.0109	0.0002	0.0025
Hummocks	0.9275		0.0086	0.0013	-0.0015	-0.0017
St. Charles	0.0127	0.3292		0.0214	0.0330	0.0216
Strawberry	0.0154	0.4550	0.0091		0.0167	0.0095
Goat Marsh	0.3936	0.4683	**0.0001**	< 0.0010		0.0020
McBride	0.2059	0.4570	0.0046	0.0040	0.1487	

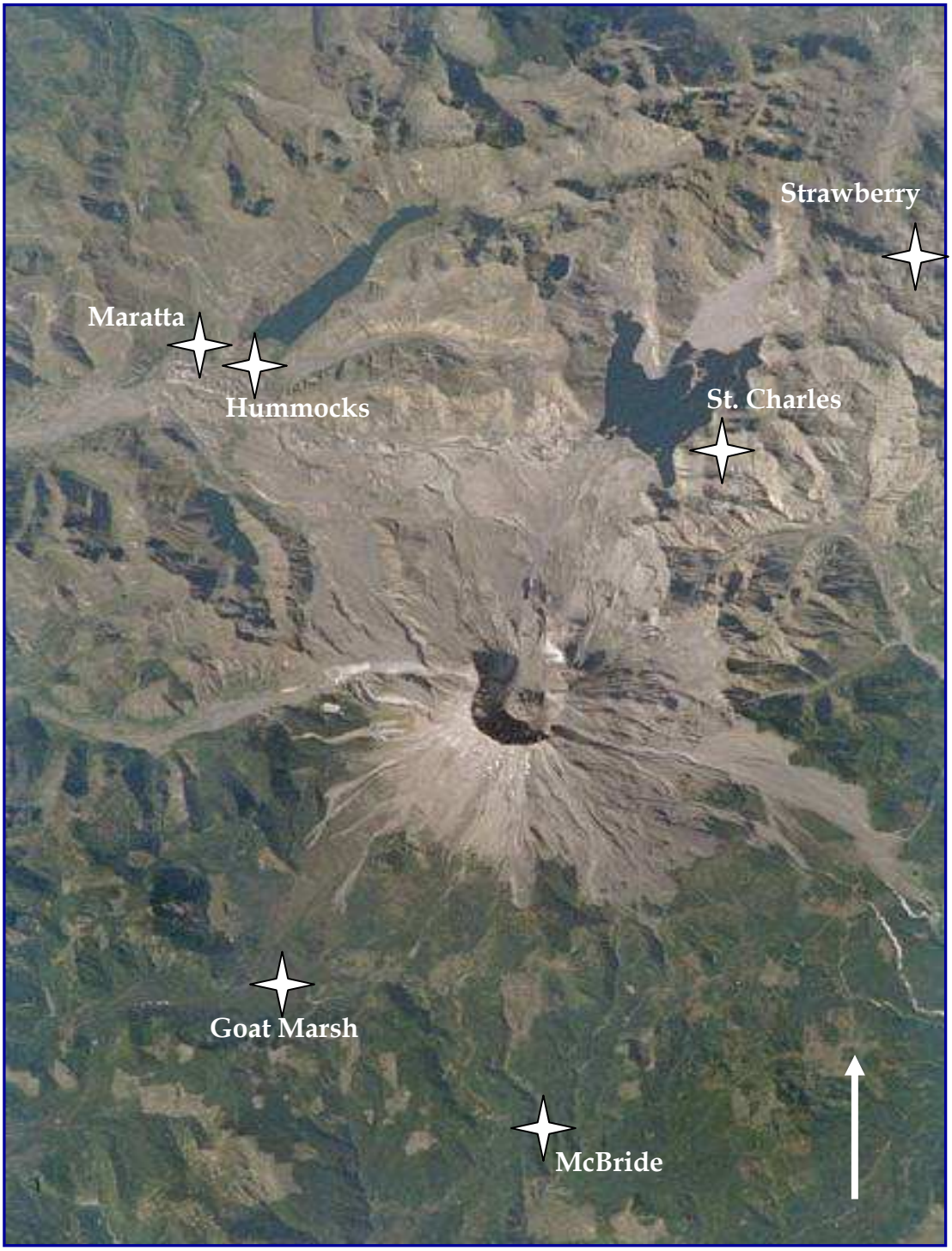

Figure 2.1. Collection sites at MSHNVM. White arrow points north. New ponds are Maratta and Hummocks in the debris-avalanche zone, recovery lakes are St. Charles and Strawberry Lakes in the blowdown-zone, and reference lakes are Goat Marsh and McBride Lakes. Linear distance between Strawberry and McBride Lake is 26.14 km.

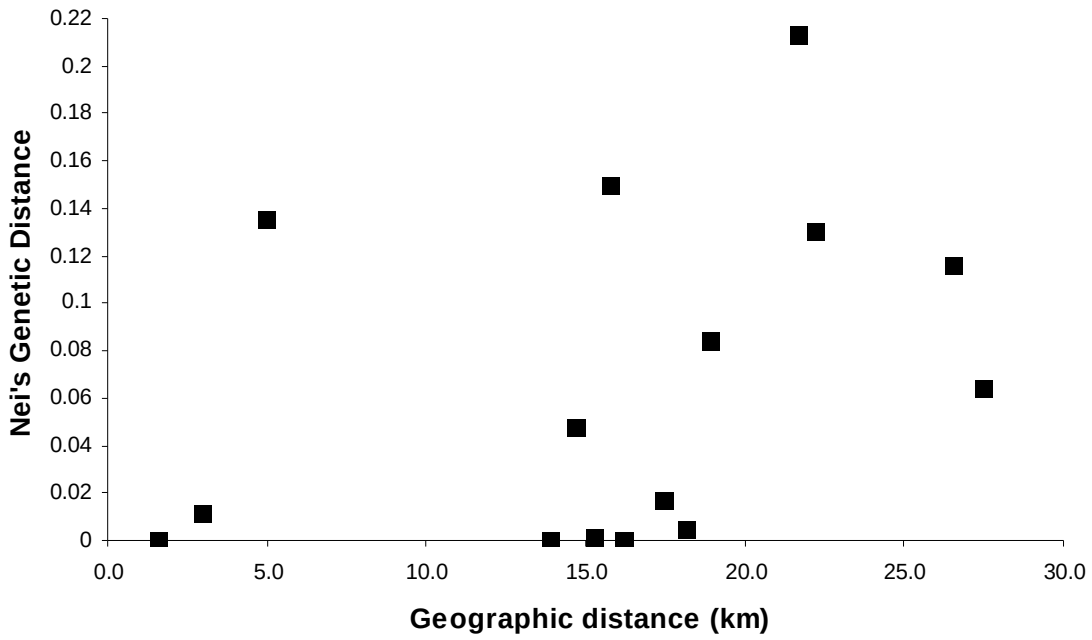

Figure 2.2. Isolation by distance in *T. granulosa* from six collection sites in MSHNVM. Negative genetic distances were converted to zero. A table of distances (geographic and Nei's is in Appendix C).

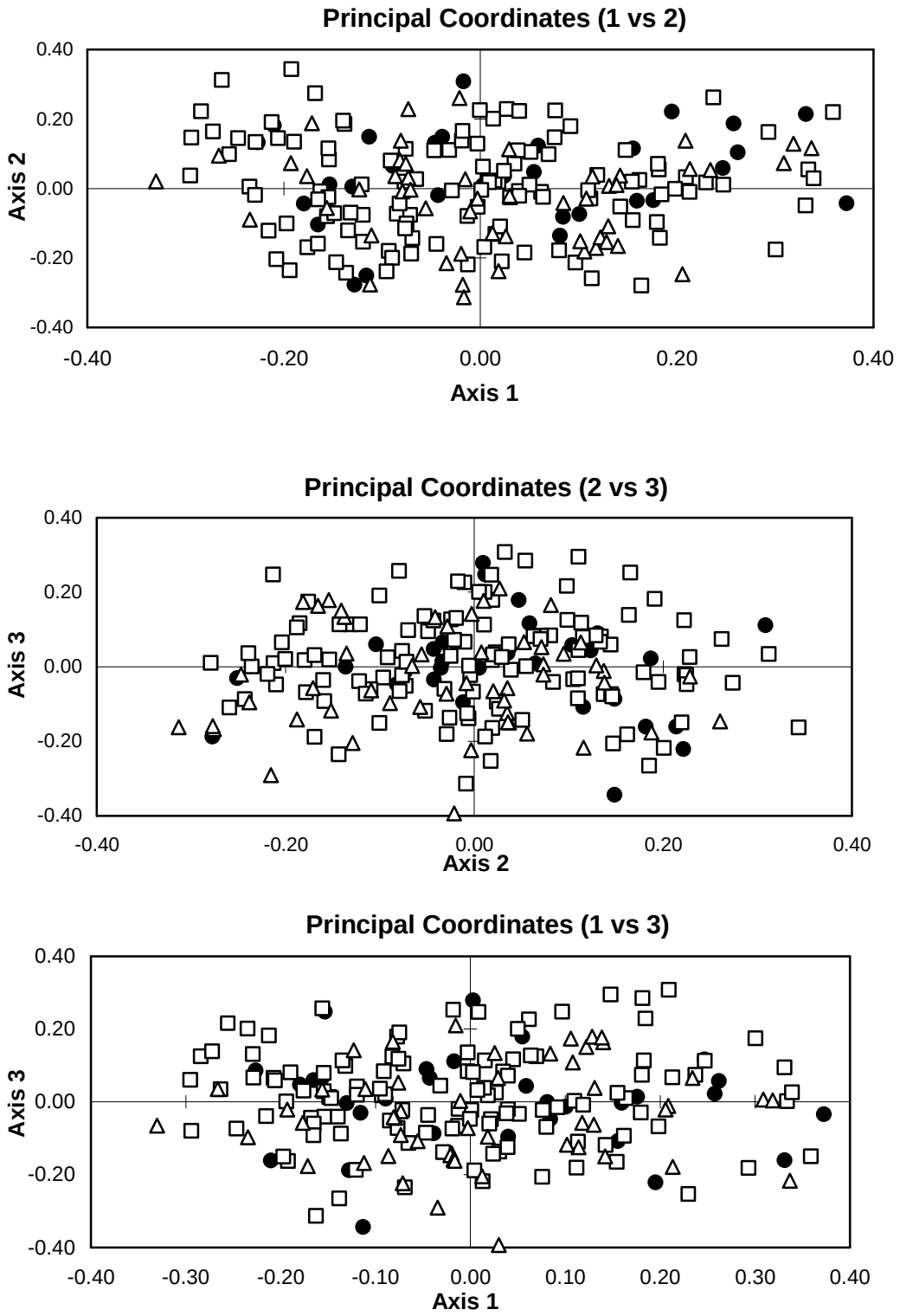

Figure 2.3. Principal components analysis for *T. granulosa* at MSHNVM by treatment. New populations are represented by filled circles, recovery populations by open triangles and reference populations by open squares.

CHAPTER 3

GENETICS OF COLONIZATION IN *AMBYSTOMA GRACILE* (CAUDATA: AMBYSTOMATIDAE) AFTER THE 1980 ERUPTION OF MOUNT ST. HELENS

Introduction

Colonization is the result of a dispersal event that leads to the establishment of an organism into an area from which it was previously absent. Factors that lead to colonization are varied. Within an organism's traditional range, there may be an environmental disturbance that creates new, suitable habitat or previously degraded habitat has been restored. Individuals may disperse into new habitats or ecosystems via invasion or range expansion. Depending on the circumstances of the colonization event and the life-history characteristics of the species, founding populations may lose alleles and genetic diversity as compared to source populations (Nei *et al.* 1975; Allendorf 1986; Whitlock & McCauley 1990). Whether founder populations gain or lose genetic variability is of great interest because genetic diversity is directly related to population fitness (Reed & Frankham 2003; Reed *et al.* 2007).

Amphibian decline is of great concern for conservationists (Mendelson *et al.* 2006) and the list of adverse factors only seems to increase (Becker *et al.* 2007). Many species are imperiled through habitat fragmentation or outright habitat destruction. Although some restoration efforts have resulted in colonization events (e.g. Pechmann *et al.* 2001; Willson & Dorcas 2003; Brodman *et al.* 2006; Petranka *et al.* 2007), the genetic impacts are unclear. For example, an island-population of tungara frogs, established 12

years prior to the study, showed evidence of a genetic bottleneck (Lampert *et al.* 2007) but in contrast, a recently (past 70 years) fragmented frog population showed low levels of population subdivision (Vos *et al.* 2001). Human-caused habitat fragmentation over a large scale (> 100 km) left a genetic signal in a widespread and common anuran, *Physalaemus cuvieri* (Telles *et al.* 2007) but the crested newt, *Triturus cristatus*, showed no genetic bottleneck in ponds colonized only decades before (Jehle *et al.* 2001). Few individuals of the frog, *Rana ridibunda*, effectively colonized Great Britain in 1935 without a strong bottleneck effect (Zeisset & Beebee 2003). The cane toad, *Bufo marinus*, rapidly spread once introduced to Australia, showed no evidence of isolation by distance after an estimated 25-35 generations (Leblois *et al.* 2000). From these and other studies (Cushman 2006 and references therein), we can see that the genetic response to colonization in pond-breeding amphibians appears to be situational, depending on the species though studies of anurans are more numerous than those of caudates. Therefore, there is no clear pattern as to whether founding populations of pond-breeding amphibians have lower genetic variability than their source populations.

Salamanders in the family Ambystomatidae are typical pond-breeding amphibians with aquatic eggs, aquatic larvae, and terrestrial adults. However, some species also reproduce as neotenes, a sexually mature adult in the aquatic larval form (Petranka 1998). The ratio of neotenes to transformed adults could have a significant impact on gene flow in an animal that breeds in discrete ponds. Ambystomatid salamanders are generally considered site-fidelic, move only short distances (usually < 1km) from breeding ponds when not reproducing, usually breed in their natal pond, have low vagility, and move slowly across a landscape (Spear *et al.* 2005; Gamble *et al.* 2007; Zamudio & Wieczorek

2007). At the genetic level, pond-breeding amphibians are considered to exist in metapopulations and migration rates between subpopulations are low (Kinkead *et al.* 2007; Petranka 2007). Based on population genetics theory, it is reasonable to expect that founding populations of ambystomatid salamanders would possess low levels of genetic diversity characterized by low levels of gene flow among populations. In at least one amphibian species, the natterjack toad, *Bufo calamita*, low genetic variability can have adverse affects on survivorship and subsequent population viability (Rowe & Beebee 2003; 2005). A theoretical simulation showed that the number of colonists moving into unpopulated habitat would have to be considerable larger than the number of migrants moving between existing populations to reduce the amount of genetic differentiation between existing populations (Wade & McCauley 1988). Therefore, although wetland restoration efforts for ambystomatid salamanders and other pond-breeding amphibians are on-going (Lehtinen & Galatowitsch 2001; Petranka *et al.* 2007), more research is needed to determine if natural colonization processes are sufficient to develop genetically diverse and robust populations.

Mount St. Helens has become a classic study site for testing ecological theories on colonization and primary succession (Dale *et al.* 2005a). The 1980 eruption created a natural experiment that generated the conditions where colonization and recovery can be studied in concert. New ponds in the debris-avalanche zone created by the initial landslide were first colonized by two anuran species within 3 years of the eruption and *Ambystoma gracile*, the Northwestern salamander, naturally colonized these ponds 9 years post eruption (Crisafulli *et al.* 2005b). A typical pond-breeding amphibian, *A. gracile* has a biphasic lifestyle that includes aquatic eggs and larvae and a terrestrial adult

form. However, *A. gracile* also has a neotenic form, common in many localities. In fact, terrestrial adults are often difficult to find(Bosakowski 1999; Grialou *et al.* 2000; Hoffman *et al.* 2003; but see Aubry 2000). Although data on presence, survival, and breeding status of *A. gracile* in lakes and ponds in all zones around Mount St. Helens are available (Crisafulli *et al.* 2005b), the amount of genetic variability in new ponds and the amount of gene flow across this disturbed landscape remains unknown. Based on the 3 – 4 year maturation time in this species, I estimate there has been only 5–10 generations since the eruption, not enough time for mutation to have an influence on genetic diversity. Therefore, any genetic effects should be due solely to colonization and subsequent gene flow.

I examined the genetic response of *A. gracile* to the 1980 eruption of Mount St. Helens. Using two molecular markers, microsatellites, and AFLPs (amplified fragment length polymorphisms), I measured genetic diversity, gene flow, and population structure to determine if genetic diversity is lower in newly colonized ponds. Based on the life-history characteristics of this pond-breeding amphibian, I predicted that newly colonized ponds would have less genetic diversity than reference populations. Populations consisting of descendents from eruption survivors would show intermediate levels of genetic diversity and reference sites would have the highest measures of genetic diversity. I also expected that populations at Mount St. Helens would show significant evidence of population structure because of the irregular and rugged landscape, low vagility and high philopatry, a characteristic of ambystomatid salamanders. Measuring the genetic diversity of amphibian populations affected by the eruption helps elucidate how or if genetic diversity is lost when a local population goes extinct and the habitat is later recolonized.

If newly colonized and recovery populations show a significant loss of genetic diversity, that would indicate that more than a few generations are required for a population to regain original levels of genetic diversity. Little to no loss of genetic diversity would be a positive indicator that *A. gracile* can quickly recover (within 25 years) from a major but infrequent environmental disturbance.

Materials and Methods

Study site

The impact of the 18 May 1980 eruption on the surrounding landscape was swift, violent, and dramatic. The eruption began with a landslide, followed by a searing blast that dramatically altered the local landscape. The initial debris-avalanche and blast oriented towards the north and north-east (Peterson 1986). Approximately 570 km^2 of forest was leveled by the blast, creating a blowdown zone, where due to the timing (snow and ice still present in the montane spring, and morning), a few plants and burrowing animals survived (Dale *et al.* 2005b). For instance, post-eruption surveys found some fish survived in 67% of the lakes within the blowdown zone (Crawford 1986). Except for approximately 5 to 25 cm of tephra fall, the landscape on the volcano's south side was relatively unaffected. Thus, several lakes on the south side have been used as reference sites to compare pre- and post-eruption effects (Wissmar *et al.* 1982; Dahm *et al.* 2005). In this study, reference populations are assumed to represent the levels of genetic diversity that would have been present across the entire area if the volcano had not erupted. Table 3.1 summarizes the study area.

Study organism

Ambystoma gracile, the northwestern salamander, has a biphasic life-cycle with terrestrial adults and aquatic larvae. However in some populations, adults do not transform but instead remain in their natal pond and upon reaching maturity, breed as gilled adults, called neotenes. Compared to most *Ambystoma*, the larvae of *A. gracile* have an extended aquatic phase (greater than 1 year) and it may take several years for adults to mature, depending on elevation and temperature (Eagleson 1976; Petranka 1998). Current Mount St. Helens populations appear to be highly neotenic, especially in the debris-avalanche zone (Crisafulli *et al.* 2005b). Females attach egg masses (40–270 eggs) to vegetation about 0.5–1 m below the water surface (Nussbaum *et al.* 1983; MacCracken 2007). Trout (*Salvelinus fontinalis*, *Oncorhynchus* spp.) prey upon adult and larval *A. gracile*, thus population densities are lower in trout-infested ponds. Rough-skinned newts (*Taricha granulosa*) also feed upon egg masses (Efford & Mathias 1969; Taylor 1983; MacCracken 2007). At metamorphosis, juvenile *A. gracile* leave the natal pond, move into the surrounding landscape and take 2–3 years to sexually mature (Eagleson 1976; Nussbaum *et al.* 1983). The life-span of a neotenic *A. gracile* is estimated to be 5 yrs (Efford & Mathias 1969). The lifespan of transformed adults is unknown but *A. macrodactylum* (long-toed salamander, a related species also found in the Pacific Northwest) can live up to 10 years (Russell *et al.* 1996). *Ambystoma macrodactylum* (spotted salamander), *A. gracile's* closest relative, can live to 32 years, although most animals from the study population were between 2 and 18 years old (Flageole & Leclair 1992).

Population sampling

I captured *A. gracile* May-June 2003 and July 2005 from ponds and lakes in the Mount St. Helens National Volcanic Monument (MSHNVM), Washington State, USA using minnow traps (set and left overnight) and dipnet (Fig. 3.1, Table 3.2). *Ambystoma gracile* were either larvae or neotenes. Collection sites (at least two per treatment) were chosen based on the probability that they would contain salamanders (Crisafulli, pers. comm.) and their accessibility within a 3 hr hike. Collection sites were assigned to treatments based on how they were affected by the eruption (Table 3.1). Maratta and Hummocks, in the debris-avalanche zone, were classified as new ponds as they did not exist prior to the eruption. Curtis, Ghost, Meta, Ryan and St. Charles Lakes in the blowdown zone where trees were toppled by the blast, were classified as recovery lakes. Goat Marsh and McBride Lakes, south of the volcano, where the impact of the eruption was minimal, were assigned as reference lakes.

In 2005, I also collected from a beaver pond, approximately 2.4 km in north of the Maratta complex. In 1997, beavers created this pond on Maratta Creek (Crisafulli, pers. comm.). Although this population is of recent origin, it was not formed by the volcano and its locality outside of the debris-avalanche precludes it from being grouped with Maratta and Hummocks. Therefore, I analyzed this pond separately to determine which of my other collection sites could be its source population. Table 3.2 provides geographic coordinates for collection sites and samples sizes.

Neither Maratta nor Hummocks are discrete lakes but instead are a collection of small ponds created by the landslide of the north slope of the volcano (debris-avalanche) during the initial stages of the eruption. Maratta is a complex of approximately 35 ponds

and the Hummocks is a complex of approximately 15 ponds within the boundary delineated by the Hummocks trail (Tr 229). I attempted to collect *A. gracile* from all localities both years except for Meta and Curtis Lakes, which were sampled only in 2003. After removing a section of tail tip, all animals were immediately released at their capture site.

DNA extraction and amplification

I collected tissue samples by removing approximately 1 cm of tail tip from each salamander. Each tissue was stored individually in 95% ethanol until return to the lab when samples were stored at -80º C. I isolated total genomic DNA by digesting approximately 0.5mm of tissue sample in 550µl of lysis buffer (50mM Tris HCl, pH 8.0, 10mM EDTA, pH 8.0, 200mM NaCl) with 11µl of Proteinase K (20 µg/ml) at 55º C followed by a modified salt-chloroform extraction and isopropanol precipitation (Mullenbach *et al.* 1989). For AFLPs, I purified DNA from this initial extraction using a Qiagen DNeasy kit starting with step 3 of Appendix A: Purification of Genomic DNA from whole or non-nucleated animal blood (DNeasy Tissue Handbook 03/2004). Incubation was followed by step 3 of Protocol: Purification of total DNA from cultured animal cells (DNeasy Tissue Handbook 03/2004). I used a Nanodrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) to measure the concentration of the purified DNA and used ddH_2O to standardize each sample to 50ng/µL.

Microsatellites: No microsatellite primers for *A. gracile* have been published. Therefore, I tested 65 primers published for other *Ambystoma.* Of those, four amplified

acceptably (Table 3.3). Details on all *Ambystoma* primers tested are in Appendix D. I used AmaD49, AmaD226 (Julian *et al.* 2003a) and AjeD23 and AjeD314 (Julian *et al.* 2003b). Each PCR was carried out in a 15µL total volume with 100ng of DNA, a final concentration of 0.5µM each primer, 0.25µM total dNTPs, and 2.0mM of $MgCl_2$. I used a PTC-200 Peltier thermal cycler (MJ Research) with the following parameters: 2 min denaturation at 94º C, 35 cycles of 1 min at 94ºC, 45 sec at 58ºC and 1.5 min at 72ºC for extension, then ending with a 5 min extension period at 72º C. PCR amplicons were separated and visualized on an ABI PRISM® 3730 DNA Analyzer by the Nevada Genomics Center, Reno, NV for all loci. I used Genemapper 3.0 (Applied Biosystems Inc.) to score each sample and rounded allele sizes to the appropriate whole number.

***AFLP*:** Because only four microsatellite primers amplified, I also used amplified fragment length polymorphism (AFLP), amplifying DNA using the protocols of Vos *et al*. (1995). I repeated 40 samples to calculate the error rate. Purified DNA (100ng) was digested for 1 hr at 37ºC with 5U of *Eco*RI (New England Biolabs) and 5U of *Mse*I (New England Biolabs) in 5µL of 10 RL buffer (100mM Tris-acetate, 100mM Mg-acetate, 500mM K-acetate, 50mM DTT, pH 7.5), with ddH_2O to a final volume of 50µL. To the restriction digest, I added 10µL of ligation mix composed of 1µL 10X RL buffer, 5pmole each of *Eco*RI adaptor (forward and reverse) and 50pmole each of *Mse*I adaptor (forward and reverse), 1.2µL of 10mM ATP (Fisher), 1U T4 DNA ligase (5 WeissU/Ul; Fermintas) and ddH_2O. The ligation plus restriction digest was incubated for another 3 hrs at 37ºC.

The *A. gracile* genome is large, C-value = 42.0 pg (human C-value = 3.5 pg) (Licht & Lowcock 1991). Thus, a pre-amplification step using a 2+2 primer combination was required. Pre-amplification with a 1+1 combination (*Eco*-A and *Mse*-A) was tried but

this yielded messy results in the selective PCR with several combinations of 3+3 and 4+4 selective primers. Preamplification was performed with 3µL of undiluted ligase samples in a total volume of 50µL with 10µL 5X PCR buffer (Promega), 1.5mM $MgCl_2$, 0.2mM dNTPs (equimolar mix of all 4), 1 U Taq DNA polymerase (GoTaq Flexi, Promega) and 0.1µM each of *Eco*-AC (5'-GAC TGC GTA CCA ATT CAC-3') and *Mse*-AC (5'-GAT GAG TCC TGA TGA GTA AAC-3'). The PTC-200 Peltier thermal cycler (MJ Research) parameters were 72ºC for 2 min, 94ºC for 30 sec, 58ºC for 30 sec, 72ºC for 1 min, then repeating 29 times from 94ºC. This preamplification PCR product was diluted 1:10 for selective PCR.

Three selective primer combinations were used: *Eco*-ACGC (5'-GAC TGC GTA CCA ATT CAC GC-3') / *Mse*-ACAG (5'-GAT GAG TCC TGA TGA GTA AAC AG-3'), *Eco*-ACGC / *Mse*-ACAC, and *Eco*-ACGC / *Mse*-ACTC. The *Eco* primer was labeled on the 5' end with 6-FAM. Each PCR consisted of 2.5µL of diluted preamplification product, 2.0µL of 5X PCR buffer (Promega), 1.5mM $MgCl_2$, 0.2mM dNTPs (equimolar mix of all 4), 0.05uM *Eco* primer, 0.2uM *Mse* primer, 0.5U Taq DNA polymerase (GoTaq Flexi, Promega) and ddH_2O to 10µL. The thermal cycler parameters were 94ºC for 2 min, then repeated 9 times: denature at 94ºC for 30 sec, anneal at 65ºC for 30 sec (reduced by 1ºC each cycle with final temperature of 57ºC), and extension at 72ºC for 1 min. Finally, there were 30 cycles of 94ºC for 30 sec, 56ºC for 30 sec, and 72ºC for 30 sec. Fragments were separated on an ABI PRISM® 3100 Genetic Analyzer by the Center for Integrated BioSystems at Utah State University, Logan, UT. After converting the sample files with the program "3730 FSA to GS converter" then

Genescan 3.7 (Applied Biosystems, Inc), I scored the gels using Genographer version 1.6 (Benham 2001).

Within-population patterns of genetic diversity

Data were analyzed by individual collection site and then by treatment. Basic population genetic diversity measures, including heterozygosity, number of alleles, genetic distances, and pairwise population Φ_{PT} values, for microsatellite and AFLP loci were calculated using GENALEX 6.1 (Peakall & Smouse 2006) employing the option to interpolate missing data when calculating genetic distances for the microsatellite loci.

Among-population patterns of genetic diversity

Due to small sample sizes (N < 5), animals from Ghost and Meta Lakes were excluded when testing for among-population patterns of genetic diversity. Beaver Pond was also excluded because it did not exist until 1997. To test for pairwise population differences, I used GENALEX 6.1. When comparing dominant and codominant markers, Φ_{PT} (an analogue of F_{ST}) is recommended by the authors (Peakall & Smouse 2006). The null hypothesis of $\Phi_{PT} = 0$ was tested using 999 permutations and significance was set at P = 0.05. I used analysis of molecular variation (AMOVA) for both microsatellite and AFLP data to look for genetic structure. Collection sites were grouped by treatment and for microsatellites, I used the codominate-genotypic option which uses Φ_{PT}-values. For microsatellites, there were 93 animals in the new treatment (Hummocks, Maratta), 120 recovery animals (Curtis, Ryan, St. Charles Lakes) and 30 reference animals (Goat Marsh and McBride Lakes). For AFLPs, there were 99 animals in the new treatment, 132 in the recovery treatment and 31 in the reference treatment. Samples sizes differ because not all

loci amplified in all animals. Significance was tested against 9999 random permutations where a significant P value ($P < 0.05$) indicates the observed value is significantly greater than the permutation value.

I also used GENALEX 6.1 to visualize population structure by treatment for both markers, performing a principal component analysis (PCA) of genotypic distances using a standardized covariance matrix with 9999 permutations. PCA makes no prior assumptions about population structure. I used the Mantel test in Tools for Population Genetic Analysis (TFPGA) version 1.3 (Miller 1997) to test for evidence of isolation by distance. Using 999 permutations, I tested for correlation between Nei's unbiased genetic distance (Nei 1978) and a geographical distance matrix for the microsatellite and AFLP data. Geographic distance, accounting for topography (longer than point to point distances) was measured with TOPO 6.0 (DeLorme, Yarmouth, ME).

Assignment tests

To determine possible source populations for animals in Beaver Pond, a breeding site colonized in 1997, and to estimate contemporary levels of gene flow, I used the population assignment test in GenAlEx for the microsatellite data and AFLPOP (Duchesne & Bernatchez 2002) for AFLPs. However, the Mount St. Helens area has more lakes and ponds where salamanders could breed than could be practically sampled. Thus, these assignments only provide a general indication of gene flow. Both programs use log-likelihoods to calculate assignments, GenAlEx using the method of Paetkau *et al*. (2004). AFLPOP allocates animals of unknown origin to their most probable source population by calculating the probability of a given genotype being present in each of the possible

populations based on dominant band frequencies. The animal is then allocated to whichever possible source population that shows the highest likelihood for its genotype. I first performed the least stringent test, minimal log-likelihood difference (MLD) set to zero, which assigns animals as soon as one likelihood is higher than all others. I repeated the test and set MLD to 0.2 which corresponds to a 95% confidence level (Yang *et al.* 2008). I also increased sensitivity by setting MLD = 1 so that an animal is allocated to a population only if its largest likelihood is 10 times as high as the next largest likelihood. Calculated P values indicate the percent of likelihoods that are less than or equal to that of the likelihood of an individual animal belonging to a specific population. The lower the P value, the less likely it is that an individual is properly allocated to a population.

Results

Sampling

I captured a total of 380 *A. gracile*. The number of animals captured by year and site is found in Appendix A. All were larvae or neotenic adults. Only one larvae appeared to be reabsorbing its gills and in the process of transforming to a terrestrial adult. *Ambystoma gracile* were abundant across the study area except for the two reference lakes, Goat Marsh and McBride Lake where animals were only found in 2005, and Ghost and Meta Lakes (Table 3.2). I also set traps in Strawberry Lake in 2005 (46°19.64N/122° 3.21W) and although *Taricha granulosa* were easily captured by dip net and minnow trap, I did not find any *A. gracile*. These collection sites with few or no *A. gracile* were populated by enough trout to attract fly fishermen (pers. obs) and in Ghost Lake, my traps filled with a large number of crayfish.

Within-population levels of genetic diversity

The microsatellite genotyped animals showed high levels of polymorphism and genetic diversity (Tables 3.3, 3.4; Appendix B). Observed heterozygosity ranged from 0.668 at McBride Lake to 0.917 at Ghost Lake. By treatment, recovery populations had the highest observed heterozygosity, 0.844 and reference lakes had the lowest at 0.711 (Table 3.5). Aje D23 was not in Hardy-Weinberg equilibrium (HWE) at Maratta ($P = 0.029$) and Ryan Lake ($P < 0.001$). AjeD314 was not in HWE at Beaver Pond ($P = 0.033$), Curtis ($P=0.004$), Ryan ($P = 0.001$), Hummocks, Maratta, McBride, and St Charles Lakes ($P < 0.001$). AmaD226 was not in HWE at McBride and St. Charles Lakes ($P = 0.030$) and AmaD49 was not in HWE at Beaver Pond ($P = 0.036$) or Maratta ($P < 0.001$).

AFLP analysis yielded 81 polymorphic loci from 269 animals (Table 3.6). *Eco*-ACGC / *Mse*-ACAG combination yielded 32 bands, *Eco*-ACGC / *Mse*-ACAC combination yielded 25 bands, and *Eco*-ACGC / *Mse*-ACTC combination yielded 24 bands. The methodological error rate was estimated to be 0.57% from the included replicates. Estimated heterozygosities (H_A) ranged from 0.161 (Meta Lake) to 0.291 (Hummocks and Goat Marsh; Table 3.5). The low H_A value for Meta and Ghost Lake are a product of low sample sizes. Removing those two sites yields a mean H_A of 0.251. Despite its recent origin (1997), inspection of microsatellite and AFLP data shows nothing unusual about the animals collected from the Beaver Pond. Heterozygosities and the percent of polymorphic loci are within the ranges of the other collection sites (Tables 3.4, 3.6).

Among-population patterns of genetic diversity

Microsatellite and AFLP data show that *A. gracile* at MSHNVM had high levels of gene flow and low levels of genetic structure, either by collection site or by treatment. With microsatellites, observed heterozygosity was highest in the recovery treatment and almost equal at new and reference sites. AFLP data showed that average heterozygosity was highest (0.298) in the new treatment and lowest in the recovery treatment (0.272; Table 3.5). Pairwise Φ_{ST} values for microsatellites and AFLP's are small (Table 3.7) ranging from 0.000 to 0.069 for microsatellites and 0.015 to 0.132 for AFLPs. Analysis of molecular variation (AMOVA) for microsatellite markers showed that 96% of the variance was found within populations (P = 0.001; Table 3.8) and for AFLPs, the amount of variance within populations was 90% (P = 0.001; Table 3.8). There was some evidence of isolation by distance by collection site only in the AFLP data (r = 0.7378, Z = 8.87, P = 0.0015) but not in the microsatellite data (r = 0.1873, Z= 84.8, P = 0.1559, Figure 3.2), likely due to the high scatter. However, PCA of the microsatellite data showed extensive overlap between treatments (Figure 3.3) indicating low levels of genetic structure. PC axis 1 explains 21.2% of the variation, axis 2 explains 20.1% and axis 3 explains 16.3% for a cumulative total of 57.4%. Similarly, with AFLPs, PCA analysis showed overlap between treatments (Figure 3.3). A plot of PC2 versus PC3 (not shown) has no pattern or grouping by treatment; many data points fell upon each other. Axis 1 explains 28.3% of the variation, axis 2 explains 16.5% and axis 3 explains 14.5% for a cumulative total of 59.4%. PC axis one most likely represents distance between ponds. I expected that treatments would be more likely to group than if collection sites and treatments were randomly located on the landscape, simply because when collection sites were grouped by

treatment, they were closer to each other than collection sites in the other treatments. Therefore, the overlap between treatments was even more surprising.

Assignment tests

Salamanders from the Beaver Pond did not have a clear signal indicating their origin but both markers indicated that Maratta and Hummocks were the most likely source populations, a reasonable result as those localities are only 2.37 km and 3.80 km away from Beaver Pond, respectively. The microsatellite data showed that of the 23 Beaver Pond animals, 13 were assigned to new ponds, 3 to Hummocks and 10 to Maratta. Surprisingly, there were 6 animals assigned to recovery lakes (3 to Curtis, 2 to Ryan and 1 to St Charles) and 4 to reference lakes (2 each to Goat Marsh and McBride). Under the least stringent conditions for assigning AFLP genotypes to a putative source population, Beaver Pond animals clearly originated from either Maratta (13 of 23; P = 0.1724 – 0.8576) or Hummocks (9 of 23; P = 0.0768 – 0.4436). One animal was assigned to Hummocks but with only a 0.8% probability so it did not assign well to any population. With MLD = 0.2, 2 animals were not allocated to any population (P = 0.001 – 0.7200), 12 were allocated to Maratta (P = 0.1622 – 0.8567), and 8 to Hummocks (P = 0.0514 – 0.4436). One animal was assigned to Hummocks but with only a 0.8% probability so it did not assign well but yet, did not meet the criteria to be unassigned. With MLD = 1, 16 animals were not allocated to any population (P = 0.1166 – 0.4886), 5 were allocated to Maratta (P = 0.1360 – 0.8600), and 2 to Hummocks (P = 0.1360 – 0.2420).

Discussion

In general, pond-breeding amphibians are thought to be slow dispersers with low vagility (Duellman & Trueb 1986; Blaustein *et al.* 1994). Additionally, the apparent preponderance of neotenes captured in breeding ponds would lead to expectations of low gene flow. Given large source populations (mostly likely in the forests surrounding Mount St. Helens), *A. gracile* was resilient in the face of a large infrequent environmental disturbance. High levels of gene flow and no loss of genetic variability in new populations was an unexpected result. However, Titus & Gaines (1991) compared gene flow of *A. gracile* in coastal populations composed of transformed adults with montane populations composed of neotenic adults using allozyme markers. They were surprised to find high levels of gene flow (F_{ST} = 0.006, coastal populations; F_{ST} = 0.010, montane populations) and proposed three hypotheses to explain their results. Their first hypothesis (and the only relevant one here) is that metamorphosis and dispersal may be more common than thought. This is congruent with the genetic evidence from Mount St. Helens. Initial colonization of the new ponds formed by the debris-avalanche could only occur through terrestrial adults. Even though subsequent conditions in the new and recovery areas would seemingly favor neotenes (Sprules 1974a), low levels of population structure strongly indicate that there are substantial numbers of terrestrial adults moving across the landscape at Mount St. Helens. Sprules (1974b) demonstrated that *A. gracile* populations could include individuals that always metamorphose, regardless of environmental conditions, those that are always neotenic and finally, those that may or may not transform, depending on environmental cues. Because colonizers at Mount St.

Helens, could only come from unaffected forest areas surrounding the affected area and blowdown zone survivors, my results agree with Sprules (1974a,b) in that variation in reproductive mode contributes to colonization and survival.

Little is known of the terrestrial ecology and behavior of *A. gracile* except that they are fossorial, rarely seen, and make extensive use of rodent burrows (Nussbaum *et al.* 1983). It is likely that dispersing *A. gracile* used Northern pocket gopher (*Thomomys talpoides*) burrows as refuges. Many individuals of this fossorial herbivore survived the blast and significantly promoted recovery of the plant community due to their burrowing activities (Andersen & MacMahon 1985). My and the allozyme data of Titus (1990) suggest that *A. gracile* may not be philopatric to their natal or breeding pond, interesting in that *A. maculatum*, sister taxon to *A. gracile*, is highly philopatric (Shaffer *et al.* 1991; Semlitsch 1998). Kinkead *et al.* (2007) studied, using AFLPs and mark-recapture methods, *Ambystoma talpoideum* and *A. maculatum* and expected to find population structure in their breeding populations because only 6 animals were detected moving between ponds (142 m maximum distance) during a 3-year period. However, the genetic data showed no evidence of population structure, even over distances of approximately 25 km. The results of my study, coupled with that of Kinkead *et al.* (2007) indicate that we still do not fully understand migration and dispersal (as defined by Semlitsch 2008) in ambystomatid salamanders.

High levels of heterozygosity and low F_{ST} values strongly indicate that, in the Mount St. Helens area, *A. gracile* appear to comprise a large, panmictic population. Unlike many other pond-breeding amphibians (Gamble *et al.* 2007), including most *Ambystoma*, these populations are not acting as a metapopulation at the scale of my study.

Instead, *A. gracile* populations around Mount St. Helens can be characterized as a single, large patchy population (Roslin 2001; Bradford *et al.* 2003). While is difficult to discern why pond-breeding amphibians move from favorable to apparently unfavorable habitats, there is evidence that some species are able to assess the condition of the breeding pond at oviposition time and if not adequate, due to factors such as the presence of predators or competitors, can switch to a different breeding site (Semlitsch 1998; Petranka *et al.* 2004).

Dispersal capability certainly plays a large role in determining the level of population structure and the amount of gene flow. At Mount St. Helens, Crisafulli *et al.* (2005b) report that some recently metamorphosed *A. gracile* dispersed at least 3.2 km in the blowdown zone, traveling over a steep valley wall, over a ridge, into the next drainage. In the Pumice Plain, a barren "moonscape," an individual salamander dispersed 3.0 km. The closest known source populations for the ponds in the debris-avalanche zone were 3.7 km away. If the colonization of the new ponds at Mount St. Helens is considered a process similar to that of an invasive species colonizing new habitat, my results suggest colonization by large numbers of animals. This may be a similar process to that of introduced *Anolis* lizards from around the Caribbean region into Florida where admixture contributed to increased genetic variation in introduced populations as compared to source populations (Kolbe *et al.* 2004; 2007). Although long distance migration of amphibians is difficult to study and thus may be underestimated for many species (Marsh & Trenham 2001), direct observation by Crisafulli *et al*. (2005b) and my genetic data show that *A. gracile* are capable of and do engage in long-distance movements.

High gene flow can also be evidence of population expansion (Newman & Squire 2001). If that were the case at Mount St. Helens, I would expect to see differences in the measures of genetic diversity, especially with respect to reference populations, unless the expansion is over a larger area than my study area. However, I consider this unlikely. First, this species was present in and around Mount St. Helens prior to the eruption. Second, populations of *A. gracile* do not appear to be declining or expanding and current distribution appears to resemble their historical range (Shaffer 2005), although demographic data on any salamander are limited. Another study, perhaps in another 20 years or so, may be warranted to ensure I have not recorded a transient response to the disturbance.

This study leads to another question: Why has *A. gracile* successfully colonized new ponds and is seemingly successful in blowdown areas while *Ambystoma macrodactylum* (long-toed salamander) has not? This species was assumed present in the area and reported to be breeding in Elk and St. Charles Lake within 3 years post eruption (Karlstrom 1986; Crisafulli *et al.* 2005b). However, I did not find any during my collecting. *Ambystoma macrodactylum* is considered a relatively common salamander, breeds earlier than *A. gracile*, and does not have a neotenic form (Petranka 1998). There is evidence that *A. macrodactylum* is capable of dispersal and migration. In rugged terrain, *A. macrodactylum* showed no isolation by distance in the eight populations sampled in northeastern Oregon and western Idaho and there was more variability within populations than between populations (Howard & Wallace 1981). In the Bitterroot Mountains of Idaho and Montana, *A. macrodacylum* showed substantial levels of genetic variation and was panmictic within basins with evidence for some gene flow on a

regional scale (Tallmon *et al.* 2000). Low altitude sites (< 1200 m) had more gene flow between them than among high altitude sites (> 1200 m) or between high and low altitude sites (Giordano *et al.* 2007). Perhaps there were far fewer *A. macrodactylum* than *A. gracile* in the Mount St. Helens area pre-eruption and therefore few animals available for colonization post-eruption (Dvornich *et al.* 1997). Although both species are common in nearby Mount Rainer National Park, they differ in breeding site preferences and large *A. gracile* larvae are capable of preying upon smaller *A. macrodactylum* larvae (Hoffman *et al.* 2003).

Amphibian colonization at Mount St. Helens appears to follow a pattern similar to that of other amphibian communities: anurans first, then pond-breeding salamanders. Four species of anuran colonized the Maratta wetlands complex in the debris-avalanche zone (new ponds) before the first salamander, *A. gracile* arrived (Crisafulli *et al.* 2005b). Anurans and salamanders easily colonized constructed ponds within a year in a North Carolina site with multiple source ponds nearby (Petranka *et al.* 2003). In a sand prairie habitat in Indiana, seven of nine anurans colonized restored areas in one year while tiger salamanders (*A. tigrinum*) took five years (Brodman *et al.* 2006). Wildfire in Glacier National Park in Montana did not negatively affect populations of *A. macrodactylum* and the Columbia spotted frog, *Rana luteiventris*, while the western toad, *Bufo boreas* colonized fire affected areas (Hossack & Corn 2007). Nine of nine anuran species colonized restored wetlands in Minnesota while only one of three salamander species was able to do so (Lehtinen & Galatowitsch 2001). In Great Britain, the two anuran species (*Bufo bufo* and *Rana temporaria*) more readily colonized newly constructed ponds than two newt species (*Triturus cristatus* and *Triturus vulgaris*) because newts were unable to

colonize new ponds found greater than 400m from existing ones (Baker & Halliday 1999). In South Carolina, some anuran species colonized new ponds (built to mitigate loss of wetlands) within a year and successfully reproduced while successful salamander reproduction took three years (Pechmann *et al.* 2001). As Petranka *et al.* (2004) noted, time is an important variable when determining the success of a colonization event. With respect to ambystomatid salamanders, colonization can take up to a decade and even if animals are observed in a breeding pond the first year, it can take 3-5 years for salamanders to mature (Petranka 1998). Therefore, colonization studies (usually conducted with respect to evaluating a habitat restoration event) must be cognizant of these time scales.

The effects of disturbance and habitat fragmentation on pond-breeding amphibians are complex. Traditionally, pond-breeding amphibians were thought to exist in metapopulations and generally did not move extensively across the landscape, although this assumption has been questioned several times (Marsh & Trenham 2001; Jehle *et al.* 2005; Cushman 2006). This study shows that *A. gracile* are resilient in the face of a major environmental disturbance. Their ability to colonize new ponds set in an seemingly inhospitable matrix is surprising, more so when it appears to have been done by a large number of individuals, either in a single pulse, or more likely, multiple times. Clearly, predicting dispersal and colonization ability based on the broad category of pond-breeding amphibian is not always reliable. Additionally, dispersal ability of the same species may be different across a continuous versus fragmented habitat; some animals, while poor dispersers in a continuous landscape, may be good dispersers through/across fragments (Marsh *et al.* 2004). Regardless of cause, the ability of *A. gracile* to colonize

the new ponds (and most likely recovery areas as well) is an encouraging result. Not all is doom and gloom in the amphibian conservation world. Future studies of amphibian colonization studies should emphasize the response of individual species at multiple scales of time and space.

Literature Cited

Allendorf FW (1986) Genetic drift and the loss of alleles versus heterozygosity. *Zoo Biology* **5**, 181-190.

Andersen DC, MacMahon JA (1985) Plant succession following the Mount St. Helens volcanic eruption: facilitation by a burrowing rodent, *Thomomys talpoides*. *American Midland Naturalist* **114**, 62-69.

Aubry KB (2000) Amphibians in managed, second-growth Douglas-fir forests. *The Journal of Wildlife Management* **64**, 1041-1052.

Baker JMR, Halliday TR (1999) Amphibian colonization of new ponds in an agricultural landscape. *Herpetological Journal* **9**, 55-63.

Becker CG, Fonseca CR, Haddad CFB, Batista RF, Prado PI (2007) Habitat split and the global decline of amphibians. *Science* **318**, 1775-1776.

Benham JJ (2001) GENOGRAPHER. Available from http://hordeum.oscs.montana.edu/genographer/.

Blaustein AR, Wake DB, Sousa WP (1994) Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. *Conservation Biology* **8**, 60-71.

Bosakowski T (1999) Amphibian microhabitat associations on a private industrial forest in western Washington. *Northwestern Naturalist* **20**, 61-69.

Bradford DF, Neale AC, Nash MS, Sada DW, Jaeger JR (2003) Habitat patch occupancy by toads (*Bufo punctatus*) in a naturally fragmented desert landscape *Ecology* **84**, 1012-1023.

Brodman R, Parrish M, Kraus H, Cortwright S (2006) Amphibian biodiversity recovery in a large-scale ecosystem restoration. *Herpetological Conservation and Biology* **1**, 101-108.

Crawford B (1986) The recovery of surviving fish populations within the Mount St. Helens National Volcanic Monument and adjacent areas. In: *Mount St. Helens: Five Years Later* (ed Keller SAC), pp. 293-296. Eastern Washington University Press, Cheney.

Crisafulli CM, Swanson FJ, Dale VH (2005a) Overview of the ecological responses to the eruption of Mount St. Helens: 1980-2005. In: *Ecological Responses to the 1980 Eruption of Mount St. Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 287-299. Springer, New York.

Crisafulli CM, Trippe LS, Hawkins CP, MacMahon JA (2005b) Amphibian responses to the 1980 eruption of Mount St. Helens. In: *Ecological Responses to the 1980 Eruption of Mount St. Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 183-197. Springer, New York.

Cushman SA (2006) Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation* **128**, 231-240.

Dahm CN, Larson DW, Petersen RR, Wissmar RC (2005) Response and recovery of lakes. In: *Ecological Responses to the 1980 Eruption of Mount St. Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 255-274. Springer, New York.

Dale V, Swanson FJ, Crisafulli CM (2005a) *Ecological Responses to the 1980 Eruption of Mount St. Helens.* Springer, New York.

Dale VH, Crisafulli CM, Swanson FJ (2005b) 25 years of ecological change at Mount St. Helens. *Science* **308**, 961-962.

Duchesne P, Bernatchez L (2002) AFLPOP: a computer program for simulated and real population allocation, based on AFLP data. *Molecular Ecology Notes* **2**, 380-383.

Duellman WE, Trueb L (1986) *Biology of Amphibians.* McGraw-Hill, New York.

Dvornich KM, McAllister KR, Aubry KB (1997) Amphibians and reptiles of Washington State: Location data and predicted distributions. In: *Washington State Gap Analysis - Final Report* (eds Cassidy KM, Grue CE, Smith MR, Dvornich KM), p. 146 Washington Cooperative Fish and Wildlife Unit, University of Washington, Seattle.

Eagleson GW (1976) A comparison of the life histories and growth patterns of *Ambystoma gracile* (Baird) from permanent low-altitude and montane lakes. *Canadian Journal of Zoology* **54**, 2098-2111.

Efford IE, Mathias JA (1969) A comparison of two salamander populations in Marion Lake, British Columbia. *Copeia* **1969**, 723-736.

Flageole S, Leclair Jr R (1992) Étude démographique d'une population de salamandres (*Ambystoma maculatum*) à l'aide de la méthode squeletto-chronologique. *Canadian Journal of Zoology* **70**, 740-749.

Gamble LR, McGarigal K, Compton BW (2007) Fidelity and dispersal in the pond-breeding amphibian, *Ambystoma opacum*: implications for spatio-temporal population dynamics and conservation. *Biological Conservation* **139**, 247-257.

Giordano AR, Ridenhour BJ, Storfer A (2007) The influence of altitude and topography on genetic structure in the long-toed salamander (*Ambystoma macrodactylum*). *Molecular Ecology* **16**, 1625-1637.

Grialou JA, West SD, Wilkins RN (2000) The effects of forest clearcut harvesting and thinning on terrestrial salamanders. *Journal of Wildlife Management* **64**, 105-113.

Hoffman RL, Larson G, L, Brokes BJ (2003) Habitat segregation of *Ambystoma gracile* and *Ambystoma macrodactylum* in mountain ponds and lakes, Mount Rainer National Park, Washington, USA. *Journal of Herpetology* **37**, 24-34.

Hossack BR, Corn PS (2007) Responses of pond-breeding amphibians to wildfire: short-term patterns in occupancy and colonization. *Ecological Applications* **17**, 1403-1410.

Howard JH, Wallace RL (1981) Microgeographical variation of electrophoretic loci in populations of *Ambystoma macrodactylum columbianum*. *Copeia* **1981**, 466-471.

Jehle R, Arntzen JW, Burke T, Krupa AP, Hödl W (2001) The annual number of breeding adults and the effective population size of syntopic newts (*Triturus cristatus, T. marmoratus*). *Molecular Ecology* **10**, 839-850.

Jehle R, Burke T, Arntzen JW (2005) Delineating fine-scale genetic unit in amphibians: probing the primacy of ponds. *Conservation Genetics* **6**, 227-234.

Julian SE, King TL, Savage WK (2003a) Isolation and characterization of novel tetranucleotide microsatellite DNA markers for the spotted salamander, *Ambystoma maculatum*. *Molecular Ecology Notes* **3**, 7-9.

Julian SE, King TL, Savage WK (2003b) Novel Jefferson salamander, *Ambystoma jeffersonianum*, microsatellite DNA markers detect population structure and hybrid complexes. *Molecular Ecology Notes* **3**, 95-97.

Karlstrom EL (1986) Amphibian recovery in the North Fork Toutle River debris avalanche area of Mount St. Helens. In: *Mount St. Helens: Five Years Later* (ed Keller SAC), pp. 331-344. Eastern Washington University Press, Cheney.

Kinkead KE, Abbott AG, Otis DL (2007) Genetic variation among *Ambystoma* breeding populations on the Savannah River site. *Conservation Genetics* **8**, 281-292.

Kolbe JJ, Glor RE, Schettino LR, Lara AC, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature* **431**, 177-181.

Kolbe JJ, Glor RE, Schettino LR, Lara AC, Larson A, Losos JB (2007) Multiple sources, admixture, and genetic variation in introduced *Anolis* lizard populations. *Conservation Biology* **21**, 1612-1625.

Lampert KP, Bernal XE, Rand SA, Mueller UG, Ryan MJ (2007) Island populations of *Physalaemus pustulosus*: history influences genetic diversity and morphology *Herpetologica* **63**, 311-319.

Leblois R, Rousset F, Tikel D, Moritz C, Estoup A (2000) Absence of evidence for isolation by distance in an expanding cane toad (*Bufo marinus*) population: an individual-based analysis of microsatellite genotypes. *Molecular Ecology* **9**, 1905-1909.

Lehtinen RM, Galatowitsch SM (2001) Colonization of restored wetlands by amphibians in Minnesota. *American Midland Naturalist* **145**, 388-396.

Licht LE, Lowcock LA (1991) Genome size and metabolic rate in salamanders. *Comparative Biochemistry and Physiology* **100B**, 83-92.

MacCracken JG (2007) Northwestern salamander (*Ambystoma gracile*) oviposition sites and hatching success in the Cascade mountains of southern Washington. *Herpetological Conservation and Biology* **2**, 127-134.

Marsh DM, Thakur KA, Bulka KC, Clarke LB (2004) Dispersal and colonization through open fields by a terrestrial, woodland salamander. *Ecology* **85**, 3396-3405.

Marsh DM, Trenham PC (2001) Metapopulation dynamics and amphibian conservation. *Conservation Biology* **15**, 40-49.

Mendelson JR, III, Lips KR, Gagliardo RW, Rabb GB, Collins JP, Diffendorfer JE, *et al.* (2006) Biodiversity: confronting amphibian declines and extinctions. *Science* **313**, 48-48.

Miller MP (1997) Tools for population genetic analysis (TFPGA) 1.3: a Windows program for the analysis of allozyme and molecular population genetic data, Computer software distributed by author (http://bioweb.usu.edu/mpmbio).

Mullenbach R, Lagoda P, Welter C (1989) An efficient salt-chloroform extraction of DNA from blood and tissues. *Trends in Genetics* **5**, 391.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583-590.

Nei M, Maruyama T, Chakroborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution* **29**, 1-10.

Newman RA, Squire T (2001) Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). *Molecular Ecology* **10**, 1087-1100.

Nussbaum RA, Brodie Jr ED, Storm RM (1983) *Amphibians and Reptiles of the Pacific Northwest.* University Press of Idaho, Moscow.

Paetkau D, Slade R, Burdens M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology* **13**, 55-65.

Peakall ROD, Smouse PE (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288-295.

Pechmann JHK, Estes RA, Scott DE, Gibbons JW (2001) Amphibian colonization and use of ponds created for trial mitigation of wetland loss. *Wetlands* **21**, 93-111.

Peterson DW (1986) Mount St. Helens and the science of volcanology: a five-year perspective. In: *Mount St. Helens: Five Years Later* (ed Keller SAC), pp. 3-19. Eastern Washington University Press, Cheney.

Petranka J (2007) Evolution of complex life cycles of amphibians: bridging the gap between metapopulation dynamics and life history evolution. *Evolutionary Ecology* **21**, 751-764.

Petranka JW (1998) *Salamanders of the United States and Canada.* Smithsonian Institution Press, Washington, DC.

Petranka JW, Harp EM, Holbrook CT, Hamel JA (2007) Long-term persistence of amphibian populations in a restored wetland complex. *Biological Conservation* **138**, 371-380.

Petranka JW, Kennedy CA, Murray SS (2003) Response of amphibians to restoration of a southern Appalachian wetland: a long-term analysis of community dynamics *Wetlands* **23**, 1030-1042.

Petranka JW, Smith CK, Scott AF (2004) Identifying the minimal demographic unit for monitoring pond-breeding amphibians *Ecological Applications* **14**, 1065-1078.

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Conservation Biology* **17**, 230-237.

Reed DH, Nicholas AC, Stratton GE (2007) Genetic quality of individuals impacts population dynamics. *Animal Conservation* **10**, 275-283.

Roslin T (2001) Spatial population structure in a patchily distributed beetle. *Molecular Ecology* **10**, 823-837.

Rowe G, Beebee TJC (2003) Population on the verge of a mutational meltdown? Fitness costs of genetic load for an amphibian in the wild. *Evolution* **57**, 177-181.

Rowe G, Beebee TJC (2005) Intraspecific competition disadvantages inbred natterjack toad (*Bufo calamita*) genotypes over outbred ones in a shared pond environment. *Journal of Animal Ecology* **74**, 71-76.

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

Semlitsch RD (1998) Biological delineation of terrestrial buffer zones for pond-breeding salamanders. *Conservation Biology* **12**, 1113-1119.

Semlitsch RD (2008) Differentiating migration and dispersal processes for pond-breeding amphibians. *Journal of Wildlife Management* **72**, 260-267.

Shaffer HB (2005) *Ambystoma gracile* (Baird, 1859) Northwestern Salamander. In: *Amphibian declines: The Conservation Status of United States Species* (ed Lannoo MJ), pp. 609-611. University of California Press, Berkeley.

Shaffer HB, Clark JM, Kraus F (1991) When molecules and morphology clash: a phylogenetic analysis of the North American Ambystomatid salamanders (Caudata: Ambystomatidae). *Systematic Zoology* **40**, 284-303.

Spear SF, Peterson CR, Matocq MD, Storfer A (2005) Landscape genetics of the blotched tiger salamander (*Ambystoma tigrinum melanostictum*). *Molecular Ecology* **14**, 2553-2564.

Sprules WG (1974a) The adaptive significance of paedogenesis in North American species of *Ambystoma* (Amphibia: Caudata): an hypothesis. *Canadian Journal of Zoology* **52**, 393-400.

Sprules WG (1974b) Environmental factors and the incidence of neoteny in *Ambystoma gracile* (Baird) (Amphibia: Caudata). *Canadian Journal of Zoology* **52**, 1545-1552.

Swanson FJ, Major JJ (2005) Physical events, environments, and geological-ecological interactions at Mount St. Helens: March 1980-2004. In: *Ecological Responses to the 1980 Eruption of Mount St. Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 27-44. Springer, New York.

Tallmon DA, Funk WC, Dunlap WW, Allendorf FW (2000) Genetic differentiation among long-toed salamander (*Ambystoma macrodactylum*) populations. *Copeia* **2000**, 27-35.

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

Telles MPC, Diniz-Filho JAF, Bastos RP, Soares TN, Guimaraes LDA, Lima LP (2007) Landscape genetics of *Physalaemus cuvieri* in Brazilian cerrado: correspondence between population structure and patterns of human occupation and habitat loss. *Biological Conservation* **139**, 37-46.

Titus TA (1990) Genetic variation in two subspecies of *Ambystoma gracile* (Caudata: Ambystomatidae). *Journal of Herpetology* **24**, 107-111.

Titus TA, Gaines MS (1991) Genetic variation in coastal and montane populations of *Ambystoma gracile* (Caudata:Ambystomatidae). *The University of Kansas Museum of Natural History Occasional Papers* **141**, 1-12.

Vos CC, Antonisse-De Jong AG, Goedhart PW, Smulders MJM (2001) Genetic similarity as a measure for connectivity between fragmented populations of the moor frog (*Rana arvalis*). *Heredity* **86**, 598-608.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, *et al.* (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**, 4407-4414.

Wade MJ, McCauley DE (1988) Extinction and recolonization: their effects on the genetic differentiation of local populations. *Evolution* **42**, 995-1005.

Whitlock MC, McCauley DE (1990) Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. *Evolution* **44**, 1717-1724.

Willson JD, Dorcas ME (2003) Effects of habitat disturbance on stream salamanders: implications for buffer zones and watershed management. *Conservation Biology* **17**, 763-771.

Wissmar RC, Devol AH, Staley JT, Sedell JR (1982) Biological responses of lakes in the Mount St. Helens blast zone. *Science* **216**, 178-181.

Yang S, Bishop JG, Webster MS (2008) Colonization genetics of an animal-dispersed plant (*Vaccinium membranaceum*) at Mount St Helens, Washington. *Molecular Ecology* **17**, 731-740.

Zamudio KR, Wieczorek AM (2007) Fine-scale spatial genetic structure and dispersal among spotted salamander (*Ambystoma maculatum*) breeding populations. *Molecular Ecology* **16**, 257-274.

Zeisset I, Beebee TJC (2003) Population genetics of a successful invader: the marsh frog *Rana ridibunda* in Britain. *Molecular Ecology* **12**, 639-646.

Table 3.1. Effects of 1980 eruption of Mount St. Helens on the local landscape (Crisafulli *et al.* 2005a; Swanson & Major 2005). The eruption also generated mudflows but these were localized to drainages and are not under consideration.

Zone	Caused by	Characterized by	Created	Survivors	Treatment
Debris-Avalanche	Collapse and landslide of volcano's north slope.	Hummocks/hills of rock. Covering of landscape by rock to 195m.	A clean slate with new lakes and ponds nonexistent prior to the eruption.	None	New
Blow down	High pressure blast, Air temps to 300ºC**.** High velocity rock and tephra moving across landscape.	Blowdown of trees, heating of lakes/ponds, heavy levels of ash and organic material into lakes/ponds.	Complex mosaic of disturbance dependent upon topography and distance from volcano.	Some (fossorial animals, fish, *T. granulosa*, *A. gracile*, underground plant parts)	Recovery
Reference	South of volcano. Not in direct path of eruptive forces.	Life as normal. Some ash/tephra fall.	No major effects.	Probably 100%	Reference

Table 3.2. Total collection of A. *gracile* by zone and treatment (Trmt) in and around the Mount St. Helens National Volcanic Monument (MSHNVM). The two letters under code are abbreviations for each collection site. Debris-Avalanche (DA), Recovery (REC), Reference (REF). Latitude, longitude, and elevation were measured with a handheld GPS. Pond designators (e.g. M5) in Maratta and Hummocks are from Crisafulli (pers.comm) and NR indicates not recorded.

	Code	Zone	Trmt	N	Lat (N) (deg. min)	Long (W) (deg. min)	Elevation (m)
Maratta	MA	DA	NEW				
M5				3	46 17.48	122 16.98	730.8
M6				36	46 17.47	122 16.97	749.8
M27				4	46 17.54	122 17.05	725.2
M28				2	NR		
M30				32	46 17.52	122 16.98	744.3
Hummocks	HU	DA	NEW				
H1				2	46 17.05	122 16.15	758.2
H6*				78	46 16.77	122 16.15	763.7
H23				21	46 16.81	122 16.22	765.2
H25				9	46 16.85	122 16.12	762.5
H26				2	46 16.91	122 16.28	745.5
Beaver Pond	BP	REC	N/A	25	46 18.76	122 12.21	939.7
Curtis Lake	CU	REC	REC	27	46 16.85	122 03.00	1107.7
Ghost Lake	GH	REC	REC	4	46 19.17	122 03.68	1154.2
Meta Lake	ME	REC	REC	4	46 19.64	122 03.21	1456.0
Ryan Lake	RY	REC	REC	32	46 21.17	122 03.70	1015.4
St Charles Lake	SC	REC	REC	69	46 19.34	122 05.23	1223.4
Goat Marsh Lake	GM	REF	REF	16	46 08.42	122 16.72	889.8
McBride Lake	MB	REF	REF	14	46 08.44	122 15.11	830.7

* I captured many more animals at this site, but collected tissue samples from 78.

Table 3.3. PCR conditions and basic data on four microsatellite loci used in *A. gracile* from MSHNVM. Number genotyped per locus (N), number of alleles (A), observed heterozygosity (H_O), expected heterozygosity (H_E), and number of migrants (Nm) based on F_{ST} values (Nm)averaged over all collection sites except for Beaver Pond.

Locus	N	Dye	Size range (base pairs)	A	H_O	H_E	F_{ST}	F_{IS}	Nm
Aje D23	245	VIC	206–280	19	0.824	0.866	0.044	0.049	5.42
Aje D314	225	6-FAM	164–300	69	0.673	0.898	0.076	0.251	3.05
Ama D226	241	6-FAM	228–370	32	0.862	0.880	0.054	0.020	4.34
Ama D49	211	NED	151–280	41	0.884	0.892	0.060	0.009	3.89

Table 3.4. Measures of genetic diversity for all collection sites at MSHNVM by microsatellite locus. Number genotyped (N), number of alleles (A), effective number of alleles (N_E), observed heterozygosity (H_O), expected heterozygosity (H_E). Over all collection sites and loci, F_{ST} = 0.057, F_{IS} = 0.084, and Nm = 4.3.

		Aje D23	Aje D314	Ama D226	Ama D49	Mean
HU	N	68	67	69	61	
	A	13	29	17	25	
	N_E	7.88	8.37	6.45	14.12	
	H_O	0.853	0.582	0.812	0.820	0.767
	H_E	0.873	0.880	0.845	0.929	0.882
MA	N	47	36	43	48	
	A	13	28	15	26	
	N_E	8.84	18.38	9.39	17.07	
	H_O	0.787	0.694	0.837	0.792	0.778
	H_E	0.887	0.946	0.893	0.941	0.917
CU	N	23	22	24	13	
	A	12	25	20	19	
	N_E	9.12	17.93	11.64	16.90	
	H_O	0.913	0.773	0.875	0.923	0.871
	H_E	0.890	0.944	0.914	0.941	0.922
GH	N	4	3	4	3	
	A	6	5	7	5	
	N_E	5.33	4.50	6.40	4.50	
	H_O	1.000	0.667	1.000	1.000	0.917
	H_E	0.813	0.778	0.844	0.778	0.803
ME	N	4	3	3	4	
	A	6	6	5	6	
	N_E	5.33	6.00	4.50	4.57	
	H_O	0.750	1.000	0.667	1.000	0.854
	H_E	0.813	0.833	0.778	0.781	0.801
RY	N	25	25	25	17	
	A	17	28	20	18	
	N_E	11.68	20.83	15.63	13.44	
	H_O	0.800	0.680	0.880	0.941	0.825
	H_E	0.914	0.952	0.936	0.926	0.932
SC	N	44	42	43	40	
	A	12	26	21	27	
	N_E	8.82	14.58	13.80	17.49	
	H_O	0.909	0.738	0.884	0.850	0.845
	H_E	0.887	0.931	0.928	0.943	0.922
GM	N	14	11	14	10	
	A	10	14	15	13	
	N_E	6.64	11.00	10.05	10.00	
	H_O	0.714	0.545	0.929	0.900	0.772
	H_E	0.849	0.909	0.901	0.900	0.890
MB	N	16	16	16	15	
	A	10	15	13	12	
	N_E	7.64	11.13	8.39	9.38	
	H_O	0.688	0.375	0.875	0.733	0.668
	H_E	0.869	0.910	0.881	0.893	0.888
BP	N	22	22	21	16	
	A	11	18	19	17	
	N_E	8.49	13.08	12.42	13.13	
	H_O	0.818	0.591	1.000	0.875	0.821
	H_E	0.882	0.924	0.920	0.924	0.912

Table 3.5. Measures of genetic diversity by treatment at MSHNVM by microsatellite and AFLP loci. Number genotyped (N), number of alleles averaged over loci (A), effective number of alleles (Ne), observed heterozygosity (H_O), expected heterozygosity (H_E), average heterozygosity (H_A) for AFLP, Fixation index (F) for microsatellite data, and percent of polymorphic loci (%P) for AFLPs. Over all treatments and loci, F_{ST} = 0.017, F_{IS} = 0.156, and Nm = 16.5 for microsatellite data. H_A over all treatments and AFLP loci = 0.284.

Trmt	N	A	Ne	H_O	H_E / H_A	F / %P
Microsatellite						
New	93	31.5	18.2	0.769	0.910	0.154
Recovery	120	27.0	12.1	0.844	0.939	0.100
Reference	30	18.0	12.2	0.711	0.912	0.217
AFLP						
New	132	2.0	-	-	0.298	100.0
Recovery	99	1.9	-	-	0.272	93.4
Reference	31	1.9	-	-	0.283	93.4

Table 3.6. Measures of genetic diversity for all collection sites at MSHNVM based on 81 AFLP loci. Number genotyped (N), number of bands (NB), average heterozygosity (H_A), standard error of estimated heterozygosity (SE), Percent Polymorphic (%P). Codes are as in Table 3.1.

Code	N	NB	H_A	SE	% P
HU	70	76	0.291	0.019	92.59
MA	62	80	0.293	0.016	98.77
CU	25	76	0.261	0.019	88.89
GH	4	55	0.191	0.023	50.62
ME	4	58	0.161	0.022	44.44
RY	26	67	0.262	0.020	86.42
SC	48	74	0.266	0.021	87.65
GM	15	73	0.291	0.019	88.89
MB	16	72	0.250	0.020	83.95
BP	23	73	0.277	0.019	87.65

Table 3.7. Pairwise population Φ_{PT} values (below diagonal) for microsatellites (top number of pair) and AFLPs (bottom number of pair). Φ_{PT} values are functionally equivalent to F_{ST} and can be directly compared between microsatellites and AFLPs. Negative Φ_{PT} values were converted to zero. P values are above the diagonal and non-significant values are in bold.

	Curtis	Ryan	St Charles	Hummocks	Maratta	Goat Marsh	McBride
Curtis		**0.392**	**0.463**	0.001	0.001	**0.191**	0.034
		0.002	0.013	0.001	0.001	0.001	0.001
Ryan	0.001		0.013	0.001	0.001	**0.142**	0.011
	0.029		0.008	0.001	0.001	0.001	0.001
St Charles	0.000	0.013		0.001	0.001	0.043	0.001
	0.015	0.015		0.001	0.001	0.001	0.001
Hummocks	0.044	0.062	0.047		0.001	0.001	0.001
	0.088	0.086	0.132		0.001	0.001	0.001
Maratta	0.023	0.020	0.020	0.042		0.016	0.001
	0.077	0.067	0.120	0.050		0.001	0.001
Goat Marsh	0.008	0.009	0.015	0.056	0.020		**0.219**
	0.052	0.063	0.075	0.086	0.062		**0.054**
McBride	0.018	0.019	0.033	0.069	0.031	0.009	
	0.094	0.107	0.114	0.120	0.091	0.019	

Table 3.8. Results of hierarchical AMOVA comparing genetic variation among the three treatments (new, recovery, reference), among collection sites within treatments and among collection sites for microsatellites and AFLPs.

Source of variation	df	SS	MS	Est. Var.	%	Statistic	Value	Probability
Microsatellites								
Among Trmt	2	24.751	12.376	0.070	2%	Φ_{RT}	0.016	0.0001
Among Pops/Trmt	4	29.564	7.391	0.096	2%	Φ_{PR}	0.022	0.0001
Within Pops	236	994.509	4.214	4.214	96%	Φ_{PT}	0.038	0.0001
Total	242	1048.824	23.981	4.380				
AFLP								
Among Trmt	2	196.527	98.264	0.930	7%	Φ_{RT}	0.069	0.0001
Among Pops/Trmt	4	107.999	27.000	0.414	3%	Φ_{PR}	0.033	0.0001
Within Pops	255	3091.958	12.125	12.125	90%	Φ_{PT}	0.100	0.0001
Total	261	3396.485	137.389	13.469				

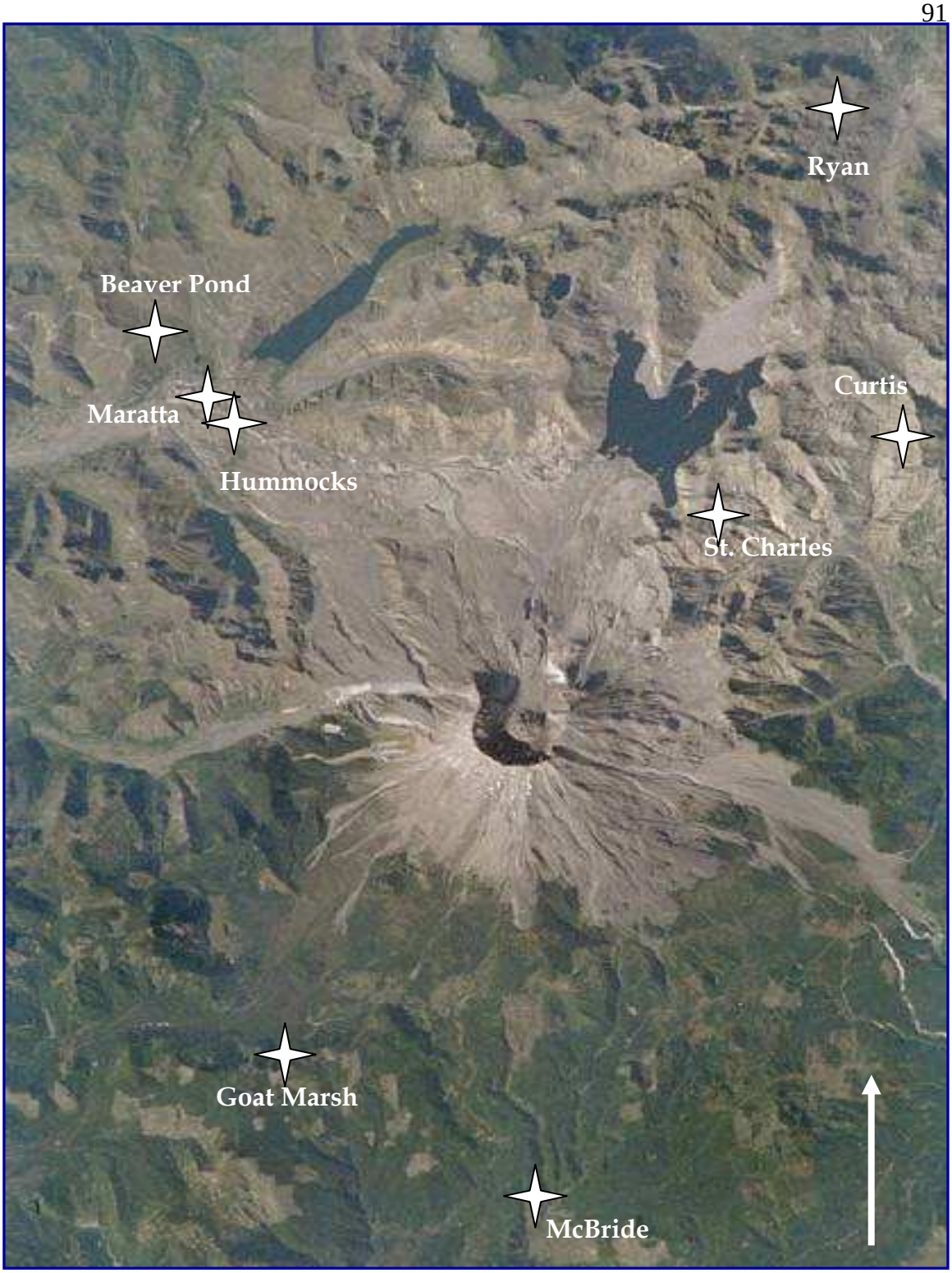

Figure 3.1. Collection sites at MSHNVM. White arrow points north. New ponds are Maratta and Hummocks in the debris-avalanche zone, recovery lakes are St. Charles and Ryan and Curtis Lakes in the blowdown-zone, and reference lakes are Goat Marsh and McBride Lakes. Linear distance between Ryan and McBride Lake is 28.04 km.

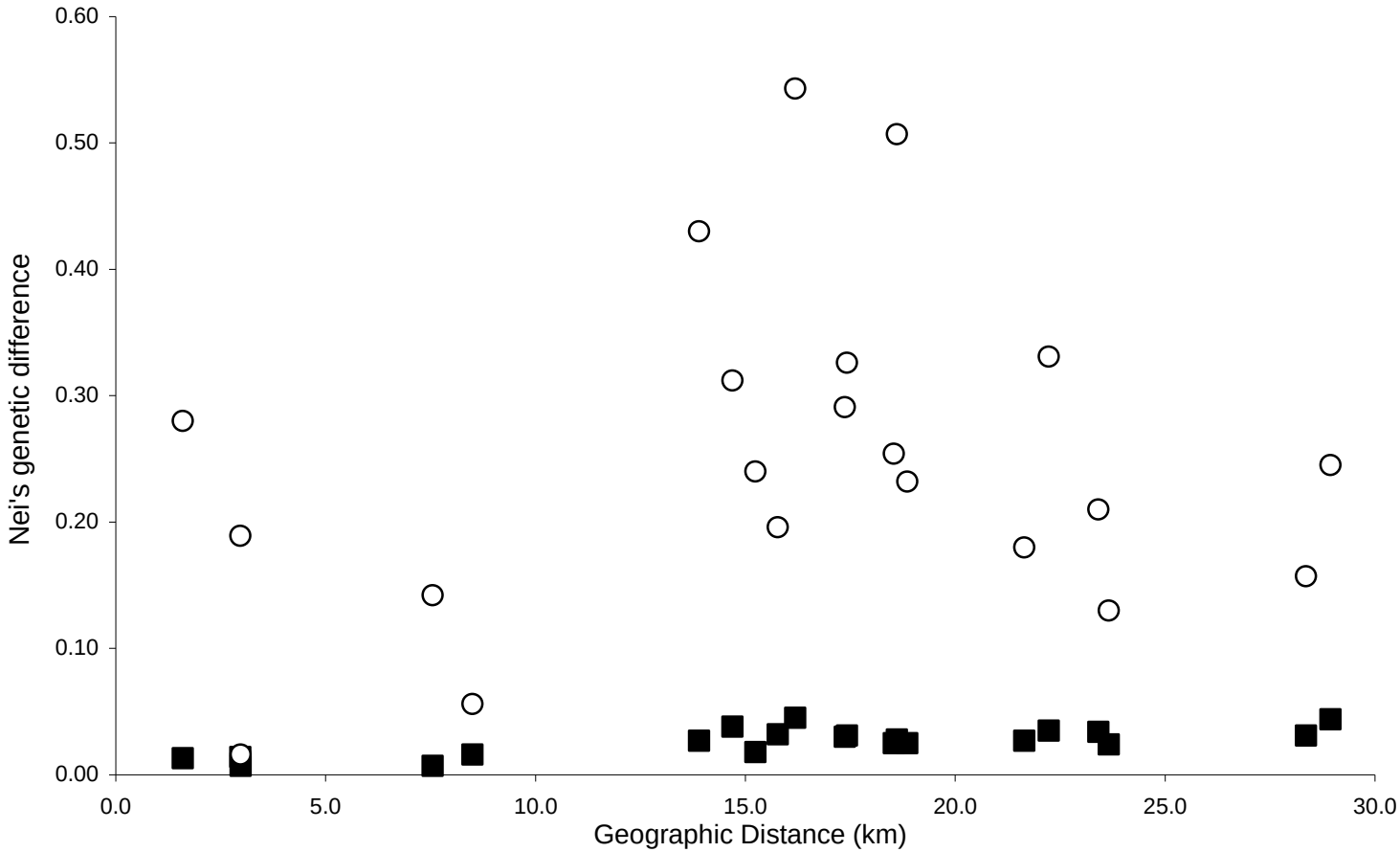

Figure 3.2. Geographic distance versus Nei's unbiased genetic distance in *A. gracile* at MSHNVM. Open circles indicates microsatellites (no isolation by distance, r = 0.1873, P = 0.1559) and solid squares indicate AFLPs (significant isolation by distance, r = 0.7378, P = 0.0015). See appendix C for a distance table (geographic and Nei's).

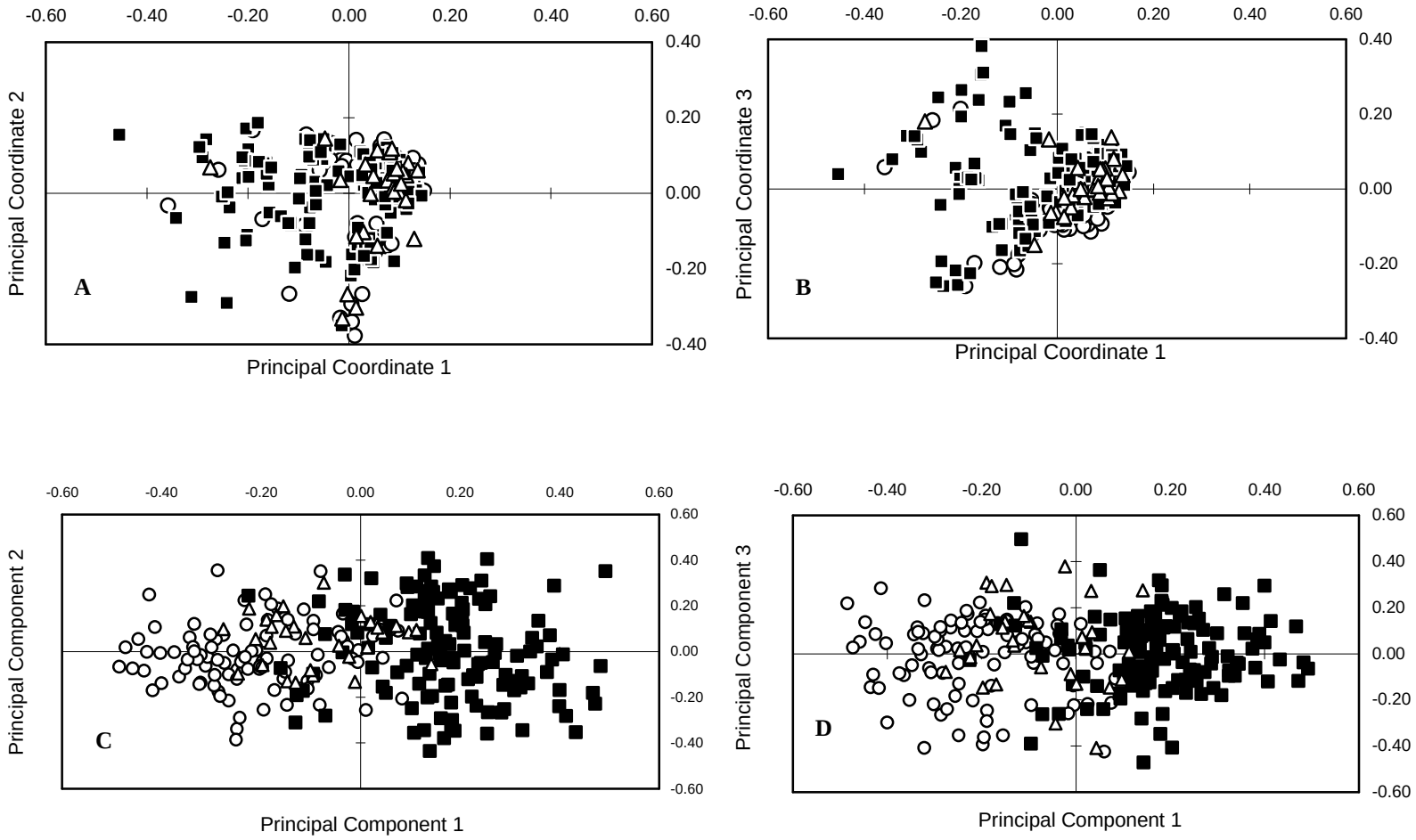

Figure 3.3. Principal component analysis for microsatellite (A,B) and AFLPs (C, D) for *A. gracile* at MSHNVM. New populations are indicated by a filled square, recovery populations by an open circle and reference populations by an open triangle.

CHAPTER 4

CONCLUSIONS

The goal of this study was to measure the population genetic response of two common amphibians, *Taricha granulosa* (rough-skinned newt) and *Ambystoma gracile* (northwestern salamander) to the 1980 eruption of Mount St. Helens. Understanding how measures of genetic diversity, gene flow, and population structure may be affected by a major environmental disturbance is important in improving our knowledge of colonization. Colonization rates and the order that plant and animal species established themselves at Mount St. Helens was more complex that present ecological theory could explain. Instead of following a clear pattern, colonization and recovery was species and situation specific (Franklin *et al.* 1985; Crisafulli *et al.* 2005b). For the purposes of my study, the eruption created a mosaic of landscape alterations that could be assigned into three treatments, which were new, recovery and reference. I predicted that in both species, populations in recently colonized habitats (composed of immigrants only), would have less genetic variation than populations from recovery area (survivors/their descendants and immigrants), or reference populations (assumed to be unimpacted by the eruption). This prediction was based on the supposition that new populations would have been colonized by few individuals and that migration rates were low. The data did not support my predictions. In newts, there were no significant differences in six measures of genetic diversity among treatments (Table 2.6). Results were similar in *A. gracile* (Table 3.6). With microsatellite markers, the observed heterozygosity of the reference lakes was lower than either new or recovering populations and with AFLPs, newly colonized

populations had a higher average heterozygosity than either of the other two treatments (Table 3.6).

The results for both species can be explained through the surprisingly high levels of gene flow, despite the rugged terrain. Gene flow, the movement of genetic material from one area to another, reduces or eliminates genetic differences among populations (Petranka 2007). Those amphibian species with higher vagility may be more adversely affected by habitat fragmentation than those that are site fidelic (Cushman 2006) because they require larger areas of suitable habitat. Additionally, habitat change may be the be the biggest threat to amphibian conservation (Gardner *et al.* 2007). Thus, the rapid recovery and minimal loss of genetic variability in colonizing populations in both these species is good news.

High gene flow can result from large numbers of animals moving across the landscape or through multiple waves of colonization. A review of *T. granulosa* activity patterns (Chapter 2, discussion) show that they are well suited to move across open areas and may in fact prefer moving across them. For both species, burrows created by northern pocket gophers (*Thomomys talpoides)* or other fossorial mammals may have created suitable refuges for salamanders unable to move during hot and dry periods (MacMahon *et al.* 1989). Interestingly, although the gophers were found within the blast zone less than a year post eruption (Andersen & MacMahon 1985), they took 12 years to colonize the debris-avalanche zone (Crisafulli *et al.* 2005a). *Ambystoma gracile* took 9 years and *T. granulosa* took 10 years (Crisafulli *et al.* 2005c). It is possible that salamander migration rates were limited by the dispersal rates of fossorial mammals because it is likely that salamanders used their burrows as refuges. Additionally, female newts mate with

multiple males (2-5) and offspring from a single clutch may have multiple fathers (Jones *et al.* 2002). While no one has tested for multiple paternity in the *A. gracile*, it is found in other species of *Ambystoma* including *A. tigrinum*, *A. maculatum*, and *A. talpoideum* (Tennessen & Zamudio 2003; Myers & Zamudio 2004; Gopurenko *et al.* 2006; Whiteman *et al.* 2006).

Genetic studies of two sympatric species can highlight how differences in life-history strategies affect gene flow and population substructure. Often, there is a key difference between species that the researchers wish to highlight. For example, comparing a specialist or generalist (Brouat *et al.* 2003), differences in dispersal ability (Tibbets & Dowling 1996; Miller *et al.* 2002; Hoehn *et al.* 2007), social structure (Brouat *et al.* 2007) or several life-history variables (Kraaijeveld-Smit *et al.* 2007). Such was the intent with this study. Efford and Mathias' (1969) study of *T. granulosa* and *A. gracile* in the same lake concluded that the two species differ greatly in their population dynamics; *T. granulosa* with low adult recruitment, and long life, and *A. gracile* with a high reproductive and growth rate and a short life span. I expected *T. granulosa* to be less philopatric to their breeding site than *A. gracile* because there was some evidence they were capable of high gene flow. Ambystomatid salamanders are generalized (although this view is being questioned (Trenham 1998; Smith & Green 2005) as highly philopatric and there was little evidence to suspect that *A. gracile* was any different. Additionally, the population of *A. gracile* at Mount St. Helens appears to be characterized by a high level of neoteny. Neotenes are not physiologically capable of moving across the terrestrial landscape because they breathe with gills. Therefore, gene flow in *T. granulosa* should be higher than *A. gracile*. Comparing sites where both animals were collected,

gene flow levels are comparable (Table 2.7, 3.7). Additionally, neither species showed evidence for population structure or isolation by distance (Figures 2.2, 2.3, 3.2, 3.3). Perhaps neoteny is not a response to a harsh terrestrial environment as suggested by Sprules (1974) or there are more terrestrial *A. gracile* moving across the landscape than originally thought. Neotenes and terrestrial forms are capable of interbreeding in many species of *Ambystoma* including *A. gracile* (Knudsen 1960; Semlitsch 1985; Licht & Sever 1991; Whiteman *et al.* 2006). Neoteny should not restrict the reproductive output of the species and gene flow can be maintained by terrestrial adults.

Finally, this study shows that the generalities encompassed in the term "pond-breeding amphibian" does not serve as good predictor variables in predicting a response to an environmental disturbance. The term pond-breeding amphibian implies high site fidelity and low vagility, resulting in low gene flow and genetic differences in subpopulations. Populations of pond breeding amphibians are often automatically classified as existing in a metapopulation type structure, an assumption not always correct or tested for (Marsh & Trenham 2001). *Taricha granulosa* and *A. gracile*, while they do breed in ponds, have shown the opposite pattern. At Mount St. Helens, populations are characterized by high gene flow which results in low population structure. Therefore, researchers should use the term pond-breeding amphibian carefully as the mental image and stereotype the term invokes is not universally applicable.

Literature Cited

Andersen DC, MacMahon JA (1985) The effects of catastrophic ecosystem disturbance: the residual mammals at Mount St. Helens. *Journal of Mammalogy* **66**, 581-589.

Brouat C, Loiseau A, Kane M, Ba K, Duplantier JM (2007) Population genetic structure of two ecologically distinct multimammate rats: the commensal *Mastomys natalensis*

and the wild *Mastomys erythroleucus* in southeastern Senegal. *Molecular Ecology* **16**, 2985-2997.

Brouat C, Sennedot F, Audiot P, Leblois R, Rasplus J-Y (2003) Fine-scale genetic structure of two carabid species with contrasted levels of habitat specialization. *Molecular Ecology* **12**, 1731-1745.

Crisafulli CM, MacMahon JA, Parmenter RR (2005a) Small-mammal survival and colonization on the Mount St. Helens volcano: 1980-2002. In: *Ecological Responses to the 1980 Eruption of Mount St. Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 199-218. Springer, New York.

Crisafulli CM, Swanson FJ, Dale VH (2005b) Overview of the ecological responses to the eruption of Mount St. Helens: 1980-2005. In: *Ecological Responses to the 1980 Eruption of Mount St. Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 287-299. Springer, New York.

Crisafulli CM, Trippe LS, Hawkins CP, MacMahon JA (2005c) Amphibian responses to the 1980 eruption of Mount St. Helens. In: *Ecological Responses to the 1980 Eruption of Mount St. Helens* (eds Dale VH, Swanson FJ, Crisafulli CM), pp. 183-197. Springer, New York.

Cushman SA (2006) Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation* **128**, 231-240.

Efford IE, Mathias JA (1969) A comparison of two salamander populations in Marion Lake, British Columbia. *Copeia* **1969**, 723-736.

Franklin JF, MacMahon JA, Swanson FJ, Sedell JR (1985) Ecosystem responses to the eruption of Mount St. Helens. *National Geographic Research* **1**, 198-216.

Gardner TA, Barlow J, Peres CA (2007) Paradox, presumption and pitfalls in conservation biology: the importance of habitat change for amphibians and reptiles. *Biological Conservation* **138**, 166-179.

Gopurenko D, Williams RN, McCormick CR, DeWoody JA (2006) Insights into the mating habits of the tiger salamander (*Ambystoma tigrinum tigrinum*) as revealed by genetic parentage analyses. *Molecular Ecology* **15**, 1917-1928.

Hoehn M, Sarre SD, Henle K (2007) The tales of two geckos: does dispersal prevent extinction in recently fragmented populations? *Molecular Ecology* **16**, 3299-3312.

Jones AG, Adams EM, Arnold SJ (2002) Topping off: a mechanism of first-male sperm precedence in a vertebrate. *Proceedings of the National Academy of Sciences* **99**, 2078-2081.

Knudsen JW (1960) The courtship and egg mass of *Ambystoma gracile* and *Ambystoma macrodactylum*. *Copeia* **1960**, 44-46.

Kraaijeveld-Smit FJL, Lindenmayer DB, Taylor AC, MacGregor C, Wertheim B (2007) Comparative genetic structure reflects underlying life histories of three sympatric small mammal species in continuous forest of south-eastern Australia. *Oikos* **116**, 1819-1830.

Licht LE, Sever DM (1991) Cloacal anatomy of metamorphosed and neotenic salamanders. *Canadian Journal of Zoology* **69**, 2230-2233.

MacMahon JA, Parmenter RR, Johnson KA, Crisafulli CM (1989) Small mammal recolonization on the Mount St Helens volcano: 1980-1987. *American Midland Naturalist* **122**, 365-387.

Marsh DM, Trenham PC (2001) Metapopulation dynamics and amphibian conservation. *Conservation Biology* **15**, 40-49.

Miller MP, Blinn DW, Keim P (2002) Correlations between observed dispersal capabilities and patterns of genetic differentiation in populations of four aquatic insect species from the Arizona White Mountains, U.S.A. *Freshwater Biology* **47**, 1660-1673.

Myers EM, Zamudio KR (2004) Multiple paternity in an aggregate breeding amphibian: the effect of reproductive skew on estimates of male reproductive success. *Molecular Ecology* **13**, 1951-1963.

Petranka J (2007) Evolution of complex life cycles of amphibians: bridging the gap between metapopulation dynamics and life history evolution. *Evolutionary Ecology* **21**, 751-764.

Semlitsch RD (1985) Reproductive strategy of a facultatively paedomorphic salamander *Ambystoma talpoideum*. *Oecologia* **65**, 305-313.

Smith MA, Green DM (2005) Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography* **28**, 110-128.

Sprules WG (1974) The adaptive significance of paedogenesis in North American species of *Ambystoma* (Amphibia: Caudata): an hypothesis. *Canadian Journal of Zoology* **52**, 393-400.

Tennessen JA, Zamudio KR (2003) Early male reproductive advantage, multiple paternity and sperm storage in an amphibian aggregate breeder. *Molecular Ecology* **12**, 1567-1576.

Tibbets CA, Dowling TE (1996) Effects of intrinsic and extrinsic factors on population fragmentation in three species of North American minnows (Teleostei: Cyprinidae). *Evolution* **50**, 1280-1292.

Trenham PC (1998) *Demography, migration, and metapopulation structure of pond breeding salamanders.* PhD. Dissertation. University of California, Davis.

Whiteman H, Krenz J, Semlitsch R (2006) Intermorph breeding and the potential for reproductive isolation in polymorphic mole salamanders (*Ambystoma talpoideum*). *Behavioral Ecology and Sociobiology* **60**, 52-61.

APPENDICES

APPENDIX A

COLLECTIONS BY LOCALITY AND YEAR

Table A.1. Collections by location and year of *Taricha granulosa* and *Ambystoma gracile* at Mount St. Helens National Volcanic Monument. NS indicates not sampled.

	Hummocks							Maratta								
2003	H1	H2B	H23	H6	H9	H25	H26	M3	M5	M6	M30	M26	M27	M28	Marratta Lake	Beaver Pond
T. granulosa	ns	ns	2	1	ns	ns	ns	ns	1	7	8	1	ns	ns	0	ns
A. gracile	ns	ns	19	42	ns	ns	ns	ns	1	27	21	0	ns	ns	1	ns
2005																
T. granulosa adult	0	0	0	3	0	0	0	0	0	3	0	ns	3	1	ns	0
T. granulosa larvae	0	0	0	0	0	0	0	0	0	0	0	ns	0	0	ns	0
A. granulosa neotene	2	0	2	36	0	9	2	0	2	9	11	ns	4	2	ns	25
A. gracile larvae	0	0	0	0	0	0	0	0	0	0	0	ns	0	0	ns	0

	Blowdown						Reference	
2003	Curtis	St. Charles	Ryan	Meta	Ghost	Strawberry	Goat Marsh	McBride
T. granulosa adult	0	7	0	0	2	ns	46	40
A. gracile	27	33	17	4	3	ns	0	0
2005								
T. granulosa adult	ns	3	0	ns	0	46	29	5
T. granulosa larvae	ns	1	0	ns	0	0	21	7
A. gracile neotene	ns	28	15	ns	1	0	16	12
A. gracile larvae	ns	8	0	ns	0	0	0	2

APPENDIX B

ALLELE FREQUENCY TABLES

Table B.1 Allele frequencies for *Taricha granulosa* and *Ambystoma gracile* from Mount St. Helens National Volcanic Monument. N indicates the number of animals genotyped at a locus. Allele sizes are in base pairs.

Taricha granulosa

Locus	size	Hummocks	Maratta	Strawberry	St Charles	McBride	Goat Marsh
Tgr 01	N	6	26	41	11	43	75
	215	0.000	0.019	0.000	0.000	0.000	0.000
	219	0.167	0.154	0.146	0.000	0.105	0.140
	223	0.000	0.038	0.061	0.182	0.035	0.047
	227	0.000	0.038	0.024	0.000	0.023	0.000
	231	0.000	0.038	0.012	0.000	0.035	0.033
	235	0.083	0.192	0.220	0.136	0.326	0.220
	239	0.083	0.231	0.220	0.091	0.233	0.287
	243	0.083	0.096	0.061	0.273	0.128	0.167
	247	0.167	0.058	0.061	0.091	0.035	0.040
	251	0.083	0.000	0.073	0.091	0.012	0.007
	255	0.333	0.077	0.061	0.045	0.035	0.053
	259	0.000	0.038	0.061	0.091	0.023	0.007
	263	0.000	0.019	0.000	0.000	0.000	0.000
	271	0.000	0.000	0.000	0.000	0.012	0.000
Tgr 02	N	6	26	37	11	40	73
	179	0.167	0.192	0.041	0.000	0.150	0.212
	183	0.000	0.000	0.027	0.000	0.025	0.014
	187	0.083	0.096	0.176	0.227	0.175	0.082
	191	0.083	0.077	0.135	0.045	0.038	0.062
	195	0.167	0.135	0.378	0.273	0.213	0.144
	199	0.000	0.038	0.027	0.000	0.013	0.007
	203	0.000	0.019	0.068	0.227	0.025	0.103
	207	0.167	0.269	0.081	0.136	0.175	0.267
	211	0.250	0.154	0.054	0.091	0.100	0.062
	215	0.000	0.000	0.000	0.000	0.000	0.007
	235	0.083	0.019	0.000	0.000	0.025	0.014
	239	0.000	0.000	0.000	0.000	0.013	0.000
	243	0.000	0.000	0.000	0.000	0.025	0.000
	245	0.000	0.000	0.014	0.000	0.000	0.000
	247	0.000	0.000	0.000	0.000	0.013	0.021
	255	0.000	0.000	0.000	0.000	0.013	0.007

Tgr 06	N	6	25	41	11	41	76
	145	0.000	0.000	0.037	0.045	0.073	0.013
	149	0.083	0.200	0.207	0.045	0.134	0.132
	153	0.083	0.080	0.061	0.045	0.134	0.145
	157	0.417	0.380	0.305	0.409	0.293	0.309
	159	0.000	0.000	0.000	0.000	0.012	0.000
	161	0.333	0.160	0.171	0.091	0.195	0.250
	163	0.000	0.000	0.037	0.000	0.000	0.000
	165	0.083	0.080	0.110	0.182	0.098	0.053
	167	0.000	0.020	0.037	0.000	0.000	0.046
	169	0.000	0.040	0.024	0.182	0.049	0.033
	171	0.000	0.000	0.012	0.000	0.000	0.000
	173	0.000	0.040	0.000	0.000	0.012	0.020

Tgr 10	N	6	26	41	11	43	75
	174	0.083	0.038	0.110	0.045	0.116	0.093
	178	0.083	0.096	0.110	0.136	0.035	0.020
	182	0.000	0.019	0.000	0.000	0.035	0.013
	186	0.250	0.173	0.122	0.182	0.267	0.213
	190	0.250	0.269	0.232	0.091	0.198	0.233
	194	0.000	0.058	0.037	0.136	0.047	0.060
	198	0.083	0.135	0.024	0.227	0.070	0.080
	202	0.167	0.154	0.268	0.136	0.151	0.167
	206	0.083	0.038	0.098	0.045	0.035	0.100
	210	0.000	0.019	0.000	0.000	0.035	0.013
	214	0.000	0.000	0.000	0.000	0.012	0.007

Tgr 14	N	6	26	41	11	41	74
	218	0.000	0.000	0.000	0.000	0.000	0.007
	222	0.083	0.077	0.061	0.000	0.000	0.027
	226	0.000	0.000	0.000	0.000	0.037	0.027
	230	0.000	0.000	0.000	0.000	0.000	0.020
	234	0.000	0.058	0.024	0.000	0.110	0.074
	238	0.083	0.019	0.085	0.045	0.061	0.068
	242	0.167	0.038	0.037	0.136	0.134	0.068
	246	0.000	0.038	0.085	0.136	0.073	0.108
	248	0.000	0.000	0.000	0.000	0.012	0.000
	250	0.000	0.096	0.049	0.045	0.024	0.047
	254	0.083	0.077	0.061	0.045	0.024	0.041
	258	0.083	0.058	0.012	0.000	0.037	0.041
	262	0.167	0.115	0.073	0.318	0.073	0.034
	266	0.083	0.058	0.024	0.000	0.012	0.027
	270	0.083	0.096	0.012	0.000	0.037	0.007

274	0.000	0.019	0.000	0.000	0.000	0.020
278	0.000	0.019	0.000	0.000	0.000	0.000
282	0.000	0.000	0.000	0.000	0.000	0.054
286	0.000	0.019	0.024	0.000	0.024	0.020
290	0.000	0.019	0.098	0.091	0.073	0.027
294	0.167	0.000	0.122	0.045	0.061	0.068
298	0.000	0.096	0.085	0.045	0.037	0.088
302	0.000	0.038	0.012	0.000	0.037	0.034
306	0.000	0.000	0.000	0.000	0.012	0.000
308	0.000	0.019	0.061	0.000	0.037	0.027
312	0.000	0.000	0.000	0.045	0.024	0.041
314	0.000	0.000	0.037	0.000	0.000	0.000
316	0.000	0.019	0.024	0.045	0.049	0.007
318	0.000	0.019	0.000	0.000	0.000	0.000
320	0.000	0.000	0.012	0.000	0.000	0.020
328	0.000	0.000	0.000	0.000	0.012	0.000

Ambystoma gracile

Locus	Size	Hummocks	Maratta	Curtis	Ryan	St. Charles	Goat Marsh	McBride
Agr 23	N	68	47	23	25	44	14	16
	206	0.000	0.000	0.000	0.000	0.000	0.000	0.031
	214	0.022	0.000	0.000	0.020	0.000	0.000	0.000
	218	0.000	0.000	0.065	0.040	0.080	0.036	0.000
	222	0.000	0.011	0.022	0.140	0.023	0.071	0.000
	226	0.154	0.149	0.043	0.060	0.102	0.179	0.000
	230	0.029	0.128	0.130	0.080	0.114	0.036	0.094
	234	0.059	0.096	0.087	0.080	0.102	0.071	0.219
	238	0.162	0.043	0.130	0.060	0.182	0.036	0.156
	242	0.074	0.149	0.087	0.060	0.114	0.214	0.125
	246	0.191	0.128	0.196	0.160	0.148	0.214	0.125
	248	0.000	0.000	0.000	0.040	0.000	0.000	0.000
	250	0.022	0.106	0.065	0.040	0.045	0.107	0.094
	254	0.140	0.106	0.043	0.060	0.023	0.000	0.031
	256	0.000	0.000	0.000	0.020	0.000	0.000	0.063
	258	0.096	0.021	0.087	0.080	0.034	0.036	0.063
	262	0.022	0.043	0.043	0.020	0.034	0.000	0.000
	266	0.000	0.011	0.000	0.020	0.000	0.000	0.000
	270	0.022	0.011	0.000	0.020	0.000	0.000	0.000
	280	0.007	0.000	0.000	0.000	0.000	0.000	0.000
Agr 49	N	61	48	13	17	40	10	15
	151	0.000	0.021	0.038	0.000	0.013	0.050	0.067
	155	0.016	0.021	0.000	0.000	0.000	0.050	0.000

159	0.082	0.063	0.077	0.059	0.013	0.050	0.000
163	0.098	0.125	0.000	0.088	0.100	0.000	0.133
166	0.041	0.042	0.077	0.000	0.038	0.050	0.067
167	0.041	0.052	0.077	0.088	0.100	0.150	0.033
171	0.115	0.073	0.077	0.059	0.063	0.050	0.067
175	0.107	0.042	0.038	0.088	0.063	0.000	0.033
179	0.016	0.063	0.038	0.059	0.025	0.000	0.133
183	0.033	0.083	0.000	0.029	0.063	0.150	0.067
187	0.000	0.021	0.038	0.029	0.000	0.000	0.000
191	0.016	0.010	0.038	0.147	0.088	0.150	0.067
192	0.000	0.000	0.038	0.000	0.063	0.000	0.000
193	0.008	0.000	0.000	0.029	0.013	0.000	0.000
195	0.107	0.021	0.077	0.029	0.013	0.050	0.033
196	0.008	0.000	0.000	0.000	0.038	0.000	0.000
199	0.008	0.010	0.000	0.000	0.050	0.050	0.000
200	0.057	0.031	0.038	0.000	0.038	0.000	0.000
203	0.008	0.000	0.038	0.000	0.000	0.050	0.167
204	0.000	0.000	0.000	0.000	0.013	0.000	0.000
207	0.008	0.021	0.077	0.029	0.013	0.000	0.000
208	0.000	0.031	0.000	0.029	0.050	0.000	0.000
210	0.000	0.010	0.000	0.000	0.000	0.000	0.000
211	0.008	0.010	0.000	0.029	0.000	0.000	0.133
212	0.033	0.010	0.000	0.000	0.025	0.050	0.000
215	0.000	0.021	0.000	0.029	0.000	0.000	0.000
216	0.008	0.000	0.077	0.000	0.025	0.000	0.000
217	0.000	0.000	0.000	0.000	0.013	0.000	0.000
218	0.000	0.000	0.000	0.000	0.000	0.100	0.000
219	0.049	0.063	0.000	0.059	0.000	0.000	0.000
220	0.016	0.073	0.000	0.088	0.025	0.000	0.000
223	0.057	0.031	0.038	0.000	0.000	0.000	0.000
224	0.000	0.000	0.038	0.000	0.000	0.000	0.000
228	0.049	0.031	0.000	0.000	0.013	0.000	0.000
232	0.000	0.000	0.000	0.029	0.013	0.000	0.000
235	0.008	0.000	0.038	0.000	0.000	0.000	0.000
237	0.000	0.000	0.000	0.000	0.013	0.000	0.000
243	0.000	0.000	0.038	0.000	0.000	0.000	0.000
258	0.000	0.000	0.000	0.000	0.025	0.000	0.000
280	0.000	0.021	0.000	0.000	0.000	0.000	0.000

Agr 314	N	67	36	22	25	42	11	16
	164	0.000	0.000	0.023	0.000	0.012	0.000	0.000
	168	0.000	0.014	0.023	0.000	0.000	0.000	0.000
	172	0.000	0.014	0.000	0.000	0.000	0.045	0.094
	176	0.007	0.000	0.045	0.000	0.000	0.000	0.000

180	0.022	0.014	0.000	0.020	0.000	0.045	0.000
184	0.000	0.000	0.000	0.000	0.000	0.000	0.156
188	0.000	0.000	0.023	0.000	0.024	0.136	0.000
190	0.000	0.000	0.023	0.000	0.000	0.000	0.000
192	0.022	0.028	0.000	0.000	0.000	0.000	0.000
195	0.000	0.000	0.000	0.000	0.000	0.000	0.031
196	0.045	0.028	0.000	0.000	0.000	0.000	0.000
200	0.082	0.028	0.045	0.000	0.036	0.000	0.000
202	0.000	0.000	0.045	0.060	0.000	0.000	0.000
204	0.007	0.000	0.000	0.000	0.000	0.000	0.000
206	0.000	0.069	0.000	0.000	0.024	0.000	0.000
208	0.112	0.000	0.023	0.000	0.000	0.045	0.000
212	0.276	0.097	0.000	0.000	0.071	0.045	0.063
214	0.000	0.028	0.023	0.000	0.024	0.000	0.031
216	0.007	0.028	0.000	0.000	0.000	0.091	0.031
217	0.045	0.014	0.000	0.000	0.000	0.000	0.000
220	0.022	0.000	0.023	0.020	0.000	0.045	0.094
221	0.007	0.014	0.091	0.000	0.048	0.000	0.000
222	0.007	0.014	0.000	0.000	0.000	0.045	0.000
224	0.000	0.000	0.000	0.000	0.000	0.000	0.125
225	0.030	0.014	0.000	0.020	0.012	0.000	0.031
226	0.000	0.000	0.000	0.020	0.000	0.000	0.000
228	0.007	0.000	0.068	0.040	0.060	0.000	0.000
229	0.007	0.014	0.023	0.020	0.012	0.045	0.000
230	0.119	0.000	0.000	0.040	0.024	0.000	0.000
232	0.022	0.056	0.045	0.020	0.167	0.045	0.000
233	0.007	0.000	0.091	0.040	0.060	0.000	0.000
234	0.000	0.000	0.023	0.060	0.000	0.000	0.000
236	0.000	0.000	0.000	0.040	0.012	0.136	0.063
237	0.000	0.042	0.045	0.040	0.000	0.045	0.000
238	0.015	0.083	0.000	0.000	0.107	0.000	0.000
240	0.000	0.000	0.045	0.000	0.024	0.091	0.000
241	0.015	0.028	0.000	0.000	0.000	0.000	0.000
242	0.000	0.000	0.000	0.020	0.000	0.000	0.000
244	0.000	0.000	0.000	0.080	0.048	0.000	0.031
245	0.007	0.097	0.000	0.100	0.000	0.000	0.094
246	0.000	0.069	0.000	0.000	0.000	0.000	0.000
248	0.000	0.014	0.023	0.000	0.000	0.000	0.031
249	0.015	0.000	0.000	0.000	0.000	0.000	0.000
250	0.015	0.000	0.000	0.020	0.000	0.000	0.000
252	0.000	0.000	0.023	0.000	0.024	0.000	0.000
253	0.000	0.000	0.000	0.020	0.000	0.000	0.094
255	0.000	0.056	0.000	0.000	0.000	0.000	0.000
256	0.000	0.000	0.000	0.020	0.000	0.000	0.031

257	0.007	0.000	0.000	0.020	0.000	0.000	0.000
258	0.015	0.000	0.000	0.000	0.000	0.000	0.000
260	0.000	0.000	0.000	0.020	0.012	0.000	0.000
262	0.000	0.000	0.000	0.000	0.000	0.136	0.000
264	0.000	0.000	0.023	0.000	0.000	0.000	0.000
265	0.000	0.028	0.000	0.000	0.000	0.000	0.000
267	0.000	0.000	0.000	0.000	0.012	0.000	0.000
268	0.000	0.000	0.114	0.060	0.048	0.000	0.000
270	0.015	0.042	0.000	0.020	0.000	0.000	0.000
272	0.007	0.000	0.023	0.040	0.048	0.000	0.000
274	0.030	0.000	0.000	0.000	0.000	0.000	0.000
276	0.000	0.042	0.023	0.040	0.024	0.000	0.000
278	0.000	0.000	0.000	0.000	0.012	0.000	0.000
280	0.000	0.000	0.000	0.020	0.000	0.000	0.000
281	0.000	0.000	0.000	0.020	0.024	0.000	0.000
285	0.000	0.014	0.045	0.060	0.036	0.000	0.000
300	0.000	0.014	0.000	0.000	0.000	0.000	0.000

Agr 226	N	69	43	24	25	43	14	16
	240	0.000	0.000	0.000	0.000	0.047	0.036	0.000
	252	0.029	0.012	0.021	0.000	0.047	0.036	0.000
	256	0.000	0.023	0.042	0.000	0.000	0.000	0.000
	260	0.014	0.000	0.063	0.040	0.012	0.000	0.000
	264	0.080	0.093	0.021	0.040	0.058	0.071	0.031
	268	0.029	0.151	0.000	0.060	0.035	0.036	0.063
	272	0.022	0.058	0.042	0.020	0.047	0.000	0.000
	276	0.283	0.163	0.146	0.020	0.163	0.036	0.063
	280	0.036	0.151	0.146	0.080	0.058	0.143	0.156
	284	0.101	0.035	0.146	0.080	0.093	0.179	0.219
	288	0.203	0.093	0.042	0.060	0.058	0.143	0.094
	292	0.000	0.070	0.000	0.040	0.047	0.071	0.031
	296	0.014	0.035	0.021	0.060	0.047	0.036	0.125
	300	0.036	0.023	0.021	0.060	0.012	0.000	0.031
	305	0.007	0.000	0.021	0.000	0.012	0.036	0.000
	309	0.000	0.000	0.042	0.020	0.081	0.071	0.094
	313	0.000	0.000	0.000	0.040	0.012	0.036	0.000
	317	0.000	0.000	0.021	0.020	0.000	0.000	0.031
	326	0.000	0.000	0.000	0.000	0.000	0.000	0.031
	330	0.007	0.000	0.000	0.020	0.000	0.000	0.000
	332	0.007	0.000	0.000	0.000	0.000	0.000	0.000
	334	0.109	0.000	0.042	0.000	0.000	0.036	0.031
	338	0.007	0.012	0.063	0.080	0.023	0.000	0.000
	342	0.000	0.000	0.000	0.060	0.000	0.000	0.000
	346	0.014	0.000	0.042	0.060	0.058	0.000	0.000

350	0.000	0.000	0.021	0.120	0.058	0.036	0.000
354	0.000	0.070	0.000	0.000	0.000	0.000	0.000
358	0.000	0.012	0.000	0.020	0.023	0.000	0.000
362	0.000	0.000	0.021	0.000	0.000	0.000	0.000
370	0.000	0.000	0.021	0.000	0.012	0.000	0.000

Agr 314	N	67	36	22	25	42	11	16
	164	0.000	0.000	0.023	0.000	0.012	0.000	0.000
	168	0.000	0.014	0.023	0.000	0.000	0.000	0.000
	172	0.000	0.014	0.000	0.000	0.000	0.045	0.094
	176	0.007	0.000	0.045	0.000	0.000	0.000	0.000
	180	0.022	0.014	0.000	0.020	0.000	0.045	0.000
	184	0.000	0.000	0.000	0.000	0.000	0.000	0.156
	188	0.000	0.000	0.023	0.000	0.024	0.136	0.000
	190	0.000	0.000	0.023	0.000	0.000	0.000	0.000
	192	0.022	0.028	0.000	0.000	0.000	0.000	0.000
	195	0.000	0.000	0.000	0.000	0.000	0.000	0.031
	196	0.045	0.028	0.000	0.000	0.000	0.000	0.000
	200	0.082	0.028	0.045	0.000	0.036	0.000	0.000
	202	0.000	0.000	0.045	0.060	0.000	0.000	0.000
	204	0.007	0.000	0.000	0.000	0.000	0.000	0.000
	206	0.000	0.069	0.000	0.000	0.024	0.000	0.000
	208	0.112	0.000	0.023	0.000	0.000	0.045	0.000
	212	0.276	0.097	0.000	0.000	0.071	0.045	0.063
	214	0.000	0.028	0.023	0.000	0.024	0.000	0.031
	216	0.007	0.028	0.000	0.000	0.000	0.091	0.031
	217	0.045	0.014	0.000	0.000	0.000	0.000	0.000
	220	0.022	0.000	0.023	0.020	0.000	0.045	0.094
	221	0.007	0.014	0.091	0.000	0.048	0.000	0.000
	222	0.007	0.014	0.000	0.000	0.000	0.045	0.000
	224	0.000	0.000	0.000	0.000	0.000	0.000	0.125
	225	0.030	0.014	0.000	0.020	0.012	0.000	0.031
	226	0.000	0.000	0.000	0.020	0.000	0.000	0.000
	228	0.007	0.000	0.068	0.040	0.060	0.000	0.000
	229	0.007	0.014	0.023	0.020	0.012	0.045	0.000
	230	0.119	0.000	0.000	0.040	0.024	0.000	0.000
	232	0.022	0.056	0.045	0.020	0.167	0.045	0.000
	233	0.007	0.000	0.091	0.040	0.060	0.000	0.000
	234	0.000	0.000	0.023	0.060	0.000	0.000	0.000
	236	0.000	0.000	0.000	0.040	0.012	0.136	0.063
	237	0.000	0.042	0.045	0.040	0.000	0.045	0.000
	238	0.015	0.083	0.000	0.000	0.107	0.000	0.000
	240	0.000	0.000	0.045	0.000	0.024	0.091	0.000
	241	0.015	0.028	0.000	0.000	0.000	0.000	0.000

242	0.000	0.000	0.000	0.020	0.000	0.000	0.000
244	0.000	0.000	0.000	0.080	0.048	0.000	0.031
245	0.007	0.097	0.000	0.100	0.000	0.000	0.094
246	0.000	0.069	0.000	0.000	0.000	0.000	0.000
248	0.000	0.014	0.023	0.000	0.000	0.000	0.031
249	0.015	0.000	0.000	0.000	0.000	0.000	0.000
250	0.015	0.000	0.000	0.020	0.000	0.000	0.000
252	0.000	0.000	0.023	0.000	0.024	0.000	0.000
253	0.000	0.000	0.000	0.020	0.000	0.000	0.094
255	0.000	0.056	0.000	0.000	0.000	0.000	0.000
256	0.000	0.000	0.000	0.020	0.000	0.000	0.031
257	0.007	0.000	0.000	0.020	0.000	0.000	0.000
258	0.015	0.000	0.000	0.000	0.000	0.000	0.000
260	0.000	0.000	0.000	0.020	0.012	0.000	0.000
262	0.000	0.000	0.000	0.000	0.000	0.136	0.000
264	0.000	0.000	0.023	0.000	0.000	0.000	0.000
265	0.000	0.028	0.000	0.000	0.000	0.000	0.000
267	0.000	0.000	0.000	0.000	0.012	0.000	0.000
268	0.000	0.000	0.114	0.060	0.048	0.000	0.000
270	0.015	0.042	0.000	0.020	0.000	0.000	0.000
272	0.007	0.000	0.023	0.040	0.048	0.000	0.000
274	0.030	0.000	0.000	0.000	0.000	0.000	0.000
276	0.000	0.042	0.023	0.040	0.024	0.000	0.000
278	0.000	0.000	0.000	0.000	0.012	0.000	0.000
280	0.000	0.000	0.000	0.020	0.000	0.000	0.000
281	0.000	0.000	0.000	0.020	0.024	0.000	0.000
285	0.000	0.014	0.045	0.060	0.036	0.000	0.000
300	0.000	0.014	0.000	0.000	0.000	0.000	0.000

APPENDIX C

DISTANCE MATRIX

Table C.1. Matrix of Nei's unbiased genetic distances and topographic distances between collection sites at Mount St. Helens National Volcanic Monument. Distances (km) are above the diagonal, Nei's unbiased genetic distances are below the diagonal. Top number is Nei's genetic distance using AFLPs for *Ambystoma gracile*, middle number is Nei's genetic distance using microsatellites for *A. gracile*, and bottom number is Nei's genetic distance using microsatellites for *Taricha granulosa*. N/A indicates not applicable. Abbreviations for collection sites are as in Table 3.2.

	HU	MA	CU	RY	SC	SB	GM	MB
HU		1.60	17.37	18.61	14.69	18.13	13.90	16.19
MA	0.013 0.280 0.000		18.54	18.86	15.77	18.92	15.24	17.42
CU	0.030 0.291 n/a	0.025 0.254 n/a		8.50	2.98	n/a	23.66	23.41
RY	0.028 0.507 n/a	0.025 0.232 n/a	0.016 0.056 n/a		7.55	n/a	28.36	28.94
SC	0.038 0.312 0.048	0.032 0.196 0.150	0.007 0.016 n/a	0.007 0.142 n/a		4.96	21.65	22.23
SB	n/a n/a 0.005	n/a n/a 0.084	n/a	n/a	n/a n/a 0.136		26.56	27.46
GM	0.027 0.430 0.000	0.018 0.240 0.002	0.024 0.130	0.031 0.157	0.027 0.180 0.213	n/a n/a 0.116		2.97
MB	0.045 0.543 0.000	0.031 0.326 0.017	0.034 0.210	0.044 0.245	0.035 0.331 0.131	n/a n/a 0.064	0.014 0.189 0.012	

APPENDIX D

ALL *AMBYSTOMA* PRIMERS

To date, there are no published microsatellite primers for *A. gracile*. I tried 65 sets of primers from five other species of *Ambystoma*: *A. maculatum* (Wieczorek *et al.* 2002; Julian *et al.* 2003a) sister species to *A. gracile*, *A. jeffersonianum* (Julian *et al.* 2003b), *A. tigrinum* (Mech *et al.* 2003), *A. macrodactylum* (Shields & Liss 2003) and *A. texanum* (Williams & Dewoody 2004; Table 1). Of these, only four amplified sufficiently and cleanly enough to use: AmaD49, AmaD226, AjeD23, and AjeD314. Details on their use are in chapter 3 of this dissertation. Other primers amplified but either showed no variation (all animals the same) or too many peaks (e.g. > 2). The genome of *Ambystoma* is large, ranging from a C- value of 21.85 pg in *A. mexicanum* to 80.7 pg in *A. laterale* (Gregory 2006). The C-value of *A. gracile* is 42.0 pg (as reference, the human C-value = 3.5 pg; Licht & Lowcock 1991). This high level of genomic complexity likely contributed to the failure of many of the primers to amplify across species or caused amplification of more than two bands.

Conditions for amplification of DNA via PCR generally follow those in chapter 3. If a primer looked promising, I attempted optimization by varying annealing temperatures and/or concentration of $MgCl_2$. Some primers were labeled with a fluorophore (HEX, 6-FAM, NED, VIC, or PET). For those, suitability for use was determined by testing a minimum of 6 samples, and a positive control (except for *A. texanum* and *A. jeffersonianum*) then examining PCR products on a 1.7% or 2% agarose gel with TBE. No two samples were from the same collection site to increase the probability of detecting variation. To visualize the PCR product, I stained the gel with either ethidium bromide or SYBR Safe™ DNA gel stain (Invitrogen, Eugene, OR). This is indicated in the table as “gel”. Promising PCR products, as indicated on the gel by one or two bands

within 150–450 base pair range) were sent to either the Nevada Genomics Center, Reno, NV or the Center for Integrated BioSystems at Utah State University, Logan, UT for separation on an ABI PRISM 3730 or ABI PRISM 3100. I used GENEMAPPER 3.0 (Applied Biosystems Inc.) to score each sample and round allele sizes to the appropriate whole number. This is indicated in the table as "ABI". For those primers that were not labeled, I tested a minimum of 6 samples, plus a positive control, and ran the PCR product out on a 1.7% or 2% agarose gel with TBE and an appropriate sized DNA ladder to approximate the band size. I also sent 8 samples of *A. gracile* DNA to Mr. Ken Jones at Genetic Identification Services (9552 Topanga Canyon Blvd. Chatsworth, CA 91311) where the *A. macrodactylum* primers were developed. None of the samples amplified. Notes in the observation column of table 1 apply to *A. gracile* samples, not the control. Any numbers relating to peaks is the fragment size in base pairs. Micropeaks refer to those peaks that look like signal but are so small that they could not be distinguished from noise.

Literature Cited

Gregory TR (2006) Animal genome size database. *http://www.genomesize.com.*

Julian SE, King TL, Savage WK (2003a) Isolation and characterization of novel tetranucleotide microsatellite DNA markers for the spotted salamander, *Ambystoma maculatum*. *Molecular Ecology Notes* **3**, 7-9.

Julian SE, King TL, Savage WK (2003b) Novel Jefferson salamander, *Ambystoma jeffersonianum*, microsatellite DNA markers detect population structure and hybrid complexes. *Molecular Ecology Notes* **3**, 95-97.

Licht LE, Lowcock LA (1991) Genome size and metabolic rate in salamanders. *Comparative Biochemistry and Physiology* **100B**, 83-92.

Mech SG, Storfer A, Ernst JA, Reudink MW, Maloney SC (2003) Polymorphic microsatellite loci for tiger salamanders, *Ambystoma tigrinum*. *Molecular Ecology Notes* **3**, 79-81.

Shields BA, Liss WJ (2003) Genetic diversity of long-toed salamanders (*Ambystoma macrodactylum*) in high-elevation lakes. *Unpublished report to Seattle City Light* **54 pp.**

Wieczorek AM, Zamudio KR, King TL, Gjetvaj B (2002) Isolation of microsatellite loci in spotted salamanders (*Ambystoma maculatum*). *Molecular Ecology Notes* **2**, 313-315.

Williams RN, Dewoody JA (2004) Fluorescent dUTP helps characterize 10 novel tetranucleotide microsatellites from an enriched salamander (*Ambystoma texanum*) genomic library. *Molecular Ecology Notes* **4**, 17-19.

Table D.1. *Ambystoma* microsatellite primers in *A. gracile*. Abbreviations used in this table are bp (base pairs), and amp (amplification).

Primer	Ref	Developed for	Testing method	Observations
AjeD03	1	jeffersonianum	ABI	all samples > 3 peaks, all small and messy
AjeD108	1	jeffersonianum	ABI	No amplification
AjeD13	1	jeffersonianum	ABI	No amplification
AjeD162	1	jeffersonianum	ABI	Poor amplification; multiple and micropeaks most samples.
AjeD212	1	jeffersonianum	ABI	Most had a 284 bp and 383 bp peak, no variation between samples
AjeD23	1	jeffersonianum	ABI	USED IN THIS STUDY / cleanest and best of the bunch
AjeD280	1	jeffersonianum	ABI	Good amplification but 60 of 78 animals homozygotes, others 2-4 peaks / Tested 1.5mM and 2.0mM MgCl2
AjeD283	1	jeffersonianum	ABI	Messy; multiple peaks all animals
AjeD294	1	jeffersonianum	ABI	> 2 peaks, all in noise range, all samples
AjeD314	1	jeffersonianum	ABI	USED IN THIS STUDY
AjeD326	1	jeffersonianum	ABI	> 2 peaks, all samples; overall messy
AjeD346	1	jeffersonianum	ABI	Ugly clumps of peaks, messy / retested: gel only, single band @ 250 bp all samples, no variation
AjeD37	1	jeffersonianum	ABI	No amplification
AjeD378	1	jeffersonianum	ABI	Some amplification, messy, spectra looked like ECG / retested, gel only, PD and 2-3 bands > 500 bp
AjeD422	1	jeffersonianum	ABI	No to poor amplification; messy
AjeD448	1	jeffersonianum	ABI	Most samples 3 peaks, but good amplification
AjeD46	1	jeffersonianum	ABI	No amplification
AjeD75	1	jeffersonianum	ABI	One large peak at 111 bp all samples, 0 to 3 additional peaks in all samples / retested, gel only, 1 band > 500 bp; no variation
AjeD84	1	jeffersonianum	ABI	Good amplification, tested 31 animals / attempted temp and MgCl2 optimization / most samples had odd set of 279 & 354 bp peaks in addition to others
AjeD94	1	jeffersonianum	ABI	Ok amplification but abnormally high amount of variation: 28 animals had 24 alleles
F104	2	macrodactylum	GEL	No amplification / primer-dimer only
F11	2	macrodactylum	GEL	No amplification / primer-dimer only
F12b	2	macrodactylum	GEL	No amplification
F136	2	macrodactylum	ABI	All samples, 1 peak @ 92 bp
F142	2	macrodactylum	GEL	No amplification
H120	2	macrodactylum	GEL	Primer-dimer only
H123b	2	macrodactylum	GEL	No amplification
H129	2	macrodactylum	GEL	No amplification

H136	2	macrodactylum	GEL	No amplification
H18	2	macrodactylum	ABI	Good amplification with 2-3 peaks/animal; no variation
H20	2	macrodactylum	GEL	No amplification
H29	2	macrodactylum	GEL	No amplification
Ama5-1	3	maculatum	GEL	Poor amplification; smear from 200-300 bp
Ama07	3	maculatum	ABI	No variation ; most with peak @ 160 bp
Ama11-2B	3	maculatum	GEL	Smear and primer-dimer
Ama12-7	3	maculatum	ABI	Messy; all with 3 - 6 peaks
Ama3-3	3	maculatum	ABI	Each animal with peaks @ 147,155,316, 321 bp ; no variation
Ama34	3	maculatum	GEL	No variation, 1 band approx 100bp all animals
Ama4-10	3	maculatum	ABI	Every animal with peaks at 157,188, 277 bp; no variation
Ama61	3	maculatum	ABI	Multiple peaks/sample
AmaC151	4	maculatum	ABI	All micropeaks , poor amplification, >3 peaks/sample
AmaC40	4	maculatum	ABI	2 peaks/sample; no variation / retested; gel only, single band @ 150 bp; no variation
AmaD184	4	maculatum	ABI	Multiple peaks/sample, noisy, some with large peak at 280
AmaD203	4	maculatum	ABI	Poor amplification; micropeaks
AmaD226	4	maculatum	ABI	USED IN THIS STUDY
AmaD287	4	maculatum	ABI	No amplification
AmaD315	4	maculatum	ABI	No amplification
AmaD321	4	maculatum	ABI	Only 1 peak in all samples / no variation
AmaD328	4	maculatum	ABI	2-3 peaks/sample; unsuccessfully tried to optimize with temperature and MgCl2 gradients, resulted in mostly primer-dimer
AmaD367	4	maculatum	ABI	No amplification
AmaD42	4	maculatum	ABI	Good amplification but no bi, tri, tetra pattern discernable after 303 animals (alleles range from 168-424 bp, 68 total alleles) / attempted temperature and MgCl2 gradients
AmaD450	4	maculatum	ABI	Amplification in only 1 sample which was messy
AmaD49	4	maculatum	ABI	USED IN THIS STUDY
AmaD95	4	maculatum	ABI	No variation and poor amplification
AmaD99	4	maculatum	ABI	Amplification, messy
Atex65	5	texanum	ABI	Some amplification; messy, some samples have 2-5 peaks; 1 sample with 3 nice peaks
ATS10-7	6	tigrinum	GEL	No amplification
ATS12-3	6	tigrinum	GEL	No amplification
ATS14-3	6	tigrinum	GEL	All samples smeared
ATS4-11	6	tigrinum	GEL	Primer-dimer only
ATS4-20	6	tigrinum	GEL	Bad PCR? or no amplification
ATS4-25	6	tigrinum	ABI	All with 3 peaks @ 232, 241, 253 bp ; no variation
ATS5-6	6	tigrinum	GEL	Poor amplification

ATS5-7	6	tigrinum	ABI	Good amplification but no variation, all with peak @ 228 bp
ATS5-8	6	tigrinum	GEL	No amplification / primer-dimer only

Reference	
1	Julian *et al.* 2003b
2	Shields and Liss 2003
3	Wieczorek *et al.* 2002
4	Julian *et al.* 2003a
5	Williams and DeWoody 2004
6	Mech *et al.* 2003

CURRICULUM VITAE

KRISTIN A. BAKKEGARD

Department of Biology
Utah State University
Logan, UT 84322–5305
kbakkegard@biology.usu.edu

EDUCATION

Ph.D.	Biology, Utah State University, Logan, May 2008. Advisor: Dr. Edmund D. Brodie, Jr.
M.S.	Zoology (ecology minor), Auburn University, Auburn, Aug 2001. Advisor: Dr. Craig Guyer
M.A.	Chemistry, Boston University, Boston, Sept 1995. Advisor: Dr. John K. Snyder
B.S.	Chemistry, United States Naval Academy, Annapolis, May 1988. Advisor: Dr. Jeffery Fitzgerald

TEACHING

- Graduate Instructor, Animal Physiology (BIOL 5600); 3 hr lecture, Utah State University, SP 2008.
- Graduate Instructor, Comparative Animal Physiology Laboratory (BIOL 5610), 2 sections, stand-alone lab, supervise 1 GTA, Utah State University, FA 2007.
- Graduate Teaching Assistant, Animal Physiology (BIOL 5600), SP 2007.
- Graduate Instructor, Comparative Animal Physiology Laboratory (BIOL 5610), 2 sections, stand-alone lab, supervise 1 GTA, Utah State University, FA 2006.
- Graduate Teaching Assistant, Comparative Animal Physiology Lab, Utah State University. FA 2005-SP 2006.
- Graduate Teaching Assistant, General Physiology (3 qtrs), Herpetology (1 qtr, 1 sem), and Vertebrate Biodiversity (1 qtr, 1 sem), Auburn University, 1998-2001.
- Tutor, General Chemistry, Auburn University Athletic Department, 1998-2000.
- Assistant Professor of Naval Science, Boston University, Boston, MA. Introduction to Naval Science (1 sem), Principles of Naval Weapon Systems (1 sem) and Surface Ship Operations (2 sem). Academic advisor for freshman and sophomore midshipmen, 1993-1995.

RESEARCH

- Utah State University dissertation: The genetics of colonization of two amphibian species after the 1980 eruption of Mount St. Helens. Study Site: Mount St. Helens, Washington State, 2001-present.
- Auburn University thesis: Activity, foraging, and body size of the Red Hills salamander (*Phaeognathus hubrichti*), 1998-2001.
- Research Assistant, Auburn University Natural History Museum, 2000.

- Boston University: Synthesized various canthinones, 1993-1995.
- U.S. Naval Academy, senior project: Synthesis of soluble tetraazaporphyrins, 1988.

PUBLICATIONS

Peer reviewed

Reed, R.N., **Bakkegard, K. A**., Desy, G. E., Plentovich, S. M. 2007. Diet composition of the invasive cane toad (*Chaunus marinus*) on Rota, Northern Mariana Islands. Pacific Conservation Biology 13: 219-222.

Bakkegard, K. A. 2007. Interactions between the Red Hills Salamander and its potential invertebrate prey. Journal of the Alabama Academy of Science 78: 1-12.

Bakkegard, K. A. 2005. Antipredator behaviors of the Red Hills Salamander, *Phaeognathus hubrichti*. Southeastern Naturalist 4: 23-32.

Bakkegard, K. A. and C. Guyer. 2004. Sexual size dimorphism in the Red Hills Salamander, *Phaeognathus hubrichti* (Caudata: Plethodontidae: Desmognathinae). Journal of Herpetology 38: 8-15.

Bakkegard, K. A. 2002. Activity patterns of Red Hills Salamanders (*Phaeognathus hubrichti*) at their burrow entrances. Copeia 2002: 851-856.

Non peer reviewed or reports

Bakkegard, K. A. and M. P. Greene. 2002. *Heterodon platirhinos*. Diet. Herpetological Review 33: 314-315.

Bakkegard, K. A. and R. M. Timm 2001. *Boa constrictor*. Diet. Herpetological Review 32: 261-262.

Bakkegard, K. A. and C. Guyer. 2000. Population estimate of Red Hills salamanders at the proposed Falkenberry Hill construction site, Monroe Co., AL. Unpubl. report to the State of Alabama Department of Transportation.

For submission

Bakkegard, K.A. Learning and avoidance of noxious prey in the Tiger Salamander (*Ambystoma tigrinum*). In prep for Animal Behaviour. Rough draft of manuscript completed.

ACHIEVEMENTS/AWARDS

Academic

- E.B. Carmichael Award, outstanding paper during 2007 in the Journal of the Alabama Academy of Science
- Department of Biology, Graduate Teacher of the Year, Utah State University (Academic Year 2005-2006).
- Tracy Storer Award in Herpetology (Best Student Poster), 2006 Joint meeting of the American Society of Ichthyologists and Herpetologists, New Orleans, LA, 2006.
- Willard L. Eccles Foundation Science Fellowship ($18,000/year). Utah State University, College of Science, 2001-2004.
- Finalist for 2000 Herpetologists' League Award for Graduate Research, 2000.
- Graduated with merit, United States Naval Academy; 1 of 47 Midshipmen to receive Superintendent's Letter of Commendation, 1988.

Military

- Flag Letter of Commendation by Assistant Secretary of the Navy (Installations and Environment), for outstanding leadership as recognized by the Sea Services Leadership Association, 2006.
- Navy League Leadership Award for highest class standing in Surface Warfare Officer Department Head class (#1 of 62), 1996.
- Navy Commendation medal (4), Navy Achievement medal (4), Navy Unit Commendation, Battle E (2), Sea Service Deployment Ribbon, National Defense Service Medal (2), Armed Forces Expeditionary Medal, Global War on Terrorism Expeditionary Medal, Overseas Service Ribbon, Armed Forces Reserve Medal (M device), Rifle Sharpshooter, Pistol Sharpshooter Ribbon.

PROFESSIONAL

Academic

- Graduate Student Representative, USU Dept. of Biology, Faculty Search Committee for Animal Physiologist, 2007-2008.
- Graduate Student Representative, USU Dept. of Biology, Graduate Programs Committee, 2005-2007.
- Reviewer: Biological Journal of the Linnean Society, 2007.
- Reviewer: Herpetological Review, 2003.
- Reviewer: Herpetologica and Journal of Herpetology, 2002.
- Assistant Curator of Herpetology, Auburn University Natural History Museum, 2001-2002.

Military

- Naval War College, College of Distant Education Command and Staff Diploma, 2007.
- Commanding Officer, Mobile Inshore Undersea Warfare Unit (MIUWU) 109, Oct 2004-Dec 2006.
- Recall to active duty with MIUWU 103 as Executive Officer to include six months in Kuwait in support of Operation Iraqi & Enduring Freedom, 2004.
- Navy Reservist: Present rank: Commander. Variety of units and billets including Operations and Executive Officer of NR CNE-C6F MPP Det 118 (Jan 07–present) and Commanding Officer of NR COMIDEASTFOR Det 109 (Oct 2000-Oct 2001), 1998 - present.
- Naval Officer on active duty. Surface Warfare qualified, served on 3 US Navy ships and as a NROTC instructor at Boston University, Boston MA, 1988-1998.

RESEARCH PRESENTATIONS

"The genetic response of *Taricha granulosa* to the 1980 eruption of Mount St. Helens" Joint meeting of the American Society of Ichthyologists and Herpetologists, New Orleans, LA, July 2006. - Poster.

"Antipredator behaviors of *Phaeognathus hubrichti* (Plethodontidae, Desmognathinae): Convergence or phylogeny" Joint meeting of the American Society of Ichthyologists and Herpetologists, Manaus, Brazil. June 2003. - Poster.

"Sexual size dimorphism in the Red Hills salamander (*Phaeognathus hubrichti*)" Joint meeting of the American Society of Ichthyologists and Herpetologists, College Station, PA. July 2001. - Oral.

"Activity of the Red Hills salamander at the burrow entrance" Joint meeting of the American Society of Ichthyologists and Herpetologists. La Paz, Mexico. June 2000. - Oral.

"Natural history and burrow use by the Red Hills salamander, *Phaeognathus hubrichti*" Alabama Department of Conservation and Natural Resources and the Alabama Natural Heritage Program. June 2000. - Oral.

"Activity of the Red Hills salamander (*Phaeognathus hubrichti*) at the burrow entrance" Joint meeting of the American Society of Ichthyologists and Herpetologists, College Station, PA. June 1999 -Oral.

GRANTS

- USU Graduate Travel Award ($300) for meeting in New Orleans, LA, 2006.
- American Museum of Natural History, T. Roosevelt Memorial Fund ($1,522), 2005.
- Sigma Xi Grant-in-Aid of Research ($750), 2004. .
- USU Graduate Travel Award ($300) for meeting in Manaus, Brazil, 2003.
- Alabama Dept. of Transportation ($9,900). A survey of Red Hills salamanders at the Falkenberry Hill truck lanes construction site. (with Dr. C. Guyer), 2000.

FIELD

- Field assistant to Dr. J.A. Campbell (U. Texas-Arlington), capturing reptiles and amphibians in Guerrero, MX for his research on the reptiles/amphibians of MX, June 2002.
- Field assistant to Dr. R.M. Austin (Piedmont College), capturing/identifying amphibians in Costa Rica for his research on the bacterial fauna living on the skin of tropical amphibians. Follow on trip to Belize, assisting lab mate in collecting *Boa constrictor*, July-Aug 2001.
- Organization of Tropical Studies (OTS), Tropical Biology, An Ecological Approach course. Emphasized field techniques used in tropical biology, Jan-Mar 2000.

COMMUNITY SERVICE

- Science Olympiad Official for "Reptiles and Amphibians", Auburn, AL, 2001.
- Reptile and amphibian talk to first grade class at Smith's Station Elementary School, Smith's Station, AL, 2000.
- Reptile, amphibian and conservation talk to Ecology Day Camp held at the Forest Ecology Preserve, Auburn, AL, 2000.
- Presentation to Fairhope Elementary School (Fairhope, AL) and various state officials on the Red Hills salamander in support of the school's effort to have the Red Hills salamander named the official state amphibian, 1999.
- Auburn Herpetological Society Rescue and Relocation team, 1999-2001.

PROFESSIONAL AFFILIATIONS

- Phi Kappa Phi, 2001-present.
- Sigma Xi, The Scientific Research Society, 2000-present
- Society for the Study of Reptiles and Amphibians, 1999-present
- American Society of Ichthyologists and Herpetologists, 1999-present
- The Herpetologists' League, 1999-present
- American Institute of Biological Sciences, 1996-present