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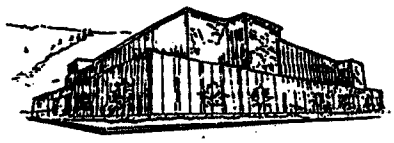
Andrew Robert Whiteley
The University of Montana



EFFECTS OF HISTORICAL AND CONTEMPORARY FACTORS ON GENETIC DIVERSITY IN THE MOUNTAIN WHITEFISH (*PROSOPIUM WILLIAMSONI*)

by

Andrew Robert Whiteley

B.A. Northwestern University 1997

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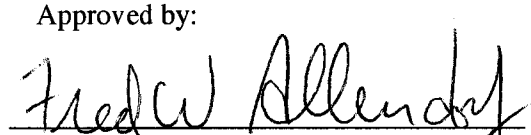

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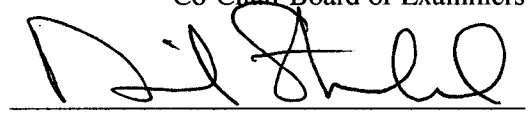

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Effects of Historical and Contemporary Factors on Genetic Variation in the Mountain Whitefish (*Prosopium williamsoni*)

Co-Chairs: Fred W. Allendorf, Paul Spruell

Historical and contemporary factors interact over different spatial scales to determine the intraspecific genetic diversity of an organism. The objective of my dissertation was to gain understanding of the interaction between these factors by examining their effects on the genetic structure of mountain whitefish (*Prosopium williamsoni*).

I examined the distribution of genetic variation across the range of mountain whitefish to explore the effects of historical factors at a large geographic scale and I compared my results with other species to learn about the species-specificity of these effects. I found evidence for five major genetic groups of mountain whitefish, which potentially reflects geographic isolation that occurred in glacial refugium. In the species surveyed, I found several examples of concordant geographic patterns of genetic differentiation that reflect similar responses to landscape features, as well as non-concordant patterns of differentiation that reflect either species-specific responses to landscape features or differences in aspects of their ecology and life history. I also found that gene flow occurred over a larger geographic scale for mountain whitefish than for other native salmonids.

On a smaller geographic scale, I examined interactions between contemporary factors by comparing the genetic structure of mountain whitefish to that of bull trout (*Salvelinus confluentus*) in the Clark Fork River, Montana. Mountain whitefish had much less genetic differentiation among local populations than bull trout, which potentially reflects differences in the physical location of spawning sites, population size, and spawning behavior.

I examined the effects of a putative snout-related trophic polymorphism on genetic subdivision to further explore the effects of contemporary factors within a single population. I examined phenotypic variation in snout morphology and tested for assortative mating for this trait in the Bitterroot River, Montana. I found continuous snout variation and subtle but consistent differences in diet associated with this morphology. I did not find evidence for assortative mating and thus found no effect of this trait on genetic subdivision.

I was supported by an NSF Ecologist, Educators and Schools fellowship for one year. Here I present a mark-recapture class investigation I created during this fellowship.

ACKNOWLEDGEMENTS

The work presented here represents the efforts of many people. I am extremely grateful to all of these people, from my advisors to those that have helped with field and lab work. I cannot possibly fully express due thanks to all of these people.

Fred Allendorf and Paul Spruell have been extremely helpful throughout my graduate career. They put up with me as I attempted several projects and provided support throughout. My dissertation committee members, Elizabeth Crone, Lisa Eby, and Doug Emlen, along with ex-committee member Andy Sheldon, have also been very helpful. Bruce Rieman (U. S. Forest Service) also provided advice, field equipment, and comments on manuscripts throughout.

I have relied on many people for help in the lab and field. I am indebted to all of them. Many members of the Wild Trout and Salmon Genetics Lab at the University of Montana helped me collect mountain whitefish and with other lab and fieldwork. This includes: Kristen Bott, Matt Boyer, Maggie Cook, Damien Cremins, Dawson Dunning, Chris Funk, Connor Jacobs, Jody Knudsen, Kathy Knudsen, Jesse Lewis, Aaron Maxwell, Aaron Martin, Jamie Martin, Luke Neraas, John Powell, Julia Powers, Kristina Ramstad, Hollie Sexton, Sara Somerville, and Zach Wilson. Kate Lindner, Ellie Steinberg, and John Wenburg provided advice along the way. The geographic distance analysis for Chapter 2 was made possible by the GIS work of Damon Holzer. John Thayer made the dietary analysis presented in Chapter 4 possible through his instruction and help with stomach analysis. Kirill Kuzishchin provided instruction for supraethmoid dissection procedures. Audrey Campbell helped with supraethmoid dissection and measurements and with scoring phenotypes. Dawson Dunning and John Powell also scored phenotypes. I am also very grateful to the many state, federal, provincial, and private sector biologists who have assisted me with sample collection or who have collected samples for me. For my ECOS experience, Jennifer Woolf was a fantastic partner and David Oberbillig and Kathleen Kennedy were inspiring

teachers to work with. Carol Brewer made the ECOS experience possible and encouraged me to use the cricket investigation for Chapter 5. She also provided comments on that manuscript. I thank the tenth grade IBES students of Big Sky High School for their enthusiastic participation in the cricket investigation. Robin Anderson of Big Sky High School beta tested this investigation and made the suggestions to use fewer crickets and mark individuals more than once. His comments also improved the investigation sheet and questions for students to answer.

I would like to extend special thanks to Alex Trillo. She helped me shape and refine ideas presented here and has inspired me as a scientist and as a human being.

Finally, I would like to thank the many mountain whitefish that I used as part of my investigations. I hope that my research can serve as a foundation for further studies that attempt to understand this understudied species.

Funding and support for my dissertation research came from Montana Fish, Wildlife & Parks, the United States Forest Service, the American Fisheries Society, the American Society of Ichthyologists and Herpetologists, the Bertha Morton Scholarship, the Five Valleys Audubon Society, and an ECOS Fellowship.

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CHAPTER 1 - Introduction

1.1 BACKGROUND

Many studies over the past 50 years have considered factors that shape the geographic partitioning of genetic variation within species (Mayr 1963; Wright 1978; Avise 2004). These factors can be either historical (acting over evolutionary time scales) or contemporary (acting over ecological time scales; Figure 1-1). Historical factors (e.g. vicariant fragmentation, extinction and recolonization, and range expansion) are often related to features of the landscape. Contemporary factors may be features of the landscape or aspects of the ecological and life history of an organism (Figure 1-1).

The role of large-scale historical factors in shaping genetic diversity is well established (Felsenstein 1982; Templeton et al. 1995; Hewitt 2000; Turgeon and Bernatchez 2001). For example, glaciation has had a major effect on the distribution of genetic variation of many plants and animals (Soltis et al. 1997; Bernatchez and Wilson 1998; Hewitt 2000). Historical factors often are responsible for causing large-scale regional genetic groups, here called cohesive genetic assemblages, that form the historical foundation and context for understanding how genetic variation is partitioned within a species. Elsewhere these large-scale regional genetic groups have been considered ESUs (Evolutionarily Significant Units), DPSs (Distinct Population Segments), or subspecies (Waples 1991; Moritz 1994; Waples 1995; Crandall et al. 2000).

Contemporary factors can shape genetic structure within cohesive genetic assemblages (Figure 1-1). Features of the local landscape (e.g. anthropogenic habitat fragmentation or non-anthropogenic features such as waterfalls) have been shown to influence the distribution of genetic variation at a small scale (Hutchison and Templeton 1999; Keyghobadi et al. 1999; Sork et al. 1999; Castric et al. 2001; Cassel and Tammaru 2003; Costello et al. 2003; Yamamoto et al. 2004). In addition, aspects of the ecology and life history of an organism (e.g. complexity of its life cycle, population size, dispersal ability, and ecological characteristics related to foraging) can

also influence how genetic variation is partitioned at a small geographic scale (Turner and Trexler 1998; McDonald et al. 1999; King and Lawson 2001; Dawson et al. 2002; Castric and Bernatchez 2004; Whiteley et al. 2004). Ecological and life history factors may also determine how genetic variation is partitioned among individuals within populations. For example, trophic polymorphisms (excessive niche-based phenotypic variation; Robinson and Schluter 2000) may lead to reproductive isolation among trophic morphs within populations (e.g. Skúlason et al. 1996; Gíslason et al. 1999; Adams and Huntingford 2004).

Most population genetic studies to date only consider a subset of the factors shown in Figure 1-1 (but see Wilson et al. 2004). Studies that examine both how all of these factors interact within particular species and that compare the effects of these factors on multiple sympatric species are needed to gain a comprehensive understanding of the evolution of patterns of intraspecific genetic variation. Ideally these studies should examine the distribution of genetic variation in organisms well-suited for understanding both historical population relationships and the effects of contemporary factors on genetic subdivision. In addition, these studies should occur in system where comparisons to other species with varying ecological and life history characteristics are possible.

1.2 RESEARCH OBJECTIVES AND FINDINGS

The main objective of my research was to gain further understanding of the interaction between historical and contemporary factors that shape intraspecific genetic diversity by examining their effects on the genetic structure of mountain whitefish (*Prosopium williamsoni*). I was also able to learn about the species-specificity of these interactions by comparing my results for mountain whitefish to previous studies of closely related species with different ecological and life history characteristics. Below are the specific objectives I address in each chapter and a brief summary of my findings.

Genetic subdivision at the range-wide scale

The objective of Chapter 2 was to examine the hierarchical distribution of genetic variation across the range of the mountain whitefish (Figure 1-2a). To determine if genetic variation was partitioned into large-scale genetic assemblages, I analyzed mountain whitefish from 62 locations using six microsatellite loci. I analyzed 29 of these 62 sites with 32 allozyme loci (14 of which were polymorphic). I also compared the patterns and scale of genetic differentiation among populations of mountain whitefish to previous data from other native fishes in northwest North America.

Mountain whitefish are especially well-suited for this type of comprehensive genetic analysis. This species occurs throughout northwest North America in most major river basins (McPhail and Lindsey 1970). In addition, this species has not been translocated within its native range and does not occur sympatrically with other *Prosopium* species in most of its range, which precludes hybridization with other species (with the exception of one population revealed during the course of my research, described in Chapter 2). Thus, it is likely that the range-wide genetic structure of mountain whitefish will reflect historical connectivity among river basins throughout northwest North America (McPhail and Troffe 2001). Furthermore, mountain whitefish are abundant and invasive sampling is unlikely to have a negative demographic influence on extant populations.

The specific questions I asked with respect to range-wide genetic subdivision were:

- What is the genetic structure of mountain whitefish in northwest North America?
- How do patterns of genetic differentiation compare among native fishes in this region?
- How does the geographic scale of genetic differentiation compare among species?

I found evidence for five cohesive genetic assemblages across the range of mountain whitefish. These assemblages are likely to be due to isolation that occurred in glacial refugium during the most recent glacial advance approximately 10,000 years ago (McPhail and Lindsey 1986). I also found evidence for reduced gene flow among major river basins but high levels of

gene flow among local populations within major river basins. I found several examples of concordant geographic patterns of genetic differentiation among species that reflect similar responses to landscape features, as well as non-concordant patterns of differentiation that reflect either species-specific responses to landscape features or differences in aspects of the ecology and life history of the fishes considered. For example, genetic patterns were largely concordant between mountain whitefish and bull trout where they co-occur across northwest North America, including concordant patterns of genetic divergence in the Snake River upstream from Hell's Canyon. However, these two species differ in their ability to disperse through saltwater, which may be responsible for differences in genetic patterns along the Pacific coast. In addition, the gene flow occurs over a much larger geographic scale for mountain whitefish than for other native salmonids. It is possible that mountain whitefish populations operate as metapopulations that occupy entire river systems (e.g. the entire Columbia River system). In contrast, other native salmonids probably have many metapopulations within the same river systems (Rieman and Dunham 2000).

Genetic subdivision at the river basin scale

The objective of Chapter 3 was to examine the effects of landscape features, ecological characteristics, and life history traits on the distribution of genetic variation within and among populations of mountain whitefish within a single river basin (Figure 1-2b). Several ecological and life history characteristics of mountain whitefish differ markedly from other co-occurring salmonids for which genetic patterns have been described, making them particularly well-suited for genetic analysis at this scale. Mountain whitefish broadcast spawn in large groups in the mainstem of larger rivers or near the mouths of tributaries to these larger rivers and have large population size (Northcote and Ennis 1994). These factors lead to the prediction that this species would have high amounts of genetic variation within local populations (spawning aggregates) and low amounts of genetic differentiation among local populations. I tested this prediction by

comparing the genetic structure of mountain whitefish to that of bull trout in the same landscape. Bull trout spawn in the headwater portion of tributary streams, generally have small population size, and spawn in small groups. Thus, I used the same ecological and life history characteristics to predict that bull trout would have much greater differentiation among local populations than mountain whitefish. I used microsatellites to analyze 11 mountain whitefish and seven bull trout sites from approximately the same location in the Clark Fork River basin (Whiteley et al. 2004). By analyzing both mountain whitefish and bull trout, I could more fully understand the effects of local landscape and ecological/life history features on the population genetic structure of each species.

The specific questions I asked with respect to genetic subdivision at the river basin scale were:

- How is genetic variation partitioned among spawning sites of mountain whitefish within the Clark Fork River?
- Can we predict the genetic structure of mountain whitefish and bull trout based on ecology and life history characteristics of each species?

As predicted, I found very low levels of genetic differentiation among spawning sites of mountain whitefish within the Clark Fork River (Whiteley et al. 2004). Genetic differentiation was much lower for mountain whitefish than for bull trout in the same landscape. I detected influences of both biological factors and landscape factors with this study. For example, I analyzed a high mountain lake site for each species. These lake sites showed increased genetic differentiation for each species. However, this pattern interacted with the biology of each species and led to comparatively less divergence of the lake site for mountain whitefish than for bull trout.

Genetic subdivision within populations

The objective of Chapter 4 was to determine if a putative trophic polymorphism related to snout morphology, where some individuals have enlarged and bulbous "pinocchios" snouts, caused genetic subdivision within populations of mountain whitefish (Figure 1-2c). Troffe (2000) and McPhail and Troffe (2001) found evidence for genetic differentiation between what they considered two trophic morphs and hypothesized that assortative mating occurs between these two forms. However, the results of Troffe (2000) were based on small sample sizes and both the nature of phenotypic variation related to snout morphology and the extent to which genetic subdivision might occur within populations due to this phenotypic variation needed further investigation. In this chapter, I examined the nature of snout phenotypic variation, analyzed stomach contents of pinocchios and nonpinocchios, and tested for assortative mating between pinocchios and nonpinocchios from the Bitterroot River.

The specific questions I asked with respect to genetic subdivision within populations and the putative trophic variation in the mountain whitefish were:

- Is there discontinuous variation in snout morphology within populations of mountain whitefish?
- Is there a difference in diet between individuals with extreme snout morphologies?
- Is there evidence of assortative mating by snout morphology?

I found that the pinocchio snout is an exaggerated trait with continuous variation within populations. Snout variation increased drastically after fish reached approximately 220mm. Individuals that grew a large snout at approximately this length appeared to continue along a growth trajectory that resulted in an extremely exaggerated snout at a larger body size. I found subtle but consistent and statistically significant differences in diets between phenotypically extreme individuals for two replicate samples. I did not find evidence for assortative mating by snout morphology in two replicate samples. The riverine landscape appeared to interact with ecological aspects of the mountain whitefish in two ways. First, food availability is probably

heterogeneous enough in time and space that it is unlikely for discontinuous variation related to foraging to evolve. Second, habitat heterogeneity in rivers is likely to prevent spatial segregation of morphs during spawning, thus preventing assortative mating, especially in a species that broadcast spawns in large groups.

1.3 ECOS FELLOWSHIP

For my final year, I was supported by an NSF sponsored ECOS (Ecologists, Educators, and Schools) Fellowship. Through this Fellowship I had the opportunity to learn more about teaching and education and to improve my teaching skills. I worked with two University of Montana students (Jennifer Woolf and Frank Janes) and two teachers from Big Sky High School in Missoula, Montana (David Oberbillig and Kathleen Kennedy). Our overall goal was to introduce more ecology and evolution into the tenth-grade general biology course taught by David and Kathleen. We created and taught many curriculum pieces about ecology, the scientific method, and sampling. We designed these investigations to lead towards an experimental prescribed burn on Department of Natural Resource and Conservation (DNRC) land near Big Sky High School. Overall, this was an incredibly rewarding experience in terms of providing me with teaching experience and knowledge of teaching philosophies as well as hands-on knowledge of how to contribute to K-12 teaching from within a university.

In Chapter 5, I discuss a mark-recapture investigation using crickets in 10-gallon aquaria that we developed to complement an existing population ecology curriculum section at Big Sky High School. I have written this activity as a manuscript for The American Biology Teacher as a How-To-Do-It piece. Briefly, we put a known number of crickets into an aquarium with cardboard egg containers with which we could easily capture the crickets. Students worked in small groups to capture and then mark crickets using non-toxic paint pens. They then released the crickets and recaptured them a short time later. They used the Lincoln-Petersen model to estimate the number of crickets in the aquarium. We developed short lectures for before this

activity and for between the capture events. This activity was very successful and has already been used by another teacher at Big Sky High School. We hope that this activity becomes part of the science curriculum at Big Sky High School and hopefully elsewhere after the resulting manuscript is published

1.4 SYNTHESIS AND SIGNIFICANCE

In summary, I considered the effects of landscape features, ecological aspects, and life history characteristics on the hierarchical genetic structure of mountain whitefish. Historical factors had strong effects on genetic subdivision of populations at the range-wide scale. Aspects of the ecology and life history of mountain whitefish had strong effects on genetic subdivision at the river basin scale. Finally, within local populations, the pinocchio snout may represent a subtle trophic polymorphism, but this phenotypic variation did not influence fine-scale genetic subdivision.

The range-wide data presented here are particularly valuable because closely related and imperiled species that co-occur with mountain whitefish have been well-studied genetically, which allowed highly informative comparisons of the patterns and the scale of genetic differentiation with previous studies. The examples of concordant and non-concordant patterns of genetic differentiation mentioned above and elaborated upon in Chapter 2 are useful for both understanding the effects of factors that shape intraspecific diversity and for informing management and conservation efforts. These comparisons will aid in defining units of conservation for native fishes in northwest North America and will help to shift management to more multispecies approaches. Most management decisions for inland native fishes are based on bull trout and westslope cutthroat trout (*Oncorhynchus clarki lewisi*). Management would benefit from consideration of other species and the data included here would aid in making the shift towards a multispecies management perspective a more informed one.

The differences in genetic structure that I observed for mountain whitefish and bull trout at a smaller geographic scale are significant for two primary reasons. First, this study shows that the genetic structure of a species can be predicted based on aspects of its ecology and life history. Other fishes that co-occur with mountain whitefish and have similar aspects of ecology and life history, such as spawning location and population size, should also have similar genetic structures. It should be possible to make similar predictions about the genetic structure of a given organism based on specific aspects of that organism that are likely to affect how genetic variation is distributed within and among populations. Second, this portion of my research allowed me to formulate a model for understanding causal factors of both neutral and adaptive divergence, as I elaborate upon in Chapter 3. For salmonids, these causal factors may be related to life-cycle complexity and habitat specificity. This model warrants further investigation because it may be of general significance for evolutionary patterns among local populations.

The pinocchio snout may represent a subtle within-population trophic polymorphism. This is significant because most examples of trophic polymorphism occur in lacustrine species. In fact, species-poor temperate lakes have become model systems for this type of phenotypic diversification (Robinson and Schluter 2000). My research suggests that heterogeneity of prey resources in time and space may explain the lack of trophic polymorphisms in riverine systems. In addition, my research suggests that it may be far more likely for variation to be maintained as within-population polymorphisms in riverine species than for this variation to become partitioned among species.

Each of the empirical components of my research (Chapters 2, 3, and 4) addressed different components of the framework shown in Figure 1-1. This framework explicitly considers what factors shape intraspecific genetic diversity and how these factors may interact within and perhaps among hierarchical levels of biological organization. While many studies have focused on one or two components of Figure 1-1, I am not aware an attempt to provide an all-encompassing framework. Thus, this framework is an important contribution to the field of

population genetics and it should aid future attempts to understand factors that shape intraspecific diversity.

My study has also provided basic information about mountain whitefish. In general, very little is known about this species (Northcote and Ennis 1994; McPhail and Troffe 2001). Mountain whitefish are often the most abundant species in rivers in northwest North America (Northcote and Ennis 1994). They are an important component of the fish community in this region and may interact with other aquatic organisms in ways that are not presently understood. In addition, they are a potential indicator of anthropogenic impacts on aquatic habitats. My study has provided a foundation for future research. An additional benefit of the data presented here is that they establish a baseline for future genetic studies of this species. If population declines occur, as have been reported in some locations (for example, this species no longer occurs in the Humboldt River, Nevada; J. Dunham, USFS personal communication), these baseline data could be used to understand the effects of population declines on the genetic structure of a common species.

Beyond empirical research, my graduate experience has broadened my perspective on the societal importance of science education. The ECOS Fellowship played an important role in the development of my educational ideas and has influenced my approach to teaching. For example, I learned the importance of including hands-on inquiry-based learning experiences as part of educational courses. Inquiry-based education promotes critical thinking in students, perhaps more so than content-based lecture approaches. I had an opportunity to create and implement inquiry-based investigations in Big Sky High School classrooms. Chapter 5 is an example of one such investigation. I have also learned how to work with schools in the local community from within a university setting and have gained valuable teaching skills from interacting with two high school teachers and their classes at Big Sky High School. I will incorporate what I have learned as an ECOS fellow in future courses that I teach.

Figure 1-1. Factors that influence intraspecific genetic diversity. Historical factors operate over evolutionary time scales and regional spatial scales. Contemporary factors operate over ecological time scales and smaller spatial scales. Both aspects of the ecology and life history of an organism interact with the local landscape to determine the influence of contemporary factors (curved arrows). Historical and contemporary factors may interact to determine the distribution of genetic variation across the range of a species.

Figure 1-2. Hierarchical analysis of genetic diversity in the mountain whitefish. I analyzed genetic variation across the range of this species (dashed line in (a)) and within the Clark Fork River in western Montana (b). At the smallest geographic scale, I analyzed the effect of potentially trophic related phenotypic variation on genetic subdivision within the Bitterroot River (c). At each geographic scale, the genetic diversity factors examined in the present study are shown.

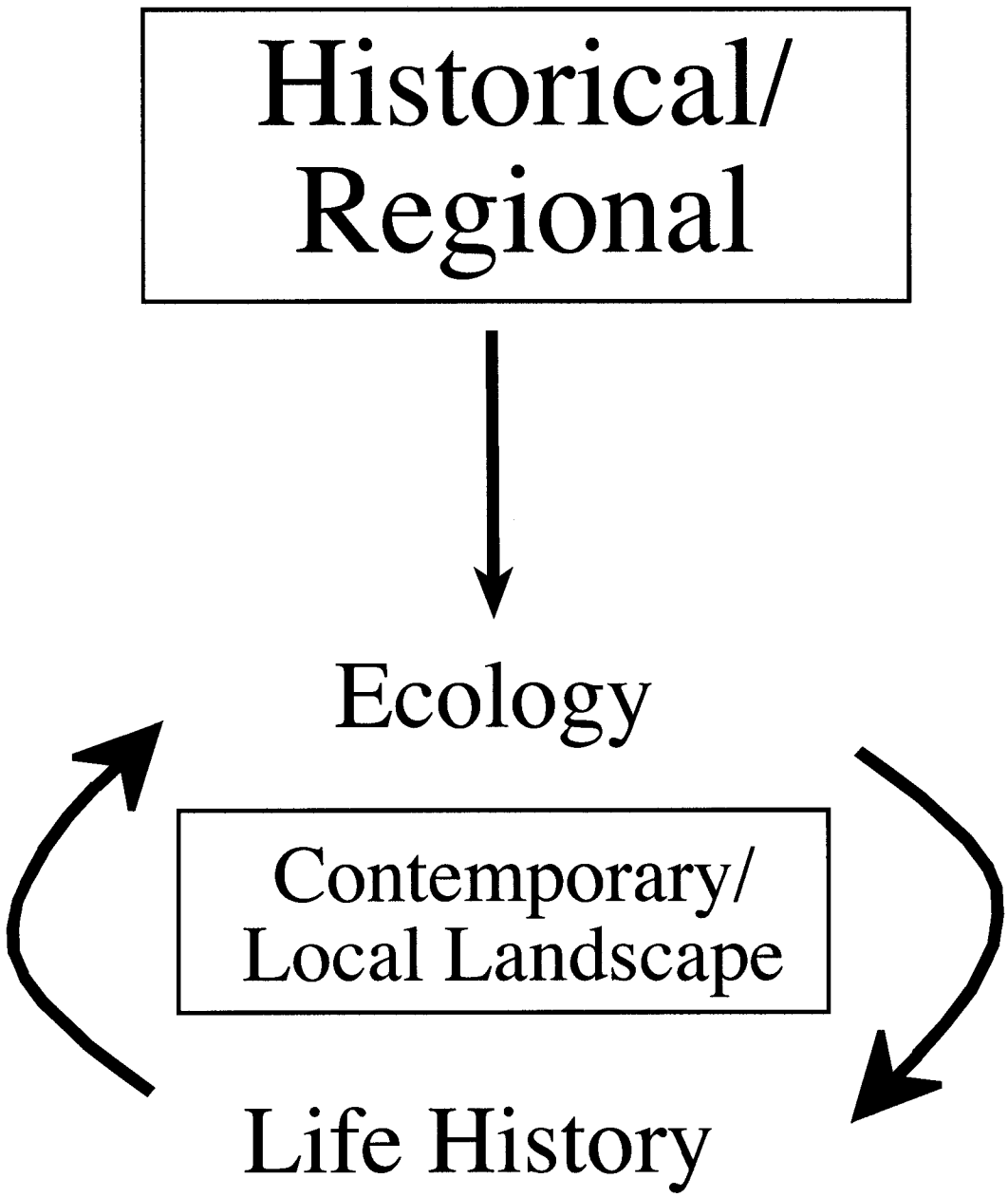
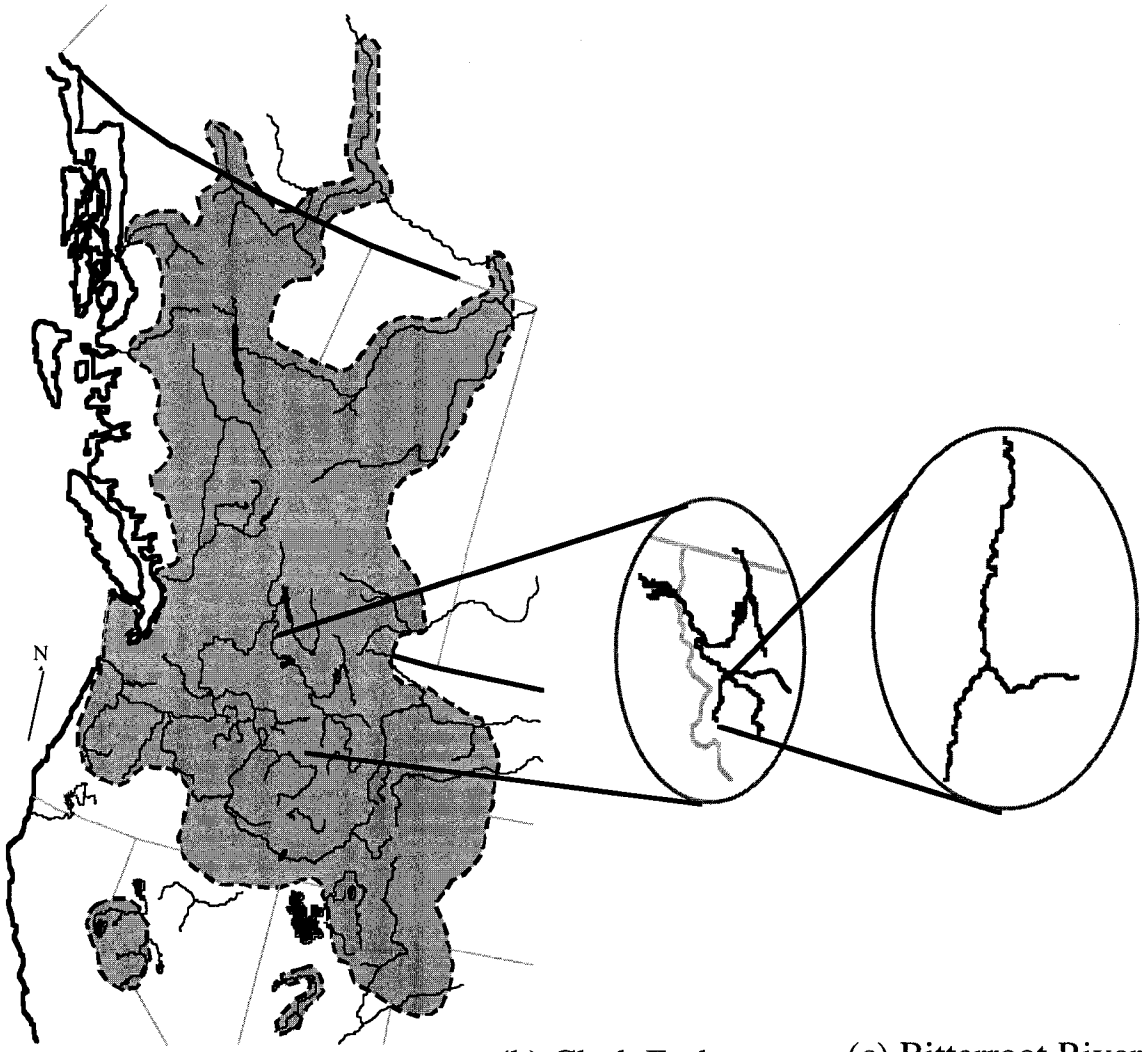


Figure 1-1



(a) Northwest North America (b) Clark Fork River (c) Bitterroot River

Genetic Diversity Factors Examined

-Vicariance
-Glaciation
-Colonization Patterns

-Landscape
-Ecological/life history differences between species

-Morphological variation
-Potential trophic polymorphism

Figure 1-2

CHAPTER 2 - Can Common Species Provide Valuable Information for Conservation?

2.1 ABSTRACT

We examined the distribution of genetic variation at allozyme and microsatellite loci across the range of the mountain whitefish (*Prosopium williamsoni*) to demonstrate the importance of genetic data for multi-species conservation approaches. The mountain whitefish is a common species that is particularly well suited for accurately revealing historical patterns of genetic structure and differs markedly from previously studied species in habitat requirements and life-history characteristics. As such, genetic data from mountain whitefish provide a useful comparison to the population genetic structure of other native fishes. Genetic variation for mountain whitefish was hierarchically distributed for both allozymes and microsatellites. We found evidence for a total of five major genetically differentiated assemblages and we observed subdivision among populations within assemblages that generally corresponded to major river basins. We observed little genetic differentiation within major river basins. Geographic patterns of genetic differentiation for mountain whitefish were concordant with other native species in several circumstances, providing information for the designation of conservation units that reflect shared historical differentiation of multiple species. Differences in genetic patterns between mountain whitefish and other native fishes provide examples where sympatric species in several river systems have different evolutionary histories. In addition, mountain whitefish populations appear to exchange genes over a much larger geographic scale than co-occurring salmonids and are likely to be affected differently by disturbances such as habitat fragmentation.

2.2 INTRODUCTION

There is a growing consensus that single species conservation efforts do not adequately protect the biological and landscape needs of multiple species within threatened ecosystems (Lambeck 1997; Roberge and Angelstam 2004). Consequently, there has been a recent trend in conservation strategies towards shifting from single-species to multi-species approaches (Lambeck 1997; Freudenberger and Brooker 2004). These efforts consider the habitat requirements of multiple species to prioritize conservation efforts (Roberge and Angelstam 2004).

Considering genetic data from multiple species in threatened ecosystems might be particularly informative for multi-species conservation approaches. To date, genetic comparisons among species have largely occurred among large-scale regional genetic groups in the context of comparative phylogeography (Avise 2004). More detailed comparisons of patterns and geographic scale of genetic differentiation at multiple hierarchical levels of biological organization (from populations through ecosystem and landscape levels) are needed to make genetic comparisons more informative for comprehensive conservation efforts.

Concordant genetic patterns for multiple species across a given region can highlight evolutionary divergence that should be conserved. For example, it might be difficult to prioritize conservation efforts for a region inhabited by moderately genetically differentiated populations of an imperiled species. However, if multiple native species are all genetically differentiated in that region, the weight of evidence suggests that an historic separation has occurred and that conservation efforts should recognize this evolutionary divergence.

Lack of concordance for multiple species in a region may reflect a) long term differences in evolutionary history of the species considered or b) differences in their ecology and life history that lead to differences in how genetic variation is partitioned within and among populations over more recent ecological time scales. If differences in genetic patterns are historical in nature, ESU

designations should reflect differences in evolutionary histories (In this paper we use ESU in its most generic sense to describe groups of populations that have a shared evolutionary history and are sufficiently genetically differentiated from other such groups to merit separate conservation efforts (*sensu* Ryder 1986; Waples 1991). We do not presume any specific functional definition (e.g. Moritz 1994). Nor are we advocating legal status for the ESUs we discuss). If differences in genetic patterns reflect contemporary aspects of ecology and life history, conservation efforts based on genetic patterns of one species may be either inadequate for another more finely subdivided species, or may be overly protective and unnecessary for a second species that is less genetically subdivided.

In addition to genetic patterns, describing the geographic scale of genetic differentiation of multiple species in the same landscape can also be important for multi-species conservation approaches. Overlaying patterns of genetic differentiation onto geographical distances among populations and comparing the resulting relationships for multiple sympatric species provides a comparison of population boundaries and the geographic scale of ecological and evolutionary processes. Inferences regarding the geographic scale of genetic differentiation can help to define habitat and area requirements for multiple species, to determine the amount and scale of connectivity necessary for population persistence, and to predict the effects of anthropogenic habitat alterations such as fragmentation.

For comparisons of both pattern and scale of genetic differentiation, it is important that the relationships among populations of species analyzed accurately reflect historical associations. For threatened and endangered species, it is often difficult to obtain large samples of all the relevant populations and regions due to the fact that these species may be extirpated in some areas, occur at low abundance where present, and sampling might put populations at even greater risk. Thus, for these species, it may be difficult to effectively reconstruct historical relationships among population. In systems where only threatened or endangered species have been studied,

multi-species conservation approaches could be considerably enhanced with comparative genetic data from species that are more likely to reflect historical genetic relationships.

Species most likely to reveal historical population relationships are widely distributed across the range of the ecosystem of interest, have not been transplanted within their native range, do not hybridize with other species, and have large populations. Wide-ranging species allow the largest possible scope of comparison. Transplantation and hybridization can obscure historical genetic patterns (Allendorf et al. 2001), as can genetic drift in recently contracted or chronically small populations. In addition, for species with large populations, it is easier to collect adequate samples and these species are amenable to invasive techniques such as allozyme analysis, which often permits direct comparison to existing data. Consequently, we suggest that abundant, widely distributed species will often provide an informative complement to genetic studies of imperiled taxa.

River systems in northwest North America have been the focus of intense conservation efforts (Policansky and Magnuson 1998; McClure et al. 2003; Mebane et al. 2003). Conservation issues range from habitat fragmentation due to a variety of sources (e.g. dams and road building) to water quality issues related to activities such as mining and forest use (e.g. Kareiva et al. 2000; Levin and Tolimieri 2001; Collins and Montgomery 2002). Genetic patterns for four salmonids with large distributions in inland freshwater systems (bull trout, *Salvelinus confluentus*; cutthroat trout, *Oncorhynchus clarki*; rainbow trout, *Oncorhynchus mykiss*; and Chinook salmon, *Oncorhynchus tschawytscha*) have been described in detail from this region and have been used in part to determine conservation and management priorities (e.g. NOAA 2003). Genetic variation is distributed hierarchically for these species across this region and all four species tend to be subdivided on a fine geographic scale, with significant genetic differences often occurring among tributaries within major river basins (Allendorf and Utter 1979; Allendorf and Leary 1988; Wenburg et al. 1998; Taylor et al. 1999; McCusker et al. 2000; Teel et al. 2000; Costello et al. 2003; Spruell et al. 2003; Taylor et al. 2003; Waples et al. 2004).

Conservation efforts for fishes in northwest North America have proceeded largely in a single-species manner. For the four species mentioned, there has not been an attempt to compare and contrast patterns of genetic differentiation. In addition, for some of these species, transplantation and anthropogenic-induced hybridization may obscure historical genetic patterns (e.g. Allendorf and Leary 1988). Genetic data from a common species with the desirable attributes for comparative genetic analyses mentioned above may be valuable as a step towards a more comprehensive conservation approach. Furthermore, because all four species tend to be genetically subdivided on a small geographic scale, they offer a limited view the geographic scale of genetic differentiation of all of the native fishes in this region. Genetic analysis of a species likely to be subdivided on a larger geographic scale will offer an alternative perspective that can broaden the scope of conservation and management planning.

The mountain whitefish (*Prosopium williamsoni*) co-occurs with the four species mentioned above and fits our criteria for a useful common species for comparative genetic analysis. Mountain whitefish have not been translocated within their native range and do not occur sympatrically with other *Prosopium* species in most of their range, precluding hybridization with other species (with the exception of one population revealed during the course of this study, described below). Mountain whitefish are abundant and invasive sampling is unlikely to have a negative demographic influence on populations. This species occurs throughout northwest North America in most major river basins (McPhail and Lindsey 1970) and has experienced the same geomorphological influences as other native fishes. Thus, it is likely that the genetic structure of mountain whitefish will reflect historical connectivity among river basins throughout northwest North America (McPhail and Troffe 2001).

The geographic scale of genetic differentiation may differ between mountain whitefish and other species examined to date, such that this species may provide a good contrast to other species in this respect as well. Mountain whitefish differ in ecological aspects from these other species because they reside and spawn primarily in larger rivers, they appear to have less habitat

specificity throughout their life cycle, and they have larger N_e (Whiteley et al. 2004). We have shown previously that mountain whitefish populations appeared to exchange genes over a larger geographic scale than bull trout in one river basin in Montana (Whiteley et al. 2004). It is possible that evolutionary processes for mountain whitefish occur at a much larger scale relative to other salmonids across northwest North America.

In this paper, we used allozymes and microsatellites to analyze the hierarchical distribution of genetic variation across the range of the mountain whitefish. We answered the following questions: What is the genetic structure of mountain whitefish in northwest North America? How do patterns of genetic differentiation compare among species? How does the geographic scale of genetic differentiation compare among species? Finally, do these data provide additional insight for management of other native fishes in northwest North America?

2.3 MATERIALS AND METHODS

Samples

We obtained samples from throughout the range of the mountain whitefish (Table 2-1; Figure 2-1). Where possible, we obtained whole fish for tissues for both allozyme analysis and for DNA extraction and subsequent microsatellite analysis. For each population sample, care was taken to include fish from multiple size classes to maximize the probability of analyzing unrelated individuals. Most sites included fish from multiple collection locations within a river. We were able to obtain samples from a wider geographic range for microsatellite analysis than for allozyme analysis, partly due to problems with international transport of whole frozen fish from Canadian sites.

Allozymes

We performed horizontal starch gel electrophoresis according to the procedures of Leary and Booke (1990) on fish collected from 29 locations (Table 1). We screened products of 32 loci

coding for enzymes from muscle, liver, or eye tissue and found evidence of genetic variation at 14 loci. We followed Shaklee et al. (1990) for nomenclature of enzymes, loci, and alleles. Enzyme Commission (EC) numbers follow IUBMBNC (1992) and are as follows: adenylate kinase (EC 2.7.4.3; *AK-1,2**), alcohol dehydrogenase (EC 1.1.1.1; *ADH**), aspartate aminotranserase (EC 2.6.1.1; *sAAT-1*, sAAT-2*, sAAT-3*, sAAT-4**), creatine kinase (EC 2.7.3.2; *CK*-A1*), cytosol nonspecific dipeptidase (EC 3.4.13.18; *PEPA-1*, PEPA-2**), glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12; *GAPDH-3,4**); glycerol-3-phosphae dehyrogenase (EC 1.1.1.8; *G3PDH-1,2**); hexosaminidase (EC 3.3.1.52; *HEX**); isocitrate dehydrogenase ($NADP^+$)(EC 1.1.1.42; *sIDHP-1,2**); L-lactate dehydrogenase (EC 1.1.1.27; *LDH-A1*, LDH-A2*, LDH-B1*, LDH-B2*, LDHC**); malate dehyrogenase (EC 1.1.1.37; *sMDH-A1,2*, sMDH-B1,2**); malic enzyme ($NADP^+$)(EC 1.1.1.40; *mMEP-1*, sMEP-1**); phosphoglucomutase (EC 5.4.2.2.; *PGM-1*, PGM-2**); superoxide dismutase (EC 1.15.1.1; *sSOD-1**) and tripeptide animopeptidase (EC 3.4.11.4; *PEPB**). Tissues were kept frozen until dissection. We used the electrophoresis buffers described in Leary et al. (1993). Stains used to reveal the position of enzymes in the gels after electrophoresis were from Harris and Hopkinson (1976) and Allendorf et al. (1977). An Arlee strain rainbow trout (*Oncorhynchus mykiss*) from the Jocko River State Fish Hatchery, Arlee, Montana (maintained by the Department of Montana Fish, Wildlife & Parks) was run on each gel as a mobility standard. We scored alleles at the malate dehydrogenase isolocus, *MDHB-1,2**, as products from two separate loci and assumed that all observed variation occurred at one locus (Leary and Book 1990).

Microsatellites

The general methods used for PCR and visualization of subsequent PCR products followed Spruell et al. (1999), Neraas and Spruell (2001), and Whiteley et al. (2004). DNA was extracted from either fin clips or liver tissue by standard methods. We visualized fluorescently-labeled PCR products on acrylamide gels and used a molecular size standard and individual fish

of known genotypes as standards for scoring. We used six of the eight loci (*COCL4*, *SSA14*, *SSA456*, *ONE8*, *SFO8-1*, and *SFO8-2*) from Whiteley et al. (2004) because these six loci could be scored reliably across the range of the mountain whitefish. PCR reagent concentrations varied among loci and are available from the authors upon request.

Data Analysis

Allele frequencies, deviations from Hardy-Weinberg expectations, linkage disequilibrium, observed (H_O) and expected (H_E) heterozygosity per locus and population, mean within-population expected heterozygosity (H_S), mean number of alleles per population, pairwise exact tests for genic differentiation, *F*-statistics and pairwise F_{ST}'s were calculated using GENEPOP 3.4 (Raymond and Rousset 1995) and FSTAT 2.9.3.2 (Goudet 1995; Goudet 2001). We used θ (Weir and Cockerham 1984) for estimates of F_{ST}. Confidence intervals (95%) for multilocus F_{ST} estimates were generated by bootstrap sampling over loci (Goudet et al. 1996). We used sequential Bonferroni adjustments to adjust multiple tests for linkage disequilibrium within populations (Rice 1989). We tested to determine if the amount of within population genetic variation (arcsine transformed H_S and mean number of alleles) detected by allozymes and microsatellites was correlated using a Spearman rank correlation test.

We calculated $F2_{ST}$ for both microsatellites and allozymes to determine if the greater genetic heterozygosity observed with microsatellites might have contributed to a downward bias in our estimate of population differentiation. With $F2_{ST}$, all loci are treated as bi-allelic by using the frequency of the most common allele and pooling the frequencies of all others (McDonald 1994; Allendorf and Seeb 2000). We used SPAGEDI (Hardy and Vekemans 2002) to calculate R_{ST} for microsatellites and to test for significant differences between R_{ST} and F_{ST}. R_{ST} values significantly greater than F_{ST} values suggest that stepwise-like mutation processes have occurred at a locus (Hardy et al. 2003). We used standard error estimates from SPAGEDI to calculate 95% confidence intervals for R_{ST}.

In the Kechika River sample, we observed microsatellite alleles outside the normal size range for alleles at several loci. This population lies within a zone of sympatry with the round whitefish (*Prosopium cylindraceum*; McPhail 1970). We used PINE-PCR (Spruell et al. 2001) to determine that these three fish were hybrids. These three fish appear to be F1's because all fragments diagnostic for both mountain whitefish and round whitefish were present in each fish. We removed these fish from subsequent analyses (Allendorf et al. 2001).

To examine range-wide patterns of population differentiation, we used principle components analysis (PCA) based on a covariance matrix using SPSS 11 (SPSS, Inc.). We excluded one allele at each locus to account for non-independence among alleles within loci for both marker types. For allozymes, the PCA is based only on loci that were polymorphic (frequency < 0.99) in at least one population. We used an analysis of molecular variance (AMOVA, Excoffier et al. 1992), performed with ARLEQUIN 2.001 (Schneider et al. 2000) to investigate how genetic variation was partitioned based on several geographical arrangements. The first arrangement was based on groups defined by PCA for both allozymes and microsatellites. The second alternative geographical arrangement was based on genetic patterns observed for the cutthroat trout. The range of the cutthroat trout overlaps with that of the mountain whitefish to a large degree (Behnke 2002). Genetic patterns from the cutthroat trout (Allendorf and Leary 1988) provided an *a priori* prediction of genetic subdivision for the mountain whitefish. We used four geographical arrangements for the mountain whitefish genetic data that correspond sites within the range of the coastal cutthroat trout (*O. c. clarki*), the westslope cutthroat trout (*O. c. lewisi*), the Lahontan cutthroat trout (*O. c. henshawi*), and finally, to be conservative, we combined the Yellowstone (*O. c. bouvieri*) and Bonneville cutthroat trout (*O. c. utah*) into the fourth group.

To further describe the scale and patterns of genetic differentiation among mountain whitefish populations, we constructed a dendrogram based on microsatellite and allozyme allele frequencies. We used PHYLIP 3.5 (Felsenstein 1993) to calculate Cavalli-Sforza and Edwards'

(1967) genetic distance (CSE) with the GENDIST module. We used the NEIGHBOR module to construct a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram. CONSENSE was used to generate a consensus tree with bootstrap values from 1000 replicate data sets created in SEQBOOT. We chose to analyze genetic divergence between populations using CSE because it is drift based, does not assume any models of mutation, and performs well in simulations of microsatellite data (Takezaki and Nei 1996).

We used BAPS 2.0 (Bayesian Analysis of Population Structure, Corander et al. 2003; Corander et al. 2004) as an alternative way to define population groups within cohesive genetic assemblages and to provide further information about the scale and patterns of population relationships. We used the group method for clustering pre-defined populations based on multi-locus tests of allele frequency differences. We chose the group clustering method because mountain whitefish populations are confined to river basins and it is not possible for gene flow to occur among many of the populations we analyzed. The only prior we used was the sample location of each individual. We ran BAPS five times for 10^5 iterations with a burn-in period of 20,000. Panmictic population groups defined by the data partitions with the highest posterior probability for microsatellites and allozymes separately were plotted onto the map of northwestern North America.

To further analyze the geographic scale of gene flow for mountain whitefish, we plotted pairwise genetic distances against pairwise geographic distances. We limited our analysis to populations in the contiguous Columbia River basin and to those within the Inland Cascadia cohesive genetic assemblage (see below). We measured river channel distances among sites using a geographic information system (GIS). We analyzed patterns with and without two sites located above impassable waterfalls (Big Wood and Bull Rivers) and one site from a high mountain lake (Doctor Lake). We used Mantel tests implemented by the program IBD (Isolation by Distance, Bohonak 2003) to test the significance of the relationship between genetic and

geographic distance matrices. Tests were performed with and without log transformation of geographic distances and using both F_{ST} and $F_{ST}/(1 - F_{ST})$ (Rousset 1997).

Based on previous work within the Clark Fork River (Whiteley et al. 2004), we predicted that we would find a discontinuity in the relationship between genetic and geographic distances when we considered sites distributed over a wider geographic range. To the left of this discontinuity, genetic distances were expected to be uniformly low (because gene flow is high). To the right of the discontinuity, the mean and variance of genetic distances were expected to be greater (because genetic drift is more influential and gene flow is reduced). We used statistical methods developed for allometric relationships in insects (Eberhard and Gutiérrez 1991) to test for a discontinuity, or "switch point", in the range of geographic distances. We used the following model: $Y = \beta_0 + \beta_1 X + \beta_2 (X - X^0) D + \beta_3 D + \varepsilon$, where, for our purposes, Y and X were pairwise F_{ST} and geographic distances, respectively (in actual measurement units); X^0 was the putative switch point; D = 0 if $X < X^0$ or D = 1 otherwise; β_i's were the regression coefficients; and ε was the random component with assumed normal distribution, mean zero, and common variance. To determine the switch point, we empirically substituted 12 different values of X^0 into the model and chose the value of X^0 that that gave the highest adjusted R^2. We then used a partial F-test to test the significant of β_3 using a stepwise regression (with the empirically determined value of X^0) implemented in SPSS 11. Significance of the β_3 term would indicate that a discontinuity in the relationship between genetic and geographic distance at the indicated switch point.

2.4 RESULTS

Variation Within Populations

Allozymes

Allozyme analysis revealed 14 polymorphic loci out of the 32 loci screened for variation. We found a total of 37 alleles for the 794 individuals analyzed from 29 sites. Mean H_S ranged

from zero to 0.048 and mean number of alleles ranged from 1.00 to 1.43 (Table 2-1). Two populations from the Bonneville Basin in Utah had no genetic variation (Bear and Weber Rivers) and two others sites that are located upstream from barrier waterfalls had highly reduced genetic variation (Big Wood River and Henrys Fork of the Snake River).

None of the polymorphic allozyme loci showed evidence of significant departures from Hardy-Weinberg proportions. Of 159 tests for genotypic disequilibrium, seven were significant ($P < 0.05$), where eight significant tests were expected by chance ($\alpha = 0.05$). There was no pattern of significant disequilibrium within any of the population samples or for any of the locus pairs across populations.

Microsatellites

For microsatellites, we observed a total of 142 alleles at six loci for the overall sample of 1769 individuals from 62 sites. H_S ranged from zero to 0.538 (Table 2-1). The mean number of alleles ranged from 1.00 to 4.83. The Big Lost and Big Wood Rivers, both of which are isolated populations, had no genetic variation. Other sites that are isolated above waterfalls (Bull River and Thutade Lake) or located in a high mountain lake (Doctor Lake) also had reduced genetic variation (Table 2-1).

Seventeen of 275 tests showed evidence for significant deviations from Hardy-Weinberg proportions with microsatellites (14 significant tests were expected by chance). No consistent patterns within loci across populations or within populations across loci were observed, except in the case of *ONE8* in populations from the upper Missouri River. Four of six sites from the upper Missouri River had significant departures from Hardy-Weinberg proportions at this locus. In each of these four cases there was a deficit of heterozygotes (positive F_{IS}), suggesting that a null allele might occur in this geographic region at this locus. However, we did not observe any

potential null homozygotes and assumed that a null allele, if present, was at low frequency and would not have a large influence on genetic patterns.

Of 643 tests for genotypic disequilibrium, 33 were significant, where 32 were expected by chance. There was no significant pattern of genotypic disequilibrium within any of the population samples or between any locus pairs across populations. After correcting for multiple tests within a population, only two tests remained significant (*SSA14* and *COCL4*, Lolo Creek and *COCL4* and *SFO8-1*, Big Spring Creek).

Comparison of Markers

Within-population genetic variation (both H_S and mean number of alleles) was significantly correlated between allozymes and microsatellites. The Spearman rank correlation ρ-value for H_S was 0.395 ($P = 0.037$) and for mean number of alleles, $\rho = 0.583$ ($P = 0.002$). Only in several populations were amounts of within-population genetic variation dissimilar between marker types. These include the Big Lost River, which had no microsatellite variation but moderate allozyme variation and Bonneville Basin sites, which had no allozyme variation but moderate microsatellite variation.

We did not find evidence for significant genotypic disequilibrium between microsatellite and allozyme loci. Of 396 total tests, 10 were significant ($P < 0.05$), where 20 were expected by chance. There was no pattern of significant disequilibrium within any of the population samples or for any of the locus pairs across populations.

Divergence among populations

Broad geographic subdivisions

Allozymes—

There was a large degree of genetic subdivision across the range of mountain whitefish with allozymes ($F_{ST} = 0.689$, 95% C.I.: 0.340, 0.863; $F2_{ST} = 0.698$, 95% C.I.: 0.343, 0.867). For

the 29 sites analyzed, principle components analysis revealed what appear to be three primary clusters of populations, or cohesive genetic assemblages (Figure 2-2a). These assemblages corresponded to the upper Missouri River, the upper Snake River, and the Cascadia region (*sensu* McPhail and Lindsey 1986 but extending further south and including populations from Nevada to northern British Columbia and Alberta; Figure 2-2a). The most genetically divergent upper Snake sites came from the Big Lost River. The one site analyzed with allozymes west of the Cascade Mountains was not differentiated from other Cascadia sites. The AMOVA based on PCA groups partitioned much more variation among groups and less variation among sites within groups than the arrangement based on cutthroat trout subspecies (Table 2-2). A dendrogram based on CSE genetic distances depicted the same three cohesive genetic assemblages as were revealed by PCA (data not shown).

Microsatellites—

The mean global F_{ST} for microsatellites was 0.369 (95% C.I. 0.343, 0.393). The mean global $F2_{ST}$ was slightly greater (0.434, 95% C.I. 0.386, 0.466). The mean global R_{ST} estimate for microsatellites (0.237) had an extremely large 95% confidence interval (-0.538, 1.012). R_{ST} was significantly greater than F_{ST} at *ONE8* (0.849 > 0.388; one-sided $P = 0.003$) and at *SFO8-1* (0.727 > 0.379; one-sided $P = 0.003$) indicating that stepwise-like mutations contributed to among-population differentiation at these two loci. The large variation in overall R_{ST} was primarily due to the low value (0.165) observed for *SSA456*.

Principle components analysis of microsatellite allele frequencies revealed five cohesive genetic assemblages for the 62 sites analyzed (Figure 2-2 b and c). The five major assemblages contained populations found in: 1) the upper Snake River, 2) the upper Missouri River, 3) rivers that lie between the Cascade Mountains and the Continental Divide and extend from Nevada to northern British Columbia and Alberta ("Inland Cascadia"), 4) rivers to the west of the Cascade Mountains, excluding the Olympic Peninsula ("Coastal Cascadia"), and 5) rivers of the Olympic

Peninsula (Figure 2-2 b and c). The AMOVA based on PCA groups partitioned more variation among groups and less variation among sites within groups than the arrangement based on cutthroat trout subspecies (Table 2-2). The UPGMA dendrogram based on CSE distances and microsatellite allele frequencies depicted the same large-scale genetic groups as our principle components analysis, but provided better resolution of differentiation within groups (see below; Figure 2-3).

Comparison of Markers—

The PCA of combined microsatellite and allozyme allele frequencies for the 29 sites analyzed with both marker types reveals three large-scale genetic assemblages. The combined PCA showed a clear separation of the upper Missouri, upper Snake, and Cascadia genetic groups (Figure 2-2d). The Coastal Cascadia site was not separated from Inland Cascadia sites for principle component axes 1 and 2 but was separated on PC 4, which explained 6% of the variation (data not shown).

Variation among populations within assemblages

Allozymes—

For allozymes, mean pairwise F_{ST} and CSE values were significantly greater in the upper Snake River group than the Cascadia or upper Missouri groups (Table 2-3). Bayesian Analysis of Population Structure (BAPS) revealed a total of eight clusters for the allozyme data (marginal posterior probability = 0.91; Table 3). All of the most likely data partitions contained eight population clusters. Of these, the two most likely data partitions (probability of 0.34 *vs.* 0.24) differ only by the placement of the Bow River. In the most likely data partition, the Bow River was placed with sites from the Columbia River. In the second most likely partition, the Bow River was placed with a different BAPS-defined cluster that consisted of sites from the Columbia

River and the Lahontan Basin in Nevada. Each PCA group had a similar number of BAPS-defined clusters (Table 2-3, Figure 2-4).

Microsatellites—

For microsatellites, pairwise F_{ST} and CSE values were greatest for the Olympic Peninsula group and lowest for the Coastal Cascadia group (Table 2-3), but statistical tests of significance were not possible because only two sites were analyzed for each of these groups. Mean pairwise F_{ST} and CSE values were significantly greater in the upper Snake River group than in the Inland Cascadia and upper Missouri groups (Table 2-3).

When we applied BAPS to the microsatellite data set, a total of 29 population clusters had the highest marginal posterior probability (0.83). The two most likely partitions of the data (probabilities of 0.54 *vs.* 0.28) only differed by the placement of the lower Clark Fork River site with 1) sites from the Fraser and Columbia Rivers in British Columbia or 2) other sites from the Clark Fork River. The Inland Cascadia PCA group had the greatest number of BAPS-defined clusters (Table 2-3) but also had the greatest number of sites (Table 2-3) and occurred over the largest geographic area (Figure 2-4b). The upper Snake River group had a large number of BAPS groups (Table 2-3) within a small geographic area (Figure 2-4b).

Geographic scale of genetic differentiation

Genetic and geographic distances were significantly correlated for allozymes (data not shown) and for microsatellites within the Columbia River system (Figure 2-5; data not shown for CSE). The mean and variance of pairwise genetic distance values increased between approximately 300 km and 500 km (Figure 2-5; allozyme data and microsatellite CSE data not shown). For the test for a "switch point" in this relationship, the X^0 value that gave the highest adjusted R^2value was 350 km (adjusted $R^2 = 0.257$). A partial *F*-test of β_3 with $X^0 = 350$ km was highly significant ($P < 0.001$). This discontinuity in the relationship between genetic and

physical distance appeared to correspond approximately to comparisons within river basins (mean geographic distance ± SE = 242 km ± 23 km) versus comparisons among river basins (mean = 1,313 km ± 32 km; Figure 2-5). Means and standard deviations of genetic distance values (pairwise F_{ST} and CSE) were less for comparisons within basins than for comparisons among basins (Table 2-4).

2.5 DISCUSSION

What is the genetic structure of mountain whitefish in northwest North America?

Distribution of genetic variation

The distribution of genetic variation we observed across the range of the mountain whitefish was influenced by historical factors at the range-wide scale while aspects of the ecology and life history of this species appeared to interact with landscape features at a smaller geographic scale (within cohesive genetic assemblages). We observed a large proportion of genetic variation partitioned among large-scale genetic assemblages and a large proportion of genetic variation within populations. Relative to other salmonid species, we observed fairly low levels of differentiation among populations within assemblages.

We observed a large range of values of within-population genetic variation (Table 2-1). Populations in the Clark Fork and Missouri Rivers consistently had the highest values. Populations with low values were usually from sites known to be physically isolated. For example, the Big Lost River is part of the isolated "sinks" basins in southeastern Idaho that flow underground before joining with the Snake River. The Big Wood River in Idaho and the Bull River and Thutade Lake in British Columbia are all isolated by barrier waterfalls.

The correlation we observed between amounts of within-population genetic variation for both marker types suggests that this variation reflects the effects of evolutionary and demographic factors on the entire mountain whitefish genome. The exception to this general pattern in the Big Lost River may be due to large N_e at the *MDHB-1,2** isolocus (Allendorf and Thorgaard 1984).

In the Bonneville Basin, more microsatellite alleles may have been retained and/or mutations may have subsequently restored variation at microsatellite loci following the founding event approximately 30,000 years ago (McPhail and Lindsey 1986).

Among population divergence

Genetic differentiation occurred in a hierarchical manner across the range of the mountain whitefish. At the broadest geographic scale, we found evidence of substantial genetic differentiation among regions (Figures 2-2, Figure 2-3) consistent with the multi-refugia hypothesis of McPhail and Lindsey (1986). The upper Snake, upper Missouri, Columbia (east of west of the Cascade Mountains), and Chehalis River on the Olympic Peninsula are all proposed refugia during the most recent continental glaciation (McPhail and Lindsey 1986).

Within major assemblages, the landscape template and hierarchical organization of river basins appeared to have shaped the geographic scale and patterns of genetic differentiation. Sites within the same or adjacent river basins tended to cluster together (Figure 2-3, Figure 2-4). The significant discontinuity in the relationship between genetic versus geographic distance at 350 km within the contiguous Columbia River (Figure 2-5), corresponded approximately to comparisons made within river basins. This pattern suggests that genes are exchanged among populations within river basins much more often than among populations in separate river basins. We also observed increased genetic differentiation among sites located within river basins but separated by geomorphic barriers. These isolated sites tended to be as differentiated from other populations in the same basin as populations in different basins were from each other (Figure 2-5). This suggests that gene flow is reduced among river basins to a similar extent as barriers reduce gene flow within river basins.

In general, we found little evidence of differentiation among sites within major river basins (Figure 2-3, Figure 2-4). An exception to this pattern occurred in the upper Snake River and on the Olympic Peninsula, where mountain whitefish populations were more finely

subdivided than elsewhere (Table 2-3). The most likely cause of this increased subdivision is natural restrictions to gene flow, either due to geomorphological discontinuities or to saltwater barriers to dispersal. The upper Snake River Plateau has a complex geomorphological history (McPhail and Lindsey 1986; Johnson 2002). In addition to the isolation of the Big Lost, the Henrys Fork site is above an impassable waterfall (Mesa Falls), and Bonneville Basin sites are currently isolated from the upper Snake River. Thus, population isolation due to the fragmented physical template might be responsible for the high genetic differentiation observed in this region. On the Olympic Peninsula, gene flow among sites may be limited because mountain whitefish apparently are not saltwater tolerant, an inference made by McPhail and Lindsey (1986) based on distributional data.

Intolerance to saltwater may explain genetic patterns for mountain whitefish in two other instances. First, we observed significant differentiation of Olympic Peninsula sites from other Columbia River sites west of the Cascade Mountains. These rivers are geographically close and we would expect greater genetic similarity if oceanic dispersal were possible. Second, the site we analyzed from the lower Fraser River (Chilliwack) grouped with other Fraser River and Columbia River sites (Figure 2-3, Figure 2-4) instead of grouping with coastal sites. This pattern is consistent with dispersal through inland freshwater dispersal routes rather than an oceanic route (McPhail and Lindsey 1986).

How do patterns of genetic differentiation compare among species?

Concordance of patterns

There are several examples of concordant patterns of genetic differentiation between mountain whitefish and other species that improve our understanding of both species. For example, mountain whitefish and bull trout populations in the Snake River upstream from Hells Canyon and downstream from Shoshone Falls exhibit concordant patterns of genetic differentiation. Bull trout populations from this region (from the Malhuer, Boise, and Jarbidge

Rivers) lie within the Inland Cascadia group but are genetically differentiated from other sites (Spruell et al. 2003). Similarly, mountain whitefish populations from this region (from the Malhuer, Boise, and Big Wood Rivers) also lie within the Inland Cascadia group but are differentiated from other sites (Figure 2-3, Figure 2-4). The three dams in this section of the Snake River (constructed between 1958 and 1967) might be responsible for these observations. However, it seems unlikely that these dams are the sole cause of these patterns, given the short time scale. The differentiation observed for each species likely predates the construction of these dams and may be due to historically reduced gene flow through Hells Canyon.

Several salmonid species in the Pahsimeroi River provide another example of parallel patterns of genetic divergence. The Pahsimeroi River is spring-dominated and differs environmentally from the Salmon River and adjacent tributaries. Populations of steelhead and Chinook salmon in the Pahsimeroi River are genetically differentiated from other populations in the Salmon River (NOAA 2003), but a history of hatchery stocking potentially confounds these among-population genetic relationships. The spring-dominated nature of this system has led others to suggest that the genetic signal of among-population differentiation of both species at this site might reflect local adaptation and historically reduced gene flow (NOAA 2003). The genetic differentiation we observed between mountain whitefish from the Pahsimeroi River and other sites in the Salmon River (Figure 2-3, Figure 2-4) provides an unusual example of genetic differentiation at a small geographic scale for the mountain whitefish. It is possible that populations of steelhead, Chinook salmon, and mountain whitefish from the Pahsimeroi River are all genetically differentiated from other nearby sites due perhaps to the environmental characteristics of this site.

Difference in patterns

Differences among species in patterns of genetic differentiation may reflect species-specific biological differences in responses to factors that can reduce gene flow. For example, an

inland/coastal genetic split corresponding to the Coast in British Columbia and the Cascade Mountains in Oregon and Washington has been observed in studies of rainbow trout (Allendorf and Utter 1979; McCusker et al. 2000), bull trout (Taylor et al. 1999; Spruell et al. 2003), cutthroat trout (Allendorf and Leary 1988), Chinook salmon (Teel et al. 2000), coho salmon (*Oncorhynchus kisutch*; Small et al. 1998), and longnose suckers (*Catostomus catostomus*; McPhail and Taylor 1999) as well as amphibians (e.g. Good 1989; Nielson et al. 2001). For fishes, there are some species-specific differences in where this split occurs (e.g. Spruell et al. 2003). Patterns for mountain whitefish from coastal sites differ in two ways from previously studied species. First, populations in the lower Fraser River belong to the coastal assemblage for other fishes (Small et al. 1998; McPhail and Taylor 1999; Taylor et al. 1999; Teel et al. 2000) rather than the inland assemblage as we observed for mountain whitefish. Second, we observed greater differentiation between sites on the Olympic Peninsula and other coastal sites than has been observed for other species (e.g. Spruell et al. 2003). Both of these observations may be due to the absence of oceanic dispersal for mountain whitefish. In both cases, biological aspects of mountain whitefish may be responsible for differences in genetic patterns and these differences have implications for conserving historical relationships among populations. For example, mountain whitefish in the lower Fraser River would belong to an inland ESU, while other species in the same river would belong to coastal ESUs.

Overall patterns of genetic differentiation for mountain whitefish differed from those of cutthroat trout subspecies, as indicated by the AMOVA (Table 2). This lack of concordance is largely due to three instances where cutthroat subspecies populations are more genetically differentiated than sympatric mountain whitefish populations. However, we also found one striking example where mountain whitefish populations are more genetically differentiated than those of a cutthroat trout subspecies.

First, the westslope cutthroat trout (*O. c. lewisi*) occurs in the Columbia River basin west of the Continental Divide and in the upper Missouri basin to the east, with the exception of the

Yellowstone River (Allendorf and Leary 1988). With allozymes, populations of westslope cutthroat trout are generally highly genetically differentiated from each other on each side of the Continental Divide, such that populations tend to be as differentiated from one another on the same side of the Divide as they are on opposite sides of the Divide (Leary et al. 1988). It is unclear if there should be one or two ESUs for this subspecies because, with allozymes, the genetic signal of regional differentiation on opposite sites of the Divide may have been obscured by genetic drift in small populations. Regional differentiation reflecting two ESUs may be observed if sequence data were collected. In the absence of sequence data, the genetic differentiation of mountain whitefish populations separated by the Divide suggests that hierarchical genetic differentiation may occur for the westslope cutthroat trout and two ESUs may exist.

Second, Lahontan cutthroat trout (*O. c. henshawi*) in the Great Basin are also a genetically differentiated subspecies (Allendorf and Leary 1988), while mountain whitefish in the Great Basin are part of the Inland Cascadia genetic assemblage. It is possible that populations of both species have been isolated from other Inland Cascadia sites for the same amount of time but differentiation of mountain whitefish populations has not occurred as rapidly due to larger N_e. Thus, mountain whitefish populations might provide a better reflection of historical relationships in this case as well.

Third, the similarity we observed between mountain whitefish populations in the Yellowstone River and the remainder of the upper Missouri River contrasts markedly with the genetic divergence of cutthroat trout subspecies in these two rivers (Yellowstone cutthroat trout, *O. c. bouvieri* in the Yellowstone River and westslope cutthroat trout in the remainder of the upper Missouri River; Allendorf and Leary 1988). In this case, two distinct cutthroat trout subspecies lie within what would be one upper Missouri mountain whitefish ESU. Biological differences, including the possibility of greater historical movement, as well as larger N_e of mountain whitefish are likely responsible for these differences.

In contrast, populations of Yellowstone cutthroat trout in the Yellowstone and upper Snake Rivers are less genetically differentiated than populations of mountain whitefish. Yellowstone cutthroat trout in these two river basins are only slightly genetically differentiated at allozyme loci (Allendorf and Leary 1988). The large degree of genetic differentiation we observed for mountain whitefish populations in these two river basins suggests that Yellowstone cutthroat trout in the Yellowstone River and upper Snake River may be more genetically divergent than indicated by allozymes and perhaps mtDNA or microsatellites would provide further resolution of population relationships.

How does the geographic scale of genetic differentiation compare among species?

For other native inland fishes studied to date, genetic variation is often partitioned among regions, among river basins within regions, among large rivers within river basins, and among tributaries within large rivers (Allendorf and Utter 1979; Allendorf and Leary 1988; Taylor et al. 1999; McCusker et al. 2000; Teel et al. 2000; Waples et al. 2001; Costello et al. 2003; Spruell et al. 2003; Taylor et al. 2003; Waples et al. 2004). Thus, relative to mountain whitefish, these other salmonid species are subdivided on a finer geographic scale and gene flow appears to extend over smaller portions of landscape. These species tend to have one, if not two, additional levels of hierarchical organization relative to the mountain whitefish. For example, bull trout and westslope cutthroat trout populations tend to be as genetically differentiated among tributaries within river basins as mountain whitefish populations are among river basins (Costello et al. 2003; Taylor et al. 2003; Whiteley et al. 2004).

Mountain whitefish populations in entire river systems may be part of one large metapopulation (*sensu* Hanski 1999). For example, the entire Columbia River basin might be one large metapopulation of mountain whitefish, while this river system probably contains many metapopulations of other salmonids. With respect to salmonid fishes, metapopulation dynamics have only been considered over much smaller geographic scales for trout, charr, and salmon (e.g.

Rieman and Dunham 2000). The same principles that have emerged from studies of other salmonids (Rieman and Dunham 2000; Dunham et al. 2003; Neville et al. *In press*) may apply to the mountain whitefish, only over much larger temporal and spatial scales.

Do these data provide additional insight for management of other native fishes in northwest North America?

Delineating conservation units requires an understanding of evolutionary relationships among populations (Waples 1995). Following this first step, it must then be determined which populations, or groups of populations, should be the focus of conservation efforts. Regions where genetic patterns for the mountain whitefish were concordant with other species, as we observed for the Snake River upstream from Hells Canyon, warrant conservation designations that reflect the independent evolutionary trajectories of the species in those regions. Regions where genetic patterns for the mountain whitefish were not concordant with other species highlight important evolutionary relationships that might not be currently recognized by conservation efforts. For example, mountain whitefish would belong to different ESUs than other species in the same river systems in several cases. These differences in genetic patterns must be considered to conserve historical relationships among populations of different species in the same systems.

Mountain whitefish populations appear to exchange migrants over a larger geographic scale than other salmonids. Management and conservation efforts should focus at the scale of river basins for this species because this is the scale at which evolutionary processes are likely to be most influential. Co-occurring salmonids should generally be managed at a finer geographic scale (i.e. tributaries within basins). Ideally, effective conservation efforts will work to protect populations of multiple species at all of these levels. Important questions to consider with respect to the geographic scale of genetic differentiation include: What demographic and evolutionary effects will habitat fragmentation (e.g. dams) have on different species? How much connectivity is needed for different species and at what scale? These questions are important for more than

mountain whitefish conservation because the scale of genetic differentiation for mountain whitefish may be similar to other unexamined fishes in this region.

Our work illustrates the importance of considering genetic data from multiple species across the same landscape and including common species in those comparisons for a more comprehensive approach to conservation. We demonstrated how similarities and differences in the scale and patterns of genetic differentiation among species can be used to highlight important evolutionary relationships, to help define species' habitat requirements, and to determine where single-species management is most likely to provide inadequate conservation of other species in an ecosystem. Appreciating these differences in the pattern and scale of genetic differentiation and evolutionary dynamics can enhance the efficacy of region-wide management and conservation plans.

Table 2-1. Genetic diversity and sample statistics for each mountain whitefish population. Populations are arranged from north to south and downstream to upstream within major rivers. *N* is sample size. H_S is average expected heterozygosity.

Location	*N*	Latitude (°N)/ Longitude (°W)	allozymes		microsatellites	
			H_S	Mean Number of Alleles	H_S	Mean Number of Alleles
Mackenzie River						
A. Liard River, BC						
1. Fort Nelson River						
a. Prophet River	19	57.7/123.4	--	--	0.215	1.83
2. Kechika River	27	59.2/127.6	--	--	0.268	3.00
a. Gataga River	21	58.6/126.9	--	--	0.220	3.00
B. Peace River						
1. Smoky River, AB						
a. Wapiti River	29	55.7/118.8	--	--	0.237	2.00
b. Kakwa River	20	54.3/119.5	--	--	0.230	2.00
2. Finlay River, BC						
a. Thutade Lake	19	56.8/127.0	--	--	0.091	1.83
3. Parsnip River, BC	18	55.2/123.1	--	--	0.310	3.50
Stikine River						
C. Klappan River, BC	15	58.0/129.7	--	--	0.186	1.67
Fraser River						
D. Chilliwack, BC	17	49.2/121.9	--	--	0.454	3.33
E. Siska Fish Wheel, BC	10	50.2/121.6	--	--	0.481	3.17
F. Thompson River, BC						
1. Bonaparte River						
a. Machete Lake	20	51.4/120.6	--	--	0.212	2.00
2. North Thompson River						
a. Eagle Creek	10	51.9/120.9	--	--	0.482	3.33
3. South Thompson River						
a. Oliver Creek	12	51.1/120.1	--	--	0.356	2.67
G. Bridge River, BC						
1. Carpenter Reservoir	25	50.9/122.5	--	--	0.329	3.50
Olympic Penninsula						
H. Hoh River, WA	23	47.8/124.2	--	--	0.165	1.83
I. N. F. Skokomish River, WA	30	47.5/123.4	--	--	0.138	1.33
Columbia River Basin						
J. Lewis River, WA						
1. Swift Reservoir	32	46.1/122.2	--	--	0.389	4.33
K. Willamette River, OR	34	46.7/123.2	0.030	1.36	0.343	4.17
L. Deschutes River, OR						
1. Warmsprings River	32	44.9/121.1	--	--	0.413	4.50
M. Walla Walla River, WA						
1. Touchet River	17	46.1/118.7	--	--	0.303	2.33
N. Snake River						
1. Clearwater River, ID						
a. Lolo Creek	21	46.4/116.2	--	--	0.190	2.17
b. S. F. Clearwater River	23	45.8/115.5	--	--	0.272	3.00
c. Lochsa River	20	46.5/114.8	--	--	0.339	3.83
2. Grande Ronde River, OR						
a. Lostine River	27	45.5/117.4	0.040	1.36	0.301	3.50
3. Salmon River, ID						
a. S. F. Salmon River	36	44.7/115.7	--	--	0.324	3.83
b. Pahsimeroi River	28	44.6/113.9	0.031	1.21	0.350	3.33
c. Salmon River at Chalis	25	44.5/114.2	0.045	1.43	0.392	4.00
4. Malhuer River, OR	26	43.9/117.0	--	--	0.361	2.83
5. Boise River, ID						
a. South Fork Boise River	20	43.4/115.6	0.031	1.21	0.460	3.33
6. Big Wood River, ID	20	43.5/114.3	0.002	1.07	0.000	1.00

Table 2-1 (continued).

Location	*N*	Latitude (°N)/ Longitude (°W)	allozymes		microsatellites	
			H_S	Mean Number of Alleles	H_S	Mean Number of Alleles
7. Snake River-Menan, ID	41	43.8/112.0	0.014	1.21	0.394	4.83
8. South Fork Snake River, ID	32	43.7/111.8	0.021	1.29	0.402	4.33
9. Teton River, ID	33	43.8/111.2	0.008	1.07	0.291	2.67
10. Henry's Fork Snake River, ID	41	44.4/111.4	0.002	1.07	0.288	2.17
O. Big Lost River, ID						
1. Lower Big Lost River	26	43.4/113.5	0.026	1.14	0.000	1.00
2. Upper Big Lost River	32	44.2/113.9	0.022	1.14	0.000	1.00
P. Yakima River, WA	22	47.2/120.9	0.038	1.29	0.350	4.17
Q. Clark Fork River, MT						
1. Cabinet Gorge Dam	16	48.1/116.1	0.030	1.21	0.462	3.50
2. Flathead River						
a. Mainstem Flathead River	30	48.4/114.2	0.024	1.21	0.501	3.17
b. Doctor Lake	22	47.2/113.5	--	--	0.343	2.17
3. Ninemile Creek	30	47.0/114.4	--	--	0.497	3.83
4. Rattlesnake Creek	91	46.9/114.0	--	--	0.522	4.17
5. Milltown Dam	20	46.9/113.9	--	--	0.522	3.33
6. Blackfoot River						
a. North Fork Blackfoot River	50	47.0/113.1	--	--	0.538	4.50
7. Rock Creek	42	46.6/113.7	--	--	0.511	3.67
8. Bitterroot River	143	46.3/114.1	0.029	1.21	0.528	4.67
R. Pend Oreille River, BC						
1. Confluence with Columbia River	20	49.5/117.7	0.037	1.21	0.431	3.50
S. Beaver Creek, BC	25	49.7/117.7	--	--	0.411	3.67
T. Kootenay River, BC						
1. Kootenay Lake	21	49.5/116.8	0.033	1.21	0.395	3.83
2. Bull River	20	49.7/115.2	0.015	1.14	0.085	1.17
Saskatchewan River						
U. Bow River, AB	24	50.0/111.7	0.025	1.14	0.214	2.17
Missouri River Basin						
V. Yellowstone River, MT	40	45.5/110.6	0.021	1.29	0.356	3.33
W. Judith River, MT						
1. Big Spring Creek	20	47.1/109.5	--	--	0.412	2.67
2. South Fork Judith River	22	46.8/110.3	0.048	1.29	0.428	2.17
X. Gallatin River, MT	21	45.9/111.5	0.030	1.36	0.490	4.17
Y. Madison River, MT	30	45.0/111.6	0.027	1.29	0.465	3.67
Z. Jefferson River, MT						
1. Bighole River	30	45.9/113.2	0.019	1.14	0.509	4.17
Bonneville Basin						
AA. Logan River, UT	34	41.8/111.8	--	--	0.269	3.50
AB. Weber River, UT	31	41.9/111.5	0.000	1.00	0.361	3.33
AC. Bear River, UT	31	40.9/110.5	0.000	1.00	0.320	2.00
Lahontan Basin						
AD. Walker River, CA, NV	33	38.2/119.1	0.013	1.21	0.090	1.33
AE. Truckee River, NV	12	39.6/119.6	0.018	1.21	0.114	1.33

Table 2-2. Analysis of molecular variance (AMOVA) for allozymes and microsatellites. The first arrangements for both allozymes and microsatellites used the patterns revealed by principle component analysis to define the AMOVA groups. The second arrangements used patterns observed for subspecies of the cutthroat trout. All variance components shown were statistically significant ($P < 0.001$).

Geographical Arrangment	Number of Groups	Variance Component	Percentage of Variation
Allozymes			
1) PCA groups	5	Among groups	65.9
		Among sites within groups	10.8
		Within sites	23.3
2) Cutthroat trout subspecies	4	Among groups	37.5
		Among sites within groups	36.6
		Within sites	25.9
Microsatellites			
1) PCA groups	5	Among groups	31.3
		Among sites within groups	14.6
		Within sites	54.1
2) Cutthroat trout subspecies	4	Among groups	23.2
		Among sites within groups	21.2
		Within sites	55.6

Table 2-3. Genetic differentiation within cohesive genetic assemblages. Allozymes revealed a general Cascadia group, while microsatellites revealed both a Coastal and an Inland Cascadia group. Mean pairwise F_{ST} and CSE values are given with standard deviations in parentheses. Superscripts denote significant differences ($\alpha = 0.05$) with Tukey's post-hoc tests. Estimates of the standard deviation for mean microsatellite genetic distances for the Olympic Peninsula and Coastal Cascadia groups were not possible because only two sites were analyzed. The number of BAPS groups are the same as shown in Figure 2-4.

PCA group	allozymes				microsatellites			
	No. Sites	pairwise F_{ST}	CSE	No. BAPS Groups	No. Sites	pairwise F_{ST}	CSE	No. BAPS Groups
Olympic Peninsula	--	--	--	--	2	0.337	0.210	2
Cascadia	16	0.194 (0.147)[a]	0.050 (0.034)[a]	3	--	--	--	--
Coastal Cascadia	--	--	--	--	2	0.020	0.120	1
Inland Cascadia	--	--	--	--	43	0.215 (0.140)[a]	0.033 (0.014)[a]	16
Upper Missouri	5	0.109 (0.096)[a]	0.037 (0.032)[a]	2	6	0.120 (0.094)[a]	0.027 (0.017)[a]	3
Upper Snake	8	0.376 (0.345)[b]	0.081 (0.083)[b]	2	9	0.317 (0.267)[b]	0.049 (0.032)[b]	7

Table 2-4. Mean pairwise genetic differentiation for within-basin and among-basin comparisons within the Columbia River basin. Number in parentheses is the standard deviation.

Population Comparisons	allozymes		microsatellites	
	F_{ST}	CSE	F_{ST}	CSE
All Populations				
Comparisons Within Basins	0.170 (0.142)	0.035 (0.026)	0.056 (0.071)	0.012 (0.009)
Comparisons Among Basins	0.186 (0.151)	0.050 (0.033)	0.194 (0.119)	0.033 (0.014)
Above Barrier and Small Lake Populations Excluded				
Comparisons Within Basins	0.130 (0.133)	0.023 (0.015)	0.037 (0.034)	0.010 (0.005)
Comparisons Among Basins	0.119 (0.095)	0.035 (0.025)	0.150 (0.078)	0.029 (0.012)

Figure 2-1. Map of mountain whitefish range (shaded area). Black circles represent locations that were analyzed with microsatellites and white circles represent sites that were analyzed with both allozymes and microsatellites.

Figure 2-2. Principle component analysis of a) allozymes, b) and c) microsatellites, and d) microsatellites and allozymes combined. Numbers in parentheses are the proportion of the variation attributable to each component.

Figure 2-3. UPGMA dendrogram based on microsatellite allele frequencies and CSE distances. Bootstrap values greater than 50% are shown. Identities of major genetic groups are shown on their respective branches.

Figure 2-4. Results from Bayesian Analysis of Population Structure (BAPS) across the range of mountain whitefish for a) allozymes and b) microsatellites. The geographic locations of cohesive genetic assemblages identified with principle components analysis are labeled and shaded grey. Each BAPS-defined group has a separate symbol and/or shading. Symbols in a) are independent of those in b).

Figure 2-5. Genetic versus geographic distance for mountain whitefish populations in the Columbia River basin. Within river basin comparisons are shown as filled circles and among river basin comparisons are shown as open circles. Geographic distance was log transformed for the lower panels. a) and c) show all populations in the Columbia River basin. In b) and d), two above barrier sites and one high mountain lake site were removed.

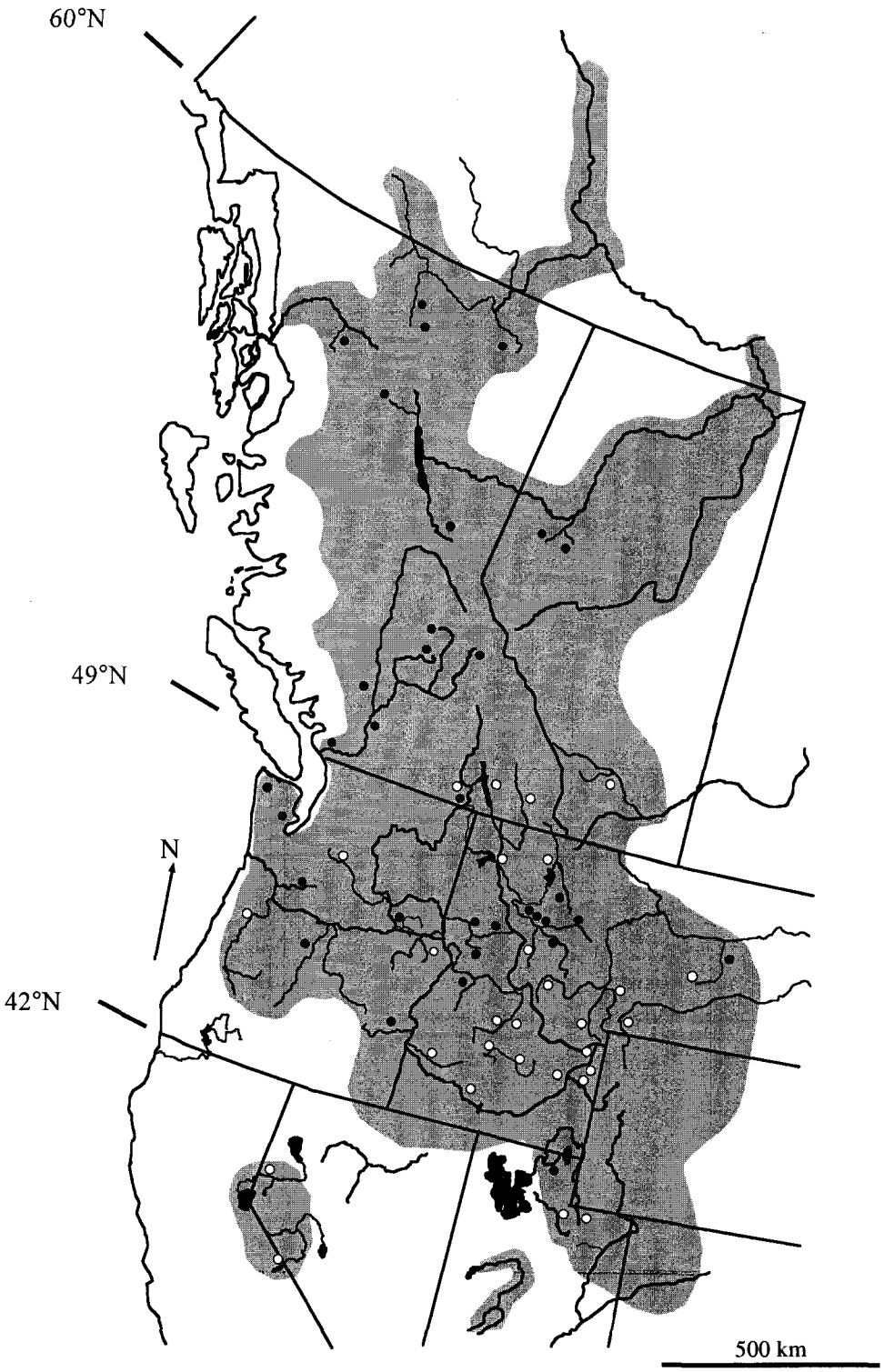
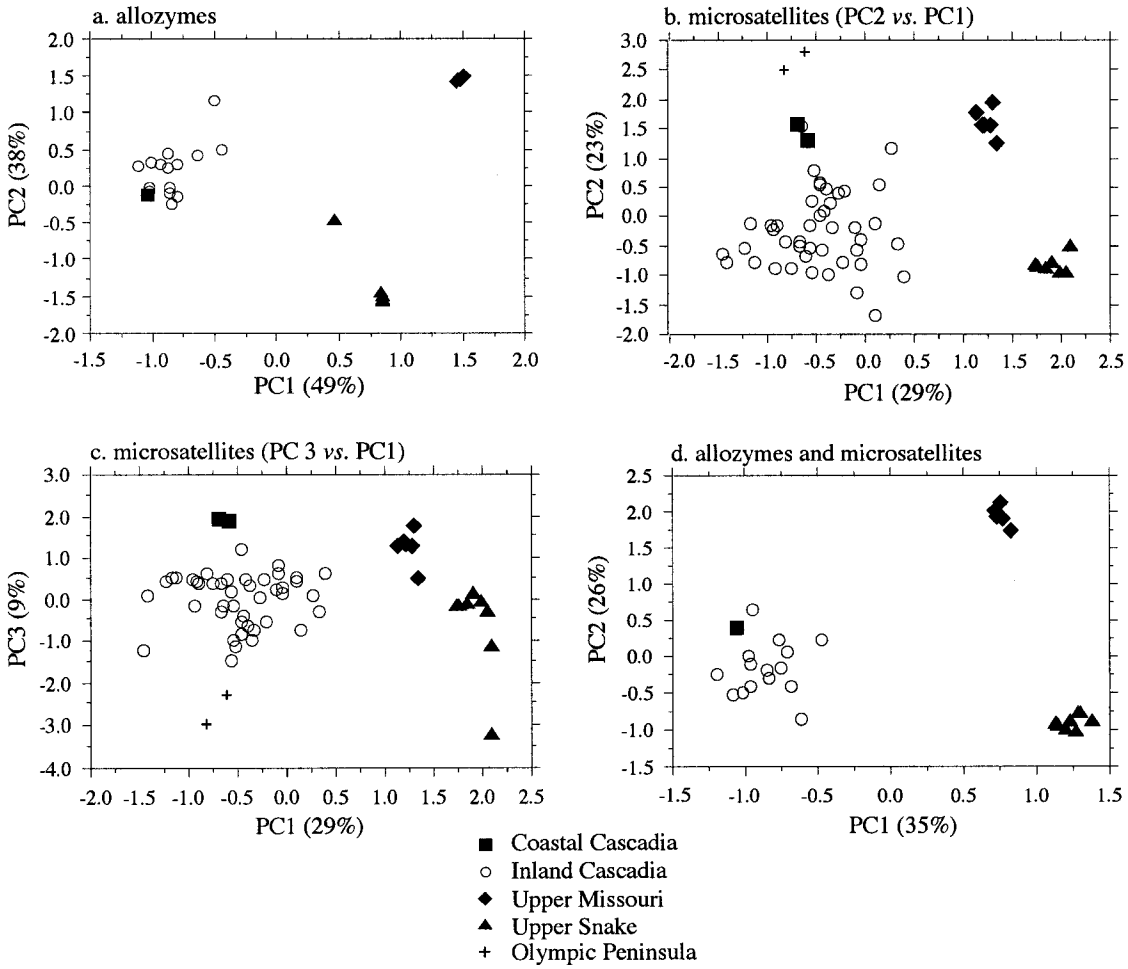


Figure 2-1

Figure 2-2



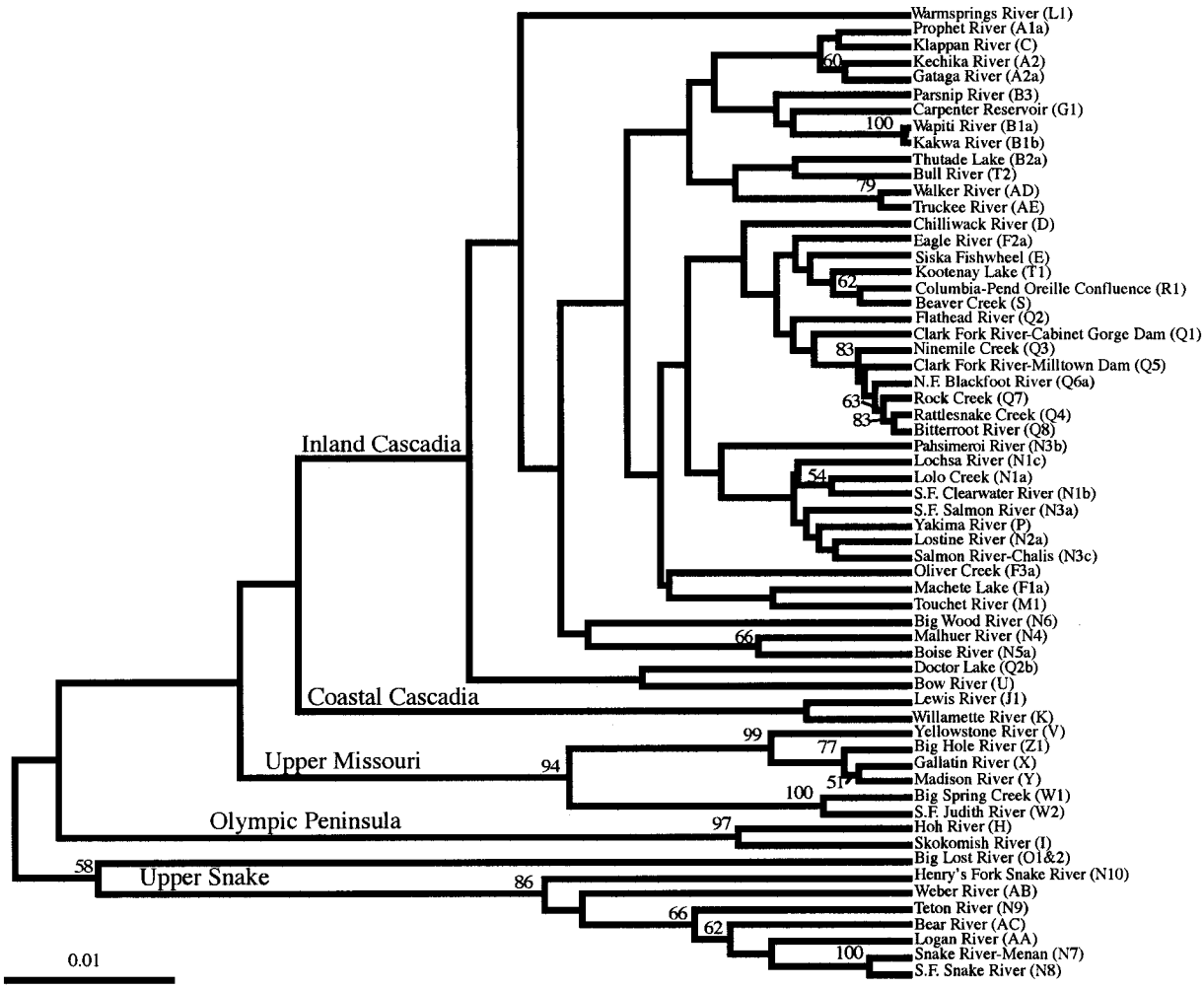


Figure 2-3

(a) allozymes

(b) microsatellites

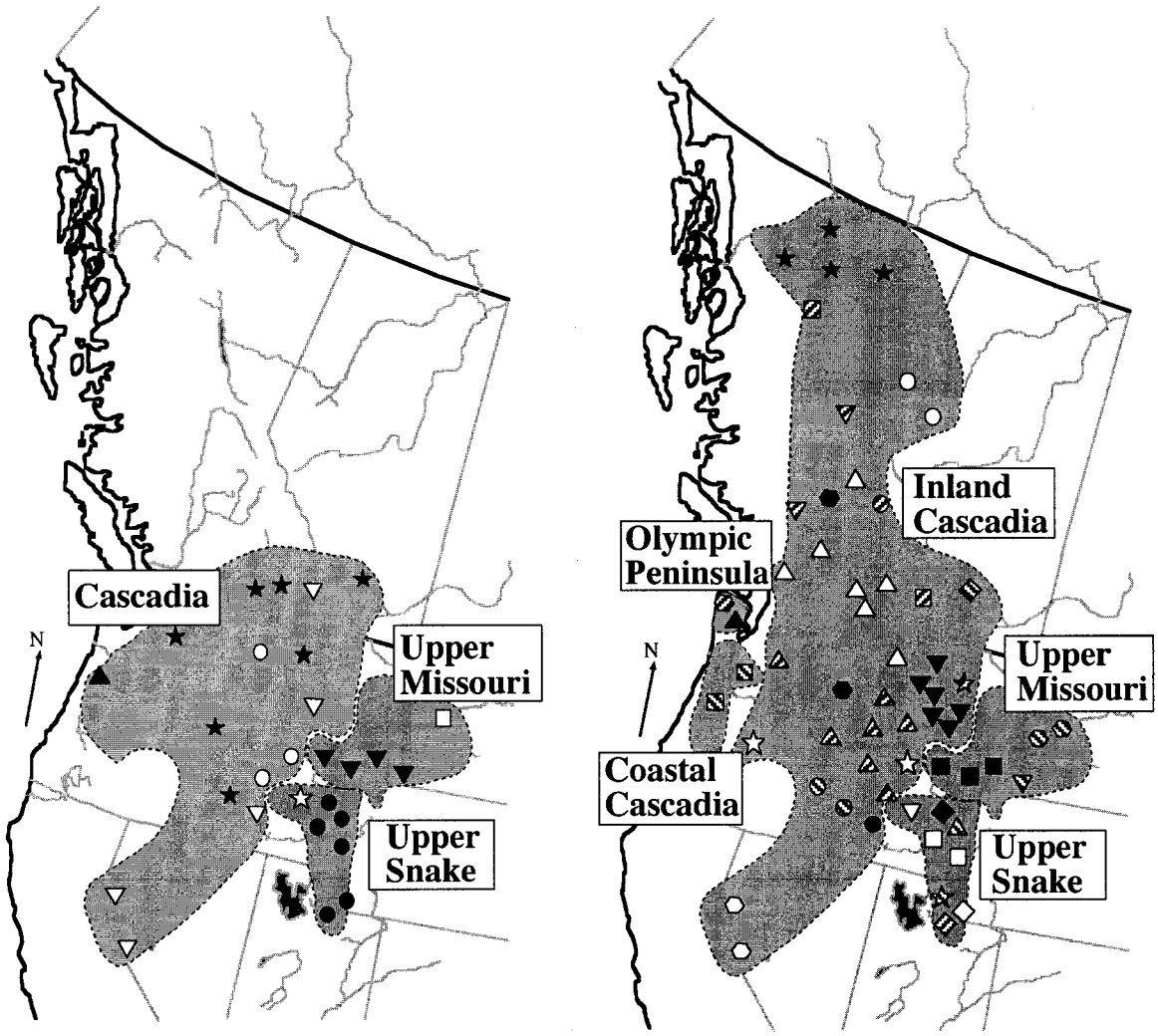
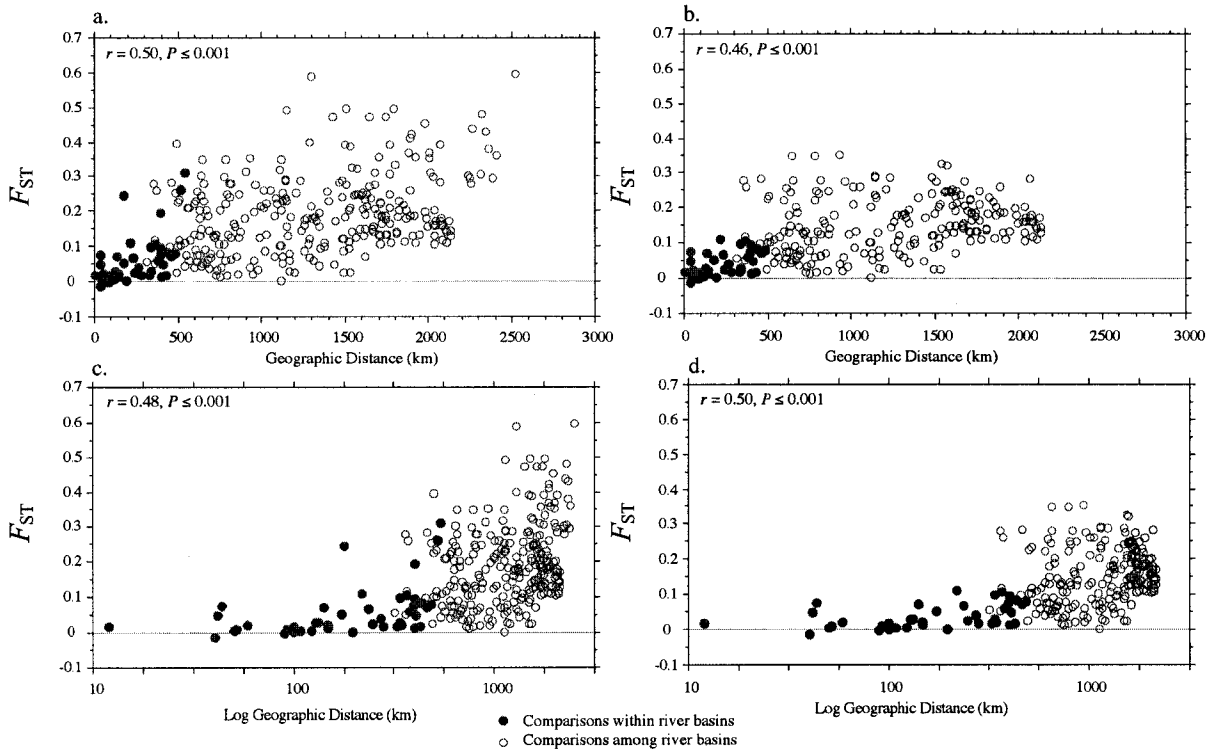


Figure 2-4

Figure 2-5



CHAPTER 3 - Ecological and Life History Characteristics Predict Population Genetic Divergence of Two Salmonids in the Same Landscape

3.1 ABSTRACT

Ecological and life history characteristics such as population size, dispersal pattern, and mating system mediate the influence of genetic drift and gene flow on population subdivision. Bull trout (*Salvelinus confluentus*) and mountain whitefish (*Prosopium williamsoni*) differ markedly in spawning location, population size, and mating system. Based on these differences, we predicted that bull trout would have reduced genetic variation within and greater differentiation among populations compared to mountain whitefish. To test this hypothesis, we used microsatellite markers to determine patterns of genetic divergence for each species in the Clark Fork River, Montana. As predicted, bull trout had a much greater proportion of genetic variation partitioned among populations than mountain whitefish. Among all sites, F_{ST} was seven times greater for bull trout (F_{ST} = 0.304 for bull trout, 0.042 for mountain whitefish) and after removing genetically differentiated high mountain lake sites for each species F_{ST} was 10 times greater for bull trout (F_{ST} = 0.176 for bull trout, 0.018 for mountain whitefish). The same characteristics that affect dispersal patterns in these species also lead to predictions about the amount and scale of adaptive divergence among populations. We provide a theoretical framework that incorporates variation in ecological and life history factors, neutral divergence, and adaptive divergence to interpret how neutral and adaptive divergence might be correlates of ecological and life history traits.

3.2 INTRODUCTION

Analyses of population genetic structure reveal groups of populations that share a common evolutionary history and the geographic scale at which evolutionary processes occur for a species (Waples 1995). Genetic divergence at a series of putatively neutral markers is often used to define management units, identify populations with unusual genetic characteristics, and identify populations with reduced genetic variation that might have reduced probability of persistence (Avise 2004). In addition, genetic differentiation observed at neutral markers can be used as an indicator of adaptive divergence among populations (Fraser and Bernatchez 2001; Morgan et al. 2001). Finally, by comparing the genetic structure of closely related species we can determine if differences in their biology lead to differences in how genetic variation is distribution within and among populations.

Historical factors (e.g. vicariant fragmentation, extinction and recolonization, and range expansion) influence patterns of the distribution of genetic variation of a species and can produce patterns similar to the effects of ongoing gene flow (Felsenstein 1982; Templeton et al. 1995; Hewitt 2000; Turgeon and Bernatchez 2001). In particular, landscape features that disrupt gene flow are often responsible for among-population genetic differentiation, or neutral divergence (Angers et al. 1999; Keyghobadi et al. 1999; Castric et al. 2001; Cassel and Tammaru 2003; Costello et al. 2003). By comparing multiple species in the same environment, the effect of common landscape-level environmental factors on genetic structure can be determined (Bermingham and Moritz 1998). In addition, comparisons of multiple species that inhabit the same landscape allow us to test hypotheses regarding factors other than physical barriers, such as ecological and life history characteristics, that might also influence neutral divergence.

A number of studies have hypothesized that ecological and life history factors such as population size, dispersal pattern, and mating system are related to population genetic divergence

through their effects on genetic drift and gene flow (e.g. Turner and Trexler 1998; McDonald et al. 1999; e.g. King and Lawson 2001; Dawson et al. 2002). There is strong support for an association between dispersal ability and neutral divergence across a wide array of taxa (Peterson and Denno 1998; Bohonak 1999). McDonald *et al.* (1999) demonstrated an association between neutral divergence and habitat related dispersal patterns along with social system in two jays in the genus *Aphelocoma*. Use of aquatic habitat explained dispersal patterns and neutral divergence among three natricine snakes (King and Lawson 2001). Dawson *et al.* (2002) noted a relationship between larval duration, habitat mediated dispersal patterns, and population size with patterns of neutral divergence in two marine gobies (Gobiidae) and many studies of marine organisms have tested for a relationship between larval dispersal ability and neutral divergence (reviewed in Bohonak 1999). In fishes residing in linear stream habitats, Turner (2001) and Turner and Trexler (1998) tested for an association between neutral divergence and life history traits in species of darters (Percidae) and Castric and Bernatchez (2004) found differences in patterns of genetic structure for two salmonids that were expected to differ in dispersal potential in the same landscape. However, the association between genetic subdivision and dispersal patterns, population size, and mating system has not been considered simultaneously in stream dwelling fishes.

Within streams, ecological and life history characteristics should have a large impact on neutral divergence. Spatial separation of reproduction sites will affect dispersal patterns because more closely situated downstream sites are more likely to be encountered by a dispersing individual. In addition, the probability of individual dispersal will be reduced if individuals must navigate through a complex environment to reach spatially separated sites to reproduce. Aspects of the mating system might act as a prezygotic isolating mechanism reducing gene flow because a dispersing individual might have a lower probability of successfully mating in systems with more complex behaviors (i.e. paired matings that involve mate choice versus group spawning without

mate choice). Other aspects of life history that might be important for dispersal are philopatry and specificity of reproductive timing (Avise 2004). Finally, populations of different sizes experiencing the same migration rate (m, defined as the proportion of individuals in each population that are from outside that population), have very different patterns of neutral divergence; larger populations will be much less divergent than smaller populations because the absolute number of migrants per generation ($N_e m$) will be larger and drift will not cause as much population divergence.

Bull trout (*Salvelinus confluentus*) and mountain whitefish (*Prosopium williamsoni*) are two species in the family Salmonidae that co-occur throughout much of western North America (Scott and Crossman 1979). Within the same river systems, these species differ markedly in spawning location, mating system, and population size and thus lie at the extreme ends of a continuum of factors that might influence patterns of dispersal and gene flow. Bull trout spawn in upstream portions of tributary streams that are generally characterized by environmental heterogeneity among locations (Rieman and McIntyre 1995; Swanberg 1997). Mountain whitefish spawn in downstream locations that are less environmentally heterogeneous (Davies and Thompson 1976; Northcote and Ennis 1994). Due to their spawning locations, dispersing bull trout must move further to spawn in adjacent tributary streams than mountain whitefish spawning in river mainstems or near the mouths of tributaries. Bull trout home to natal spawning sites with high precision (McPhail and Baxter 1996; Spruell et al. 1999; Neraas and Spruell 2001). There is some evidence that mountain whitefish return to experimental release sites within the same season (Liebelt 1970) and that they home to spawning locations (Pettit and Wallace 1975). Bull trout spawning migrations must be closely matched to environmental conditions such as seasonally reduced stream flow (Pratt 1992), while there is little evidence of such habitat specificity for mountain whitefish. Bull trout females choose dominant males and the pair spawn in a nest, or redd, often with one to several satellite males involved (Stearley 1992). Mountain

whitefish spawn in groups without digging redds (Northcote and Ennis 1994) and appear to have less complex mating behavior (Brown 1952, Appendix B). Finally, bull trout have small population sizes (Swanberg 1997) while mountain whitefish populations are often very large (Northcote and Ennis 1994). These combined factors should lead to less gene flow among populations of bull trout than mountain whitefish.

In this paper, we compared neutral molecular divergence among populations of bull trout and mountain whitefish from the Clark Fork River, Montana. We predicted *a priori* that mountain whitefish would have greater within-population genetic variation and reduced neutral divergence among populations. We tested this hypothesis by describing the genetic structure of each species using microsatellite markers. We also tested for common landscape factors that influence the distribution of genetic variation in each species. The same ecological and life history factors that allowed us to predict relative amounts of neutral divergence are also consistent with differences in likelihood of local adaptation. We use our results to suggest a general framework for the interactions among ecological and life history factors, neutral divergence among populations, and divergence among populations in traits likely to be important for local adaptation (adaptive divergence).

3.3 MATERIALS AND METHODS

Study Location

The Clark Fork River forms a portion of the headwaters of the Columbia River and has three major tributaries: the Blackfoot, Bitterroot, and Flathead Rivers (Figure 3-1). Bull trout and mountain whitefish occur throughout the Clark Fork River system, including some high mountain lakes. Several dams occur in this system and three are most relevant to fish dispersal in this study. Milltown Dam is located at the confluence of the Blackfoot and Clark Fork Rivers and has blocked upstream movement of both species since 1907 (Schmetterling 2001). Turbines and predatory fish in the upstream reservoir impede downstream movement of juveniles and adults of

both species, although downstream movement of adult bull trout has been observed (Swanberg 1997). Kerr Dam is located at the outlet of Flathead Lake and has blocked upstream fish movement since 1938 and Hungry Horse Dam is located where the South Fork of the Flathead River joins the Flathead River and has blocked upstream movement of fish since 1951.

Sample Collection

Spawning groups of mountain whitefish (Figure 3-1) were collected in 2000 and 2001 by electrofishing. In one case (Rattlesnake Creek, W2a and W2b; Table 3-3) we collected spawning mountain whitefish from the same location in both 2000 and 2001. Care was taken to sample ripe adult fish that appeared to be spawning in the vicinity with the exception of the Flathead River sample (W9), where non-spawning adults were collected from the mainstem Flathead River. Bull trout juveniles were collected in tributary streams (Figure 1) in 1998 and 1999 by electrofishing. Bull trout typically reside in their natal streams for at least one to three years after which they either migrate to larger rivers or lakes or remain in their natal or closely associated stream (Dunham and Rieman 1999; Nelson et al. 2002). By restricting the bull trout collections to juveniles, it is highly likely that each site contained individuals from their natal stream. In addition, it is unlikely that juveniles move between sites at the scale of the comparisons made in our study. For both species, care was taken to minimize the occurrence of siblings or the representation of single cohorts in each sample. In general, the samples were distributed across at least three age classes. Both species were collected from the same tributary in two cases (B2, W2 and B3, W4; Figure 3-1). Fin tissue was collected and stored in 95% ethanol until DNA extraction.

Microsatellites

The general methods used for PCR and visualization of subsequent PCR products followed Spruell *et al.* (1999) and Neraas and Spruell (2001). The seven variable microsatellite

loci used for bull trout (*SCO19*, *FGT3*, *SSA456*, *SSA311*, *SFO18*, *BT73*, and *OGO2*, and *ONE*μ7) were described in Spruell *et al.* (1999) and Neraas and Spruell (2001). DNA was extracted from each fin clip by standard methods. All PCRs were performed using an MJ thermal cycler. We visualized fluorescently-labeled PCR products on acrylamide gels and used individual fish of known genotypes as standards for scoring.

The following microsatellites were optimized for use for mountain whitefish: *COCL4*, *SSA14*, *SSA456*, *ONE8*, *FGT25*, *BT73*, *SFO8-1*, and *SFO8-2* (Table 3-1). We confirmed disomic Mendelian inheritance for all eight loci using three mountain whitefish families, each with 10 offspring. Parents for these families were collected in 2000 from site W2 (Figure 3-1). For *SSA456, FGT25,* and *BT73,* the following thermal cycler profile was used: 93°C for 3m, 92°C for 1m, variable annealing temperature (listed in Table 3-1) for 1m, and 72°C for 1m, with the number of cycles listed in Table 1. For the remaining loci, we used variations of the following touchdown PCR profile (Don et al. 1991): 96°C for 5m, 94°C for 10s, variable initial annealing temperature for 35s (Table 3-1), and 72°C for 1m for seven cycles during which the annealing temperature was decreased 1°C per cycle. At the lower annealing temperature listed in Table 3-1, a variable number of cycles (Table 3-1) were performed with the following profile: 94°C for 10s, variable annealing temperature for 35s, and 72°C for 1m. A final extension period of 72°C for 10m was used for all profiles.

Data Analysis

Allele frequencies, deviations from Hardy-Weinberg expectations, genotypic linkage disequilibrium, observed (H_O) and expected (H_E) heterozygosity per locus and population, mean within-population expected heterozygosity (H_S), mean allelic richness per population, pairwise exact tests for genic differentiation, *F*-statistics and pairwise F_{ST}'s were calculated using GENEPOP ver. 3.4 (Raymond and Rousset 1995) and FSTAT ver. 2.9.3.2 (Goudet 2001). We used θ (Weir and Cockerham 1984) for estimates of F_{ST}. Confidence intervals (95%) for

multilocus F_{ST} estimates were generated by bootstrap sampling over loci (Goudet et al. 1996). We used F_{ST} instead of R_{ST} because F_{ST} estimates are more conservative when relatively few microsatellite loci are used (< 20) and populations have diverged recently (Gaggiotti et al. 1999). We adjusted the results from tests for conformation to Hardy-Weinberg proportions and genotypic linkage disequilibrium for multiple tests using the sequential Bonferroni procedure (Rice 1989). We determined the average number of loci for which we could reject the null hypothesis that allele frequency distributions were the same between populations (determined using pairwise exact tests for genic differentiation from GENEPOP ver. 3.4) at the $P < 0.05$ and $P < 0.001$ levels for both species.

We used PHYLIP ver 3.5 (Felsenstein 1993) to calculate Cavalli-Sforza and Edwards' (1967) genetic distance (CSE) with the GENDIST module and to construct a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram using the NEIGHBOR module. CONSENSE was used to generate a consensus tree with bootstrap values from 1000 replicate data sets created in SEQBOOT. We chose to analyze genetic divergence between populations using CSE because it is drift based, does not assume any models of mutation, and performs well in simulations of microsatellite data (Takezaki and Nei 1996).

We used Mantel tests with 5000 replicates to compare matrices of both CSE distance and pairwise F_{ST} estimates to a matrix of geographic distance using the program Isolation By Distance (IBD, Bohonak 2003). We considered the relationship between genetic and physical distance with and without high mountain lake sites for each species because the differentiation we observed for these sites appeared to be due to factors other than geographic distance alone. We estimated river distances among sample locations using digital topographic maps from National Geographic TOPO! ver. 2.7.4.

3.4 RESULTS

Bull trout

We analyzed bull trout from seven locations at seven microsatellite loci. There were six river sites (B1-B6; Figure 3-1, Table 3-2) and one high-mountain lake site (Trout Lake, B7; Figure 3-1, Table 3-2). Average within-population expected heterozygosity (H_S) ranged from 0.073 to 0.394 and mean allelic richness ranged from 1.1 to 2.8 (Table 3-2). The location with the least amount of genetic variation was Trout Lake (H_S = 0.073, allelic richness = 1.1). Meadow Creek (B4) had the highest heterozygosity (0.441) and Rock Creek (B3) had the highest allelic richness (2.8).

We did not detect any significant departures from Hardy-Weinberg proportions (P > 0.05) for bull trout. Three tests for genotypic disequilibrium yielded *P*-values less than 0.05. There was no pattern of significant disequilibrium within any of the population samples or for any of the locus pairs across populations and none of the differences was significant after sequential Bonferroni correction (0.05/21 comparisons per population sample with seven loci).

Variation in allele frequencies and thus genetic differentiation among bull trout sample locations was pronounced (F_{ST} = 0.304, 95% C.I. 0.212-0.382; Table 2, Table 4). The high mountain lake (Trout Lake, B7) was the most genetically differentiated site (Figure 2A). Even with this site excluded, bull trout had a large proportion of genetic variation partitioned among sites. F_{ST} for the six river sites (B1-B6) was 0.176, (95% C.I. 0.131-0.213; Table 3-4). For tests of homogeneity of population allele frequencies at the seven loci analyzed, on average 5.2 loci were statistically significantly different at the $P < 0.05$ level and on average 3.6 loci were significantly different at the $P < 0.001$ level. When the Trout Lake sample (B7) was removed, an average of 5.1 loci were statistically significantly different at the $P < 0.05$ level and 3.4 loci were statistically significantly different at the $P < 0.001$ level. When we combined *P*-values for the

exact tests for population differentiation from all seven loci, all pairwise comparisons were highly significant ($P < 0.0001$).

The average geographic distance between sites B1-B6 was 261.4 km ± 26.9 km SE and the average distance between these six sites and Trout Lake was 559.8 km ± 44.7 km SE (Figure 3-3A). We found a significant relationship between pairwise CSE values and geographic distance for bull trout ($r = 0.80$, $P \leq 0.001$) when all comparisons were considered (Figure 3-3A). When Trout Lake was removed, the relationship between pairwise CSE values and geographic distance was not significant ($r = 0.39$, $P \leq 0.19$). Results were similar if pairwise F_{ST} was used as the genetic distance metric ($r = 0.74$, $P \leq 0.04$ for all comparisons; $r = 0.26$, $P \leq 0.25$ when Trout Lake was removed) or when geographic distances were log transformed (data not shown).

Mountain whitefish

We used eight microsatellites to analyze mountain whitefish from 10 locations (Table 3-3). There were nine river sites (W1-W9; Figure 3-1, Table 3-3) and one high-mountain lake site (Doctor Lake, W10; Figure 3-1). We detected greater genetic variation within populations of mountain whitefish than bull trout. H_S ranged from 0.403 to 0.580 and mean allelic richness per population ranged from 2.5 to 5.2 (Table 3-3). Doctor Lake had the lowest allelic richness and the lowest H_S. We detected the greatest heterozygosity at site W7 (0.580) and the greatest allelic richness at the site W5 (5.2).

All mountain whitefish population samples conformed to Hardy-Weinberg proportions ($P > 0.05$ for all exact tests). Five tests for genotypic disequilibrium had P-values less than 0.05. When we corrected (Rice 1989) for the 28 comparisons made for each population (0.05/28 comparisons per population sample with eight loci) none of the tests was significant. In addition, no pattern was evident for genotypic disequilibrium either within a sample or for a pair (or pairs) of loci.

Allele frequencies were relatively homogeneous among mountain whitefish sample sites (Table 3-3) and genetic differentiation among sites was low (F_{ST} = 0.042, 95% C.I. 0.028-0.061; Table 4). As was observed in bull trout, the high mountain lake (Doctor Lake, W10) was the most genetically divergent site (Figure 3-2). Differentiation among sites was reduced when Doctor Lake was excluded (F_{ST} = 0.018, 95% C.I. 0.012-0.028; Table 3-4).

Theoretical (Hedrick 1999) and empirical studies (O'Reilly et al. 2004; Olsen et al. 2004) have shown that estimates of genetic differentiation among populations using *F*-statistics might be biased low when highly polymorphic loci are used. We calculated estimates of $F2_{ST}$ to determine if the greater number of alleles and higher heterozygosity we observed for mountain whitefish relative to bull trout might have contributed to the lower F_{ST} estimates we observed for mountain whitefish. With $F2_{ST}$, all loci are treated as bi-allelic by using the frequency of the most common allele and pooling the frequencies of all others (McDonald 1994; Allendorf and Seeb 2000). Estimates of $F2_{ST}$ for mountain whitefish were only slightly higher than estimates of F_{ST}. For all sites, $F2_{ST}$ was 0.046 (95% C.I. 0.037-0.058) and for sites W1-W9, $F2_{ST}$ was 0.019 (95% C.I. 0.009-0.032). *BT73,* in particular, was highly variable in mountain whitefish (mean H_E = 0.89). To determine if this locus had a disproportionate effect on our estimates of F_{ST}, we also treated this locus as bi-allelic, without doing so for the remaining loci. This measure led to a slight increase in overall F_{ST} for all mountain whitefish sites (F_{ST} = 0.048 (95% C.I. 0.033-0.065) but not for the F_{ST} estimate for sites W1-W9 (F_{ST} = 0.019, 95% C.I. 0.011-0.029).

The mean number of loci at which population allele frequencies were statistically significantly different between population pairs for the eight loci analyzed was 3.3 ($P < 0.05$) and 2.0 ($P < 0.001$). When Doctor Lake was excluded, an average of 2.6 loci were statistically significantly different between population pairs at the $P < 0.05$ level and an average of 1.2 loci were statistically significantly different at the $P < 0.001$ level. We were unable to reject the null

hypothesis of identical allele frequency distributions for 15 of the 55 pairwise comparisons when all loci were combined ($P > 0.05$).

Despite the low level of genetic differentiation, the mountain whitefish dendrogram shows evidence of spatial structure (Figure 3-2B). The Flathead River site (W9) and the lake site (W10) were genetically divergent from sites W1-W8 and these eight sites clustered closely together (Figure 3-2B). There were no statistically significant differences in allele frequencies among samples W2a, W2b, and W3; nor among sites W5, W6, and W7. These sites were pooled into two groups for Figure 3-2B. The mountain whitefish dendrogram (Figure 3-2B) showed a similar overall topology as the bull trout dendrogram (Figure 2A), though on average CSE distances were substantially less for mountain whitefish (see below).

The geographic scale of the population comparisons for mountain whitefish was similar to the scale for bull trout. The average geographic distance between sites W1-W9 was 202.8 km ± 26.4 km SE. The average pairwise distance between sites W1-W9 and Doctor Lake was 651.5 km ± 22.6 km SE. We found a significant relationship between pairwise CSE values and geographic distance ($r = 0.88$, $P \leq 0.003$) when all comparisons were considered for mountain whitefish (Figure 3-3B). When Doctor Lake was removed, the relationship remained significant ($r = 0.83$, $P \leq 0.039$). There was a break in geographic distance between sites W1-W8 and site W9 (Figure 3-3B). The relationship between pairwise CSE values and geographic distance was not significant when only considering sites W1-W8 ($r = 0.08$, $P \leq 0.35$). Results were highly similar if pairwise F_{ST} was used as the genetic distance metric ($r = 0.79$, $P \leq 0.005$ for all comparisons; $r = 0.79$, $P \leq 0.009$ when Doctor Lake was removed; $r = 0.15$, $P \leq 0.24$ among sites W1-W8 only) or when geographic distances were log transformed (data not shown).

Comparison of genetic distances between species

Average CSE distances were approximately five times greater for bull trout than for mountain whitefish (Figure 3-4; mean CSE for mountain whitefish was 0.035 ± 0.004 SE and for bull trout was 0.192 ± 0.025 SE). With the high mountain lakes excluded, mean CSE for mountain whitefish was 0.024 ± 0.002 SE and for bull trout was 0.129 ± 0.014 SE. Results for pairwise F_{ST} were similar. Mean pairwise F_{ST} for mountain whitefish was 0.059 ± 0.012 SE, while mean pairwise F_{ST} for bull trout was 0.284 ± 0.045 SE. With the high mountain lakes excluded mean pairwise F_{ST} for mountain whitefish was 0.023 ± 0.005 SE, while mean pairwise F_{ST} for bull trout was 0.179 ± 0.029 SE.

3.5 DISCUSSION

We used ecological and life history characteristics of bull trout and mountain whitefish to predict that bull trout would have greater population substructure in the same river system. We were able to control for the effects of historical factors by analyzing both species in the same river system. We found substantial differences in neutral divergence, suggesting that ecological and life history factors, through their effects on the probability of dispersal, are responsible for these results. Reduced gene flow, and perhaps reduced population size and founder effects in high-mountain lakes served as a proximate factor shaping the distribution of genetic variation in a similar manner for each species.

Based on the genetic differentiation we observed we predict that bull trout have greater among-population adaptive divergence than mountain whitefish. The same ecological and life history characteristics that affect neutral divergence for these species might also affect adaptive differences among populations. We combined our results for neutral divergence with predicted differences in adaptive divergence in a framework where ecological and life history characteristics are the driving factors.

Neutral Divergence

Bull trout

We found large differences in allele frequencies among bull trout populations. The degree of genetic differentiation among bull trout populations found in this study is similar to that found in previous studies of bull trout performed at similar geographic scales (within river basins). For example, Costello *et al.* (2003) estimated F_{ST} values of 0.24 and 0.23 for two river systems in British Columbia. Our F_{ST} was also similar to what has been found for other bull trout populations in Montana and Idaho (Spruell et al. 1999; Kanda and Allendorf 2001; Neraas and Spruell 2001). The large F_{ST} we observed is also similar to other inland salmonid species that tend to use headwater habitats (Currens et al. 1990; Angers et al. 1999; Bouza et al. 1999; Carlsson and Nilsson 1999; Taylor et al. 2003).

While the high degree of neutral divergence we observed for bull trout populations might be somewhat exaggerated due to the tendency for reduced variation within populations to inflate measures such as F_{ST} (Hedrick 1999), the pronounced differentiation observed is likely due to the fact that bull trout occur in small subpopulations that are prone to drift and have reduced gene flow because they home with high precision (McPhail and Baxter 1996, Spruell *et al.* 1999, Neraas and Spruell 2001). Ecological and life history characteristics also apparently contribute to neutral divergence in this species. Dispersal probabilities for bull trout are probably low due to the location of spawning sites far upstream in heterogeneous locations that can be difficult to access (both in time and space). It is the product of the proportion of individuals in each subpopulation that are from outside the subpopulation (m) and the effective population size (N_e) that determine F_{ST} (Mills and Allendorf 1996). Small population size will enhance the effect of low individual bull trout dispersal probability on F_{ST} because both N_e and m will be small.

Mountain whitefish

For mountain whitefish, we found that the vast majority of genetic variation occurs within populations with little differentiation occurring among populations. Genetic differentiation among mountain whitefish populations was substantially lower than that observed for bull trout and the reduced differentiation did not appear to be due to greater within-population variation we observed for mountain whitefish. Two non-mutually exclusive hypotheses could explain the genetic patterns we observed for this species: 1) reduced gene flow and little drift due to large N_e or 2) at least moderate gene flow among spawning groups.

We were able to address the first hypothesis because habitat fragmentation by a dam allowed us to estimate N_e for mountain whitefish in this system. Milltown Dam has been a barrier to upstream fish movement in the mainstem of the Clark Fork River since 1907 (Schmetterling 2001). In addition, very few mountain whitefish are able to pass downstream due to turbines and high abundance of predatory fish in the upstream reservoir. We observed very little genetic differentiation among sites located on either side of this dam (among sites W1-W8 $F_{ST} = 0.006$; 95% C.I. 0.002-0.010). The N_e consistent with the observed neutral divergence (F_{ST}) of isolated populations separated for t generations can be determined with the approximation:

$F_{ST} \approx 1\text{-}e^{-t/2Ne}$

(Waples 1998). We used $t = 25$ because we assume the average generation length of mountain whitefish is four years and we assume no gene flow has occurred for approximately 100 years (since the dam was installed). Our assumption of complete isolation might be violated, but gene flow should at least be very close to zero over this time frame. For our observed $F_{ST} = 0.006$, our estimate of N_e is approximately 2000. These data are consistent with large populations that do not diverge at neutral markers because of drift and thus, hypothesis 1 is consistent with low neutral divergence observed.

However, elevated gene flow also appears to be an important factor that prevents allele frequencies from diverging among sites W1-W8 (hypothesis 2). There is very little genetic divergence among sites W1-W8 (mean pairwise F_{ST} for the 28 comparisons among sites W1-W8 is 0.008 ± 0.002 SE) but increased genetic divergence between these sites and the more geographically distant Flathead River site (Figure 3; mean pairwise F_{ST} for the eight comparisons between sites W1-W8 and site W9 is 0.076 ± 0.004 SE). N_e probably does not differ between each of the sites W1-W8 and the Flathead River site (W9). On the other hand, gene flow is likely reduced by geographic distance and the presence of Flathead Lake, a 495 km^2 natural lake. Therefore, N_e is not apparently large enough to prevent divergence among mountain whitefish populations when gene flow is reduced over what are likely longer periods of time. If there were little to no gene flow among sites W1-W8 (hypothesis 1), we would expect as much differentiation among these eight sites as we observed between these sites and site W9. Thus, it appears that reduced drift due to large N_e contributes to the lack of neutral divergence observed for mountain whitefish but high gene flow also prevents genetic divergence.

The combined effects of the ecological and life history factors we have considered (proximity of spawning locations, low complexity of intervening habitat, relative environmental homogeneity of spawning sites, large N_e, and group spawning behavior) appear to lead to the substantial differences in among-population divergence we observed between bull trout and mountain whitefish. Dispersing mountain whitefish are more likely to successfully spawn at non-natal sites (due to the proximity of sites, low complexity of intervening habitat, and their group spawning behavior). In addition, for a given m, F_{ST} will be lower in mountain whitefish than bull trout due to greater N_e of the former. Thus, even if mountain whitefish home at the same rate as bull trout (i.e. m is equal), we would expect to see less differentiation among populations of mountain whitefish.

Nonequilibrium conditions

An alternative explanation for the differences in F_{ST} we observed between bull trout and mountain whitefish is that neither species has reached equilibrium between drift and gene flow. Most natural groups of populations are probably not at equilibrium (McCauley 1993; Hutchison and Templeton 1999; Turgeon and Bernatchez 2001; Kinnison et al. 2002; Ramstad et al. 2004). If nonequilibrium conditions prevail, values of F_{ST} could fluctuate, leading to misguided interpretations about the relative values of F_{ST}. However, given the substantial differences we found, it is highly unlikely that the F_{ST} distributions for these two species would overlap

In addition, populations of each species might not be at equilibrium, but both species should be at a similar point in their progression to equilibrium. It is likely that the Clark Fork basin either served as a glacial refugium for both species or was founded by both species approximately 10,000 years ago, after the continental glaciers receded (McPhail and Lindsey 1986). Thus, both species would have had equal time in which to proceed toward equilibrium. Differences in population size effect time to equilibrium, with larger populations taking longer to achieve equilibrium (Crow and Aoki 1984). This factor is of little consequence within the realistic ranges of N_e for these species in this basin (1000 or more). Thus, even if mountain whitefish exist at an N_e that is an order of magnitude larger than that of bull trout, the effect of this larger population size on time to equilibrium is negligible (Crow and Aoki 1984). Finally, it is unlikely that unusual population dynamics have occurred in this particular river basin because our results are consistent with those observed in other regions for bull trout (Spruell et al. 1999; Kanda and Allendorf 2001; Neraas and Spruell 2001; Costello et al. 2003) and mountain whitefish (ARW unpublished data).

Additional factors

Physical barriers interact with biological factors to influence amounts of gene flow. Fragmentation due to dams can reduce gene flow and cause neutral divergence in stream systems

(Neraas and Spruell 2001). Milltown Dam has reduced movement of bull trout and whitefish in this system for approximately 100 years but has not served as a proximate factor shaping the distribution of genetic variation of either species, probably because these two species lie at opposite ends of the spectrum of population genetic structure. Drift appears to be the dominant factor shaping bull trout genetic structure and overwhelms any reduction in gene flow caused by the dam. Mountain whitefish populations appear to be too large to have increased neutral divergence due to dams over this time scale.

Founding events and reduced gene flow in high mountain lakes appear to act as proximate factors with similar impacts on the genetic structure of each species. The high-mountain lake sites of both species have reduced genetic variation (Table 3-2, Table 3-3) and are genetically divergent (Figure 3-2, Figure 3-3). Bull trout site B7 is separated by one dam and mountain whitefish site W10 is separated by two dams and thus, increased geographic distance and anthropogenic-induced fragmentation by dams might be responsible for these results. However, fragmentation by dams is probably not the only responsible factor, given our results for Milltown Dam. In addition, it is possible that the genetic patterns observed for these lake sites are due to past stocking events. However, bull trout and mountain whitefish are not typically the focus of stocking efforts and for these two species there are no records of stocking either of the lakes considered in this study. Anthropogenic intervention does not appear to be a likely explanation for these data. Other studies of salmonids have found that small high-mountain lakes can influence genetic structure (e.g. Castric et al. 2001) and founding events can cause increased genetic divergence (Hedrick 1999). Both high-mountain sites in our study share characteristics of founding effects (a reduced number of alleles that are a subset of the alleles present in nearby populations). It is likely that historical events associated with the founding of these lakes and subsequent reduced gene flow due the high probability of geomorphological discontinuities at high elevation have contributed to our observations.

Neutral versus adaptive divergence

Reduced gene flow provides conditions favorable to local adaptation if selective differences occur among populations (Lenormand 2002) and both theoretical (Haldane 1948; Slatkin 1973; Felsenstein 1976; Endler 1977; Slatkin 1978; García-Ramos and Kirkpatrick 1997; Hendry et al. 2001) and empirical data (King and Lawson 1995; Storfer et al. 1999; Hendry et al. 2002) suggest that gene flow can constrain adaptive divergence. In addition, empirical results suggest that estimates of neutral divergence from molecular markers (F_{ST}) provide conservative estimates of Q_{ST}, or among-population divergence in adaptive traits (Pfrender et al. 2000; Morgan et al. 2001) Based on our microsatellite data, we would predict that bull trout populations are more locally adapted than mountain whitefish populations in the Clark Fork River, as long as selection acting on bull trout populations is strong enough to overwhelm drift. On the other hand, selection would not need to be strong to overwhelm drift in large mountain whitefish populations, but high gene flow could prevent local adaptation from occurring at this geographic scale. Thus, while mountain whitefish might be adapted at a larger geographic scale (among river basins), within river basins we predict that neutral divergence estimates from molecular markers are correlated with adaptive divergence among populations for these two species.

This system offers some additional insights into the relationship between neutral and adaptive divergence. Neutral divergence and adaptive divergence will be positively correlated in some circumstances. However, adaptive divergence can occur in the absence of neutral divergence (e.g. Mopper et al. 2000). It is possible that both types of divergence are actually covariates of other factors and instead of focusing directly on the relationship between neutral and adaptive divergence, we might increase our understanding by focusing on other factors that actually cause differences in both types of divergence. Causal factors might lead to a reduced probability of dispersal and therefore increased neutral divergence. In addition, the same factors might lead to increased adaptive divergence. In this case, neutral and adaptive divergence would be positively correlated. This general framework could explain why adaptive and neutral

divergence are negatively correlated in some instances. For example, a factor or set of factors might lead to increased adaptive divergence *and* increased dispersal and gene flow and thus reduced neutral divergence.

With respect to bull trout and mountain whitefish, the same ecological and life history characteristics (mating location, mating system, length and extent of stage-specific migrations, and population size) that we used to predict neutral divergence for these species might cause both neutral and adaptive differences among populations. Bull trout have more extensive migrations than mountain whitefish, migrating from rearing to adult feeding habitats and back to spawning habitats in headwater portions of streams. There are more opportunities for disruptions that prevent the completion of this life cycle for bull trout than in the comparatively simple migration and life history pattern of mountain whitefish. In addition to their effects on dispersal potential and thus neutral divergence, these ecological and life history aspects should lead to greater local adaptation of bull trout populations. Once neutral divergence and adaptive divergence arise due to the ecology and life history of an organism, these two elements of genetic structure can interact. For example, increased adaptive divergence might lead to further increases in neutral divergence due to reduced success of migrant genotypes (Ehrlich and Raven 1969; Futuyma and Peterson 1985; Endler 2000; Mopper et al. 2000).

Empirical evidence for an association between local adaptation with ecological and life history factors such as mating system, migration, and/or population size is required to test this framework, as are more data on genetics and life history for a wider variety of species. This framework should apply to a wide array of taxa and mountain whitefish and bull trout offer just one opportunity to test these predictions. Our framework appears to be consistent with observations for other salmonids where there is evidence for local adaptation (e.g. Wood 1995; e.g. Koskinen et al. 2002). For example, Allendorf and Waples (1996) suggested that the high degree of local adaptation observed among populations of sockeye salmon (*Oncorhynchus nerka*) is due to the number of habitats they occupy at various life stages and the complexity and length

of migrations between these habitats. Thus, complexity of migration patterns and of the overall life cycle might lead to adaptive differences among populations of this species. These same factors might lead to a reduced probability of dispersal and subsequent gene flow and thus the high F_{ST} commonly observed for this species (Wood 1995). Finally, adaptive differences among populations might contribute to reduced reproductive success of migrant individuals, acting to ratchet populations to greater neutral divergence.

Much recent debate has centered on whether adaptive or neutral differences among populations should be used for the purpose of defining conservation units (Crandall et al. 2000; McKay and Latta 2002). To understand the relationship between adaptive and neutral divergence, we suggest that more effort should be placed on the identification of factors that directly influence both types of divergence. Variation in ecological and life history factors, when causally associated with adaptive and neutral divergence, might be valuable both as a predictor of neutral divergence and a surrogate for measures of adaptive variation. Understanding the association between ecological and life history variation and neutral and adaptive divergence might allow us to define conservation units more effectively for a broad array of taxa.

Table 3-1. Locus names, number of alleles, size range, annealing temperature, and number of cycles for mountain whitefish microsatellites.

Locus	Number of Alleles	Size Range (bp)	Annealing Temp (°C)[1]	Number of Cycles[2]	Reference
COCL4	3	146-152	57-51	7,29	L. Benatchez pers. comm. 2000
SSA14	5	167-175	57-51	7,29	O'Reilly et al. 1996
SSA456	15	138-232	52	30	O'Reilly et al. 1996
ONE8	6	178-190	60	30	Scribner et al. 1996
FGT25	4	170-180	57-51	7,26	Sakamoto et al. 2000
SFO8-1	3	158-164	55-49	7,31	Angers et al. 1995
SFO8-2	2	195-197	55-49	7,31	Angers et al. 1995
BT73	51	146-280	55	32	Estoup et al. 1993

[1]A range of temperatures indicates a touchdown PCR was used, where the annealing temperature was decreased 1° per cycle for seven cycles starting at the higher temperature. The remainder of the cycles were performed at the lower annealing temperature.

[2]The first number represents the number of cycles where the annealing temperature was decreased 1° per cycle. The second number is the number of cycles at the lower annealing temperature. The total number of cycles is the addition of both numbers.

Table 3-2. Allele frequencies for bull trout in the Clark Fork River. Sample size (*N*), average expected heterozygosities (H_S), and mean allelic richness are shown.

Sample Number	Sample Location	Basin	*SSA456*		*SSA311*		*OGO2*					*FGT3*					
			**157*	**159*	**112*	**120*	**150*	**154*	**156*	**158*	**162*	**165*	**167*	**169*	**171*	**173*	**175*
B1	Thompson River	Clark Fork	0.238	0.762	0.107	0.893	--	0.553	0.447	--	--	0.698	0.093	0.093	--	--	0.116
B2	Rattlesnake Creek	Clark Fork	0.740	0.260	0.135	0.865	0.250	0.231	0.462	0.019	0.038	0.904	--	--	--	0.096	--
B3	Rock Creek	Clark Fork	0.250	0.750	0.318	0.682	0.118	0.206	0.662	--	0.015	0.758	0.030	0.091	--	--	0.121
B4	Meadow Creek	Bitterroot	0.538	0.462	0.481	0.519	0.200	0.380	0.400	0.020	--	0.500	0.269	--	0.038	0.192	--
B5	Monture Creek	Blackfoot	0.569	0.431	0.207	0.793	0.417	0.017	0.550	--	0.017	0.672	0.103	--	--	0.224	--
B6	Copper Creek	Blackfoot	0.857	0.143	0.714	0.286	--	--	1.000	--	--	0.946	--	--	--	0.054	--
B7	Trout Lake	Flathead	0.486	0.514	--	1.000	--	--	1.000	--	--	--	1.000	--	--	--	--

Sample Number	Sample Location	Basin	*ONEμ7*		*SFO18*		*SCO19*					*N*	H_s	Mean Allelic Richness
			**218*	**244*	**150*	**156*	**174*	**200*	**202*	**206*	**216*			
B1	Thompson River	Clark Fork	0.895	0.105	0.962	0.038	0.157	0.800	0.043	--	--	43	0.318	2.4
B2	Rattlesnake Creek	Clark Fork	1.000	--	0.783	0.217	0.462	0.462	--	--	0.077	29	0.344	2.4
B3	Rock Creek	Clark Fork	1.000	--	0.691	0.309	0.448	0.397	0.052	0.017	0.086	34	0.402	2.8
B4	Meadow Creek	Bitterroot	1.000	--	0.940	0.060	0.435	0.391	0.174	--	--	27	0.441	2.6
B5	Monture Creek	Blackfoot	1.000	--	0.786	0.214	0.192	0.712	0.019	--	0.077	30	0.381	2.5
B6	Copper Creek	Blackfoot	0.880	0.120	1.000	--	0.019	0.981	--	--	--	30	0.146	1.7
B7	Trout Lake	Flathead	1.000	--	1.000	--	1.000	--	--	--	--	39	0.073	1.1

Table 3-3. Allele frequencies for mountain whitefish in the Clark Fork River. Sample size (*N*), average expected heterozygosity (H_S), and mean allelic richness are shown. W2a and W2b are from the same location but were collected in successive years. For presentation purposes, the most common allele at *BT73* (**206*) is shown and the frequencies of all other alleles at this locus were combined.

Sample Number	Sample Location	Basin	*ONE8*						*SSA14*					*FGT25*				*SFO8-1*			*SFO8-2*		*COCL4*		
			**178*	**180*	**182*	**184*	**186*	**190*	**167*	**169*	**171*	**173*	**175*	**170*	**176*	**178*	**180*	**158*	**162*	**164*	**195*	**197*	**146*	**150*	**152*
W1	Ninemile Creek	Clark Fork	--	0.050	0.567	0.383	--	--	--	0.050	0.467	0.217	0.267	0.083	--	0.117	0.800	0.317	0.517	0.167	0.917	0.083	0.810	0.155	0.034
W2a	Rattlesnake Creek 2000	Clark Fork	--	0.108	0.578	0.304	--	0.010	0.080	0.020	0.540	0.070	0.290	0.067	--	0.048	0.885	0.385	0.442	0.173	0.875	0.125	0.647	0.255	0.098
W2b	Rattlesnake Creek 2001	Clark Fork	--	0.192	0.564	0.244	--	--	0.026	0.066	0.513	0.171	0.224	0.064	--	0.038	0.897	0.372	0.462	0.167	0.923	0.077	0.641	0.295	0.064
W3	Clark Fork River-Milltown Dam	Clark Fork	--	0.150	0.725	0.125	--	--	0.100	0.100	0.450	0.175	0.175	0.050	--	0.050	0.900	0.289	0.395	0.316	0.806	0.194	0.775	0.175	0.050
W4	Rock Creek	Clark Fork	--	0.155	0.571	0.274	--	--	0.060	0.036	0.429	0.071	0.405	0.134	--	0.061	0.805	0.464	0.452	0.083	0.866	0.134	0.679	0.190	0.131
W5	Bitterroot River-Hamilton, MT	Bitterroot	--	0.083	0.708	0.208	--	--	0.014	0.125	0.431	0.153	0.278	0.167	--	0.069	0.764	0.386	0.514	0.100	0.871	0.129	0.736	0.167	0.097
W6	W.F. Bitterroot River	Bitterroot	--	0.085	0.659	0.256	--	--	0.024	0.061	0.537	0.134	0.244	0.085	--	0.037	0.878	0.293	0.500	0.207	0.878	0.122	0.732	0.207	0.061
W7	E.F. Bitterroot River	Bitterroot	--	0.116	0.616	0.267	--	--	0.047	0.058	0.523	0.128	0.244	0.151	--	0.093	0.756	0.291	0.512	0.198	0.780	0.220	0.651	0.279	0.070
W8	N.F. Blackfoot River	Blackfoot	0.010	0.184	0.643	0.153	0.010	--	0.160	0.080	0.440	0.130	0.190	0.020	--	0.060	0.920	0.350	0.510	0.140	0.860	0.140	0.720	0.220	0.060
W9	Flathead River	Flathead	--	--	0.700	0.300	--	--	0.050	0.033	0.700	0.083	0.133	0.367	0.017	0.067	0.550	0.700	0.133	0.167	0.650	0.350	0.517	0.433	0.050
W10	Doctor Lake	Flathead	--	--	1.000	--	--	--	--	0.364	0.114	--	0.523	--	--	0.432	0.568	0.136	0.045	0.818	0.773	0.227	0.364	0.636	--

Sample Number	Sample Location	Basin	*SSA456*															*BT73*		*N*	H_s	Mean Allelic Richness
			**138*	**158*	**160*	**162*	**166*	**186*	**200*	**206*	**210*	**220*	**222*	**224*	**228*	**230*	**232*	**206*	1-**206*			
W1	Ninemile Creek	Clark Fork	0.283	0.083	0.483	--	0.017	0.017	--	--	0.033	0.067	--	--	--	--	0.017	0.204	0.796	30	0.533	5.1
W2a	Rattlesnake Creek 2000	Clark Fork	0.317	0.067	0.567	--	0.019	--	--	--	0.019	--	--	0.010	--	--	--	0.177	0.823	51	0.533	4.7
W2b	Rattlesnake Creek 2001	Clark Fork	0.355	0.039	0.513	--	--	--	--	0.013	0.013	0.066	--	--	--	--	--	0.205	0.795	39	0.532	4.9
W3	Clark Fork River-Milltown Dam	Clark Fork	0.500	0.050	0.425	--	0.025	--	--	--	--	--	--	--	--	--	--	0.147	0.853	20	0.530	4.3
W4	Rock Creek	Clark Fork	0.155	0.095	0.667	--	--	--	--	--	0.036	0.024	--	0.024	--	--	--	0.207	0.793	42	0.539	4.9
W5	Bitterroot River-Hamilton, MT	Bitterroot	0.306	0.069	0.500	--	--	0.014	0.014	--	0.014	0.014	0.056	0.014	--	--	--	0.097	0.903	36	0.547	5.2
W6	W.F. Bitterroot River	Bitterroot	0.378	0.171	0.402	0.012	--	--	0.012	--	--	0.024	--	--	--	--	--	0.134	0.866	41	0.531	5.1
W7	E.F. Bitterroot River	Bitterroot	0.329	0.122	0.488	--	--	--	--	--	--	0.049	--	0.012	--	--	--	0.119	0.881	43	0.580	4.9
W8	N.F. Blackfoot River	Blackfoot	0.390	0.090	0.400	--	--	--	--	--	--	0.040	0.010	0.040	0.010	0.010	0.010	0.151	0.849	50	0.538	5.1
W9	Flathead River	Flathead	0.367	0.017	0.517	--	0.100	--	--	--	--	--	--	--	--	--	--	0.217	0.783	30	0.555	4.2
W10	Doctor Lake	Flathead	0.182	--	0.818	--	--	--	--	--	--	--	--	--	--	--	--	0.523	0.477	22	0.403	2.5

Table 3-4. Genetic differentiation of bull trout and mountain whitefish populations. The high mountain lake site excluded for bull trout was Tout Lake (B7) and for mountain whitefish was Doctor Lake (W10). The exact tests column contains results of tests for genic differentiation and is presented as the percentage of loci at which allele frequencies are statistically significantly different ($P < 0.05$). See text for 95% confidence intervals for estimates of F_{ST}.

Population Groups	bull trout		mountain whitefish	
	F_{ST}	Exact Tests (%)	F_{ST}	Exact Tests (%)
All sites	0.304	74.3	0.042	41.3
High mountain lake excluded	0.176	72.9	0.018	32.5

Figure 3-1. Sample locations of bull trout (black circles) and mountain whitefish (grey squares) in the Clark Fork River, Montana. Sample numbers correspond to Table 2 and 3.

Figure 3-2. UPGMA dendrogram based on Cavalli-Sforza and Edwards (1967) chord distances for (A) bull trout and (B) mountain whitefish in the Clark Fork River. There were no statistically significant differences in allele frequencies among samples W2a, W2b, and W3; nor among sites W5, W6, and W7. We pooled these sites into two groups for (B). Bootstrap values > 50% are shown for bull trout in (A). All bootstrap values were greater than 50% for the mountain whitefish dendrogram (B) but, for presentation purposes, are not shown.

Figure 3-3. Isolation by distance analysis of A) bull trout and B) mountain whitefish in the Clark Fork River. Pairwise Cavalli-Sforza and Edwards (1967) chord distances (CSE) are plotted against pairwise geographic distances for all sample sites for each species. Comparisons that include high mountain lake sites (Trout Lake, B7, in A and Doctor Lake, W10, in B) are shown as filled circles.

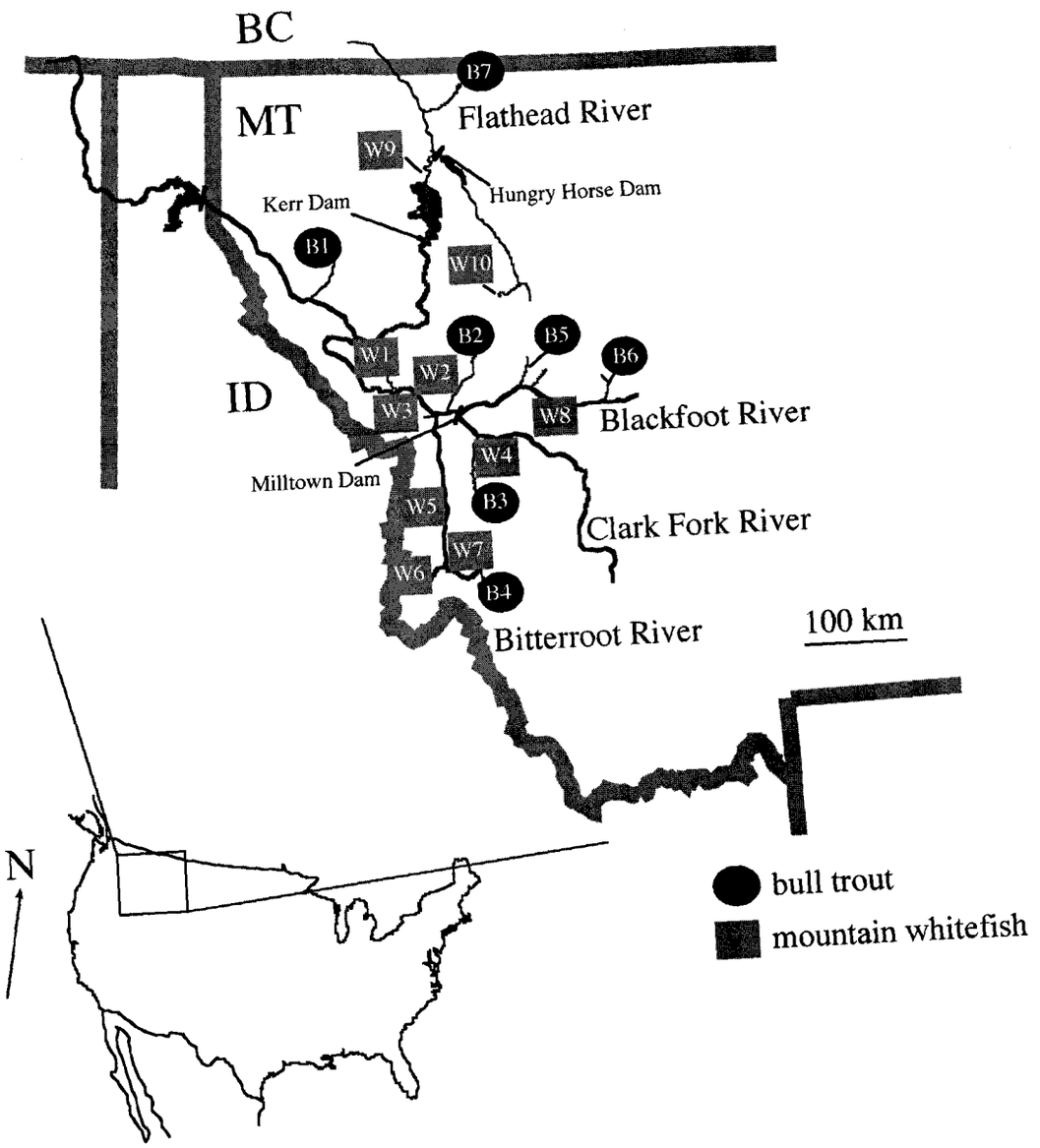


Figure 3-1.

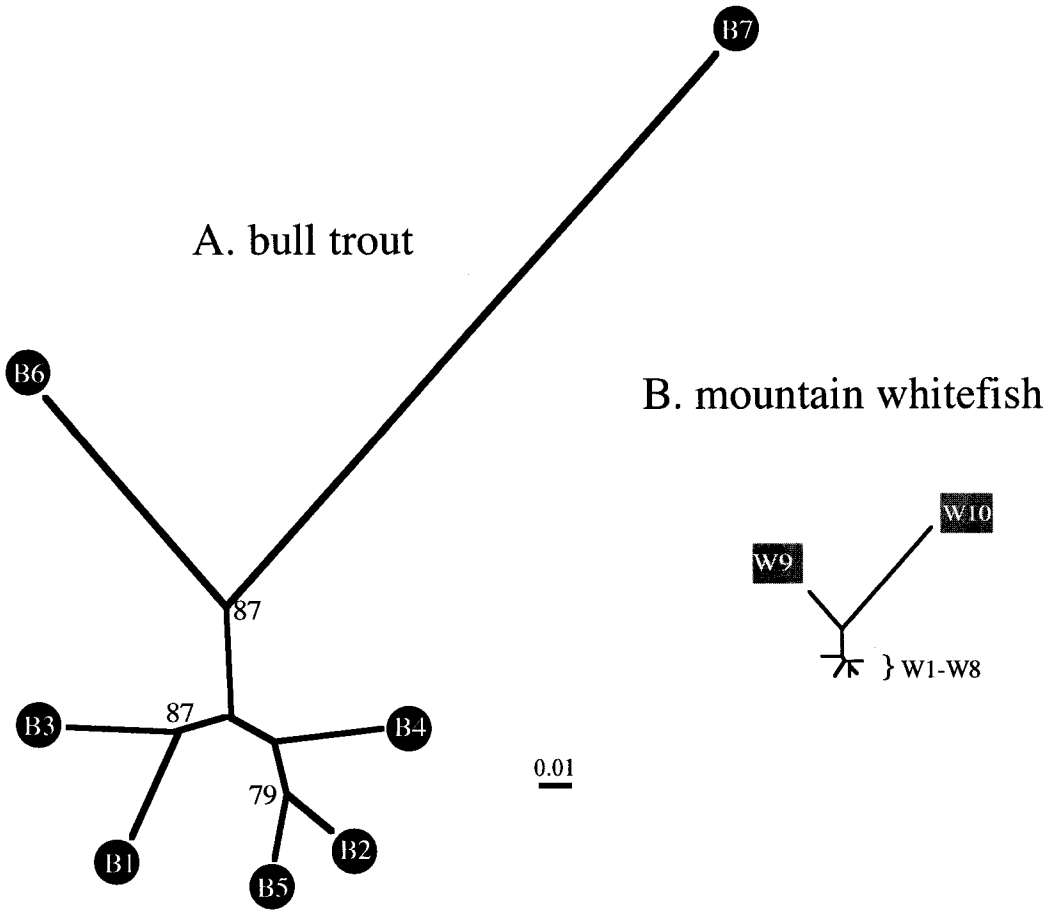


Figure 3-2.

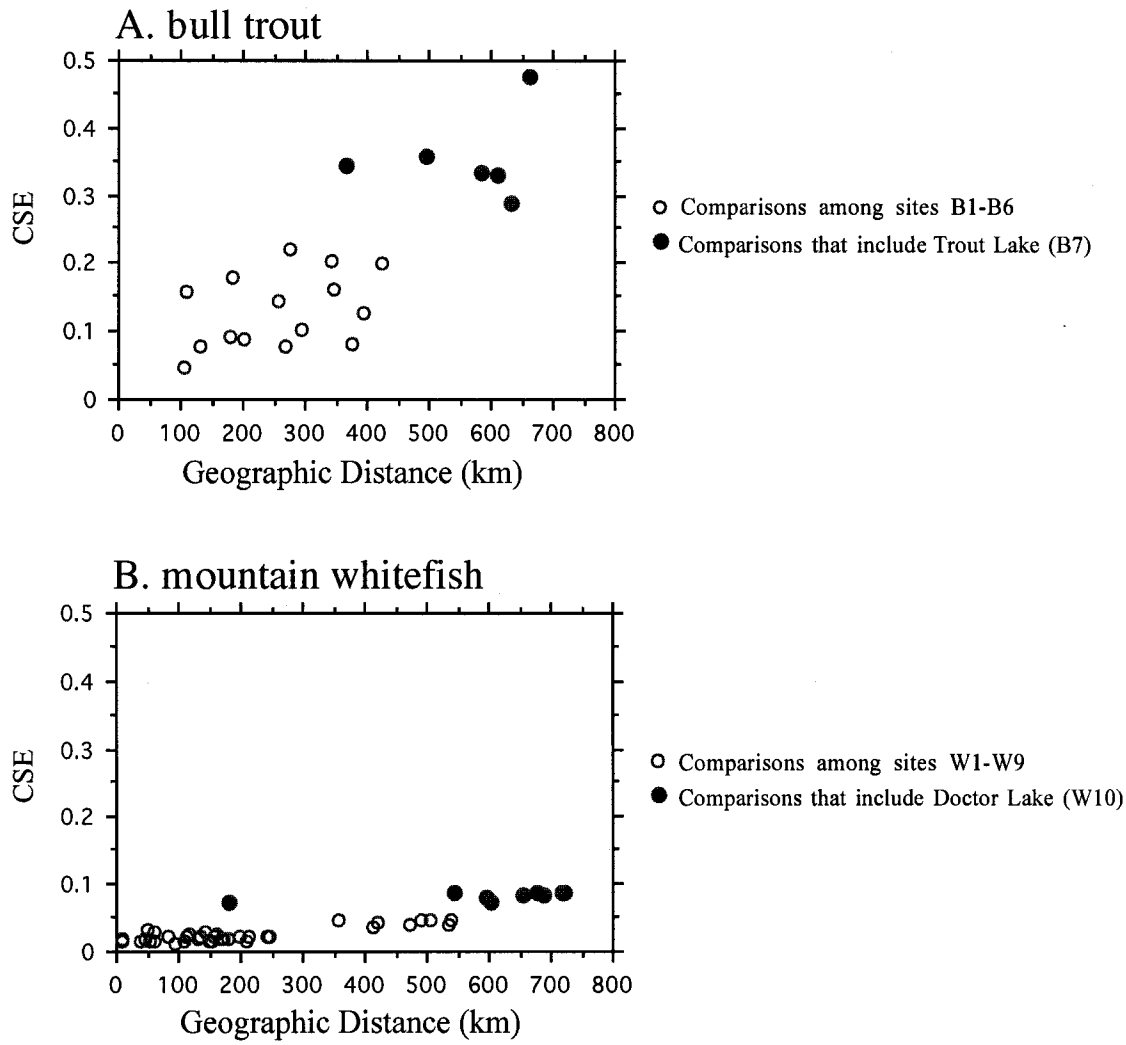


Figure 3-3.

CHAPTER 4 - Morphological, Dietary, and Genetic Analysis of a Potential Trophic Polymorphism in a Riverine Fish Species

4.1 ABSTRACT

Northern temperate lakes have become model systems for the investigation of sympatric speciation due to trophic polymorphisms. Many examples of niche-based phenotypic variation occur in temperate lakes, while northern rivers offer few such examples. The mountain whitefish (*Prosopium williamsoni*) has been hypothesized to exhibit a rare example of reproductively isolated trophic morphs in a northern riverine fish species. I found that variation in snout size and shape increased dramatically with body size, with pinocchios (individuals with large bulbous snouts) at one extreme and nonpinocchios at the other. I found subtle but consistent differences in diet between individuals with extreme snout morphologies. I found no evidence of assortative mating within populations at seven microsatellite loci. Together, these results suggest that the snout morphology of mountain whitefish is a continuous trait where individuals at extremes of the morphological continuum feed on different prey items. Differences in diet between pinocchios and nonpinocchios may be slight because of the lack of distinct foraging habitats within rivers. The lack of assortative mating may be due to the explosive mating system of this species. This study highlights the importance of ecological factors for promoting phenotypic diversification due to trophic morphology.

4.2 INTRODUCTION

Trophic polymorphisms, within-population niche-based variation in feeding structures, are hypothesized to reduce intraspecific competition (McLaughlin et al. 1999; Swanson et al. 2003) and play a role in speciation (Wimberger 1994; Skúlason et al. 1999; Robinson and Schluter 2000). For trophic, or resource polymorphisms, phenotypic variation is often discontinuous in nature but need not be (Robinson and Schluter 2000) and alternate morphs often have accompanying differences in growth rate, age at maturity, and mating strategies (Skúlason and Smith 1995). In addition, trophic polymorphisms may be the outcome of genetic polymorphisms or adaptive phenotypic plasticity (Robinson and Wilson 1994; Robinson and Wilson 1996; Smith and Girman 2000). Trophic polymorphisms occur in all classes of vertebrates and may be more common than historically appreciated (Wimberger 1994; Skúlason and Smith 1995; Smith and Skúlason 1996).

Several authors have discussed models for the translation of within-population phenotypic variation related to trophic polymorphisms to variation that occurs among species (West-Eberhard 1986; Wimberger 1994; Skúlason et al. 1999; Adams and Huntingford 2004). These models hypothesize that subtle behavioral and/or morphological variation within populations can become increasingly specialized and under the right conditions can lead to reproductive isolation and potentially the fixation of alternate traits between species (West-Eberhard 1986; Wimberger 1994; Skúlason et al. 1999). A key factor of these models is the stability and location of feeding habitats. If pronounced and persistent ecological differences occur among feeding habitats, subsequent behavioral and morphological specialization to these habitats are more likely (Wimberger 1994; Skúlason et al. 1999). Reproductive isolation may occur for purely ecological reasons if positive assortative mating occurs within these distinct feeding habitats (Wimberger 1994; Skúlason et al. 1999; Smith and Girman 2000). Otherwise,

for reproductive isolation to occur, assortative mating by phenotype must occur without spatial separation.

Fishes in general offer extraordinary examples of trophic polymorphisms. These range from cichlids in the African Rift Lakes (e.g. Danley and Kocher 2001) and lakes in Nicaragua (e.g. Wilson et al. 2000) to salmonids and sticklebacks (*Gasterosteus aculeatus*) in northern temperate postglacial lakes (Robinson and Wilson 1994; Robinson and Schluter 2000). In fact, species-poor northern lakes have become model systems for examining this type of phenotypic variation (Robinson and Schluter 2000). In these lakes, shallow littoral margins and deeper open-waters offer stable and spatially separated habitats in which the whole continuum of divergent biological units, from within-species variation represented by slightly different phenotypes to distinct species, can be found (reviewed by Robinson and Schluter 2000). Trophic polymorphisms within these relatively simple environments have improved our understanding of the ecological causes of phenotypic diversification and adaptive radiation (Robinson and Schluter 2000; Robinson and Parsons 2002).

Northern temperate rivers offer very few examples of trophic polymorphisms (Robinson and Wilson 1994; Robinson and Schluter 2000). In one of two such examples, recently emerged brook trout exhibit alternative foraging behaviors without any morphological differentiation (McLaughlin and Grant 1994; McLaughlin et al. 1999; McLaughlin 2001). In another example, reproductively isolated lenok (*Brachymystax lenok*) morphs (Osinov et al. 1990) have differences in trophic structures (gill rakers and position of the mouth) that suggest they may also have differences in diet (Kondrashov and Mina 1986; Smith and Skúlason 1996). It is possible that phenotypic differences between morphs arose in isolation rather than sympatrically (Osinov et al. 1990).

The paucity of examples of trophic polymorphisms in northern riverine fishes may be due to the greater temporal and spatial variability of benthic and limnetic resources in rivers than in lakes (McLaughlin et al. 1999). Adopting alternative foraging tactics may not be an effective

means to reduce intraspecific competition in this environment (however see Swanson *et al.* 2003 for an example in a spring-fed pool system). Furthermore, it may be unlikely for prezygotic reproductive isolation to occur among alternately specialized trophic morphs without spatially separated feeding locations.

The mountain whitefish (*Prosopium williamsoni*) potentially provide a third example of a trophic polymorphism in a northern riverine fish. Some individuals of this species have large cylindrical snouts (Figure 4-1a), which was originally hypothesized to be a sexual dimorphism present in males (Evermann 1892). Troffe (2000) and McPhail and Troffe (2001) negated this hypothesis and suggested the hypothesis that "pinocchio" mountain whitefish use their exaggerated snouts to overturn rocks to feed on benthic invertebrates. Troffe (2000) provided preliminary evidence for differences in foraging behavior and for reproductive isolation between what he classified as discrete morphs. Populations of this species occur at high densities (Whiteley et al. 2004) and this could lead to strong selection for traits that reduce intraspecific competition, such as those related to trophic specialization.

If substantiated, the results of Troffe (2000) would represent an example of a trophic polymorphism where morphs have become reproductively isolated under unlikely conditions. The conditions are unlikely because a) the spatial and temporal heterogeneity of resources in rivers and b) both spawning location and spawning behavior of this species make it unlikely for positive assortative mating by snout morphology to occur. Mountain whitefish spawn in large aggregates in rivers or near the mouths of tributary streams (Davies and Thompson 1976; Northcote and Ennis 1994). There does not appear to be any spatial segregation of individuals by snout morphology and the probability of assortative mating is further reduced because mountain whitefish are broadcast eggs over the substrate and many males fertilize the eggs of a single female. Given these conditions, natural selection would have to be very strong to lead to discrete reproductively isolated trophic morphs of mountain whitefish, especially when frequency-dependent selection or phenotypic plasticity would be able to maintain diversity in the absence of

reproductive isolation. Thus, further investigation is needed to confirm the results of Troffe (2000), especially since the morphological, behavioral, and genetic results of this study were based on small sample sizes and genetic variation was examined at only one locus with small effective population size (mitochondrial DNA).

My objectives in this paper were to provide further investigation of phenotypic variation in snout morphology of the mountain whitefish, to test the hypothesis that this variation is associated with a trophic polymorphism, and to test the hypothesis that morphs are reproductively isolated within populations. I addressed these objectives by asking the following questions: Is there discontinuous variation in snout morphology within populations of mountain whitefish? Is there a difference in diet between pinocchios and nonpinocchios? Is there evidence of assortative mating by snout morphology?

4.3 MATERIALS AND METHODS

Sample collection

Mountain whitefish were collected from Rattlesnake Creek ($N = 135$) in Missoula, Montana and the Bitterroot River near Stevensville, Montana ($N = 225$; Table 4-1). Fish were collected with a backpack electrofisher or with a boat electrofisher. I sampled a total of three locations, two of which were sampled multiple times (Table 4-1). Specific subsets of these animals where used for morphological, diet, and genetic analyses, as detailed below.

Morphology

I captured digital images of all individuals the day they were collected with a digital camera mounted on a tripod. Standard length was used as a measure of overall body size and the sex of all individuals was determined by inspection of internal organs.

I developed a method (hereafter referred to as the "snout index") to quantify phenotypic variation in snout size and shape by measuring the area of the snout and part of the forehead

region. This method was designed to capture not only the size of the bulbous cylindrical snout of pinocchios but also the inward sloping forehead (Figure 4-1a). For nonpinocchios, this method measured the small snout and convex forehead region immediately adjacent to the snout (Figure 1b). The steps used for this method of quantification were as follows (Figure 4-2a): First, I placed a landmark at the tip of the snout and where the operculum meets the ventral lateral margin. Second, I connected these landmark points with a straight line (L1). Third, I drew a line (L2) at a right angle to L1 and tangential to the anterior orbit of the eye. Fourth, I drew a straight line (L3) from the landmark at the tip of the snout to the bisection of L2 and the dorsal lateral margin. Fifth, I bisected L3 with line L4. Sixth, I bisected the anterior half of L3 with line L5. I measured two areas (A1 and A2). A1 was the area between the landmark at the tip of the snout and L5. A2 was the area between L5 and L4. If these areas lay anterior to L3 they were positive and they were negative if they lay posterior to L3. I determined the value of the snout index by subtracting A2 from A1. This value tended to be positive for pinocchios and negative for nonpinocchios. For the example of this method in Figure 4-2a, the majority of A1 would occur anterior to L3. Note that a small portion of A1 also would lie posterior to L3. In this case, A1 would consist of the positive area anterior to L3 less the negative area posterior to L3. A2 in Figure 4-2a would be negative and the snout index would have a positive value. All steps for this method were performed with Image J ver. 1.23 (available at http://rsb.info.nih.gov/ij).

A subjective classification procedure was used to verify that snout index effectively quantified variation in snout size and shape. All individuals were subjectively classified into three phenotypic categories based on external morphology. I used the nomenclature "pinocchio", "intermediate", and "nonpinocchio" instead of "pinocchio" and "normal" used by Troffe (2000) and McPhail and Troffe (2001) because there were clearly individuals with intermediate snout phenotypes that could not be classified as either "pinocchio" or "normal". Three people in addition to the author scored phenotypes of individuals from digital photographs based on the characteristics listed in Figure 4-1. Individuals were conservatively classified as intermediate if

they did not match these characteristics and if there was any question about their phenotype. Phenotypic classification was based on digital images of heads without taking overall body size into account. For the final phenotypic classification, individuals were considered either pinocchios or nonpinocchios if three out of four people scored them as such. Otherwise they were classified as intermediate. I determined proportions of each phenotypic class for all fish combined and all fish except immature individuals because immature individuals were only classified as normal or intermediate and thus might skew the overall proportions.

I used statistical methods developed for allometric relationships in insects (Eberhard and Gutiérrez 1991) to test for a discontinuity, or "switch point", in the range of body sizes (standard lengths) in the relationship between snout size and body size for males and females separately. The first step was to test for significant nonlinearity in the relationship between the snout index and body size. I performed a partial *F*-test by fitting the following model:

$Y^* = \alpha_0 + \alpha_1 X^* + \alpha_2 X^{*2} + \varepsilon$ (1), where Y^* was the natural log of body size (standard length, mm); X^* was the natural log of the snout index(mm^2), where I added 10 to each value to make all values positive; α_i were the regression coefficients; and ε was the error with assumed normal distribution, mean zero, and common variance (Eberhard and Gutiérrez 1991). Significant difference of α_2 from zero indicated that the relationship between the snout index and body size was significantly nonlinear and that further tests for a switch point were justified.

I used the following model to determine the most likely switch point and to test for a discontinuous relationship at that point: $Y = \beta_0 + \beta_1 X + \beta_2 (X - X^0) D + \beta_3 D + \varepsilon$ (2), where, Y and X were values of the snout index and body size, respectively (in untransformed measurement units); X^0 was the putative switch point; $D = 0$ if $X < X^0$ or $D = 1$ otherwise; β_i's were the regression coefficients; and ε was the random component with assumed normal distribution, mean zero, and common variance. To determine the switch point, I empirically substituted 10 different values of X^0 into the model and chose the value of X^0 that gave the highest adjusted

R^2. I then used a partial F-test to test the significant of β_3 using a stepwise regression (with the empirically determined value of X^0) implemented in SPSS 11 (SPSS Inc.). If β_3 was not significant, this would indicate that the relationship was not discontinuous at the switch point. To test for a change in linear slope at the switch point (without a discontinuity at the switch point), I used the following model: $Y = \beta_0 + \beta_1 X + \beta_2 (X - X^0) D + \varepsilon$ (3), where the terms were the same as defined above. If the β_2 term was significant in this model, this would indicate that a change in slope occurred at the switch point and that the switch point was significant (Eberhard and Gutiérrez 1991).

To investigate the relationship between external snout morphology and underlying bone structure, I measured the supraethmoid of all individuals (Figure 4-2b). The supraethmoid lies at the tip of the snout and provides attachment points for the cartilage and other tissues within the snout. I suspected that this bone would be larger in pinocchios relative to nonpinocchios because a) it appeared to be larger in x-rays of pinocchio individuals relative to nonpinocchios (data not shown) and b) the base width of the supraethmoid is a diagnostic character used to distinguish the sharp-snouted morphotype of the lenok from the blunt-snouted morphotype (Kondrashov and Mina 1986; Alekseyev 1995; Alekseyev et al. 2003). The external morphology of the sharp-snouted lenok appears to be very similar to the morphology of pinocchio mountain whitefish and thus I predicted that the supraethmoid would be wider in pinocchios than nonpinocchios. I dissected supraethmoids from frozen fish and prepared and cleaned them using trypsin according to Mayden and Wiley (1984). I used Image J ver. 1.23 to measure the length of the supraethmoid as well as its width at its base (Figure 4-2b).

To characterize growth patterns of the supraethmoid, I compared the allometric relationship of the supraethmoid with other body structures. The other structures I measured were the lengths of the pectoral, pelvic, and anal fins. I expected these structures to have an allometric slope approximately equal to one because they were predicted to grow in direct

proportion to body size (Eberhard et al. 1998). I regressed the natural log of supraethmoid base width, supraethmoid length, pectoral fin length, pelvic fin length, and anal fin length on the natural log of standard length. I generated 95% confidence intervals from the standard error of the regression coefficients from each regression. I excluded the only two immature individuals from this allometric analysis because they were outliers.

I also tested for a correlation between the external snout measure and supraethmoid base width for males and females separately by using residuals from regressions of each trait on standard length. For the snout index I used a polynomial regression. For females I used a second order polynomial regression because the coefficient of the (standard length)^2 term was highly significant ($P = 0.0037$) but the coefficient of the (standard length)^3 term was not significant ($P = 0.532$) when I used a third order polynomial regression. For males I used a third order polynomial regression because the coefficient of the (standard length)^3 term was highly significant ($P = 0.0025$). For the regression of supraethmoid base width on standard length, I used natural log transformed lengths for both variables. I used a parametric test for the correlation analysis.

To examine whether there might be physiological costs associated with the pinocchio snout, I tested for a tradeoff between size-adjusted weight and size-adjusted snout index values. I used residuals from a regression of natural log transformed weight versus the natural log of standard length and the residuals from a polynomial regression of snout index on body size. Due to sampling constraints mentioned above, I only analyzed males from Rattlesnake Creek for this analysis ($N = 88$). I again used a third order polynomial regression for snout index versus body size. The coefficient for the (standard length)^3 term was highly significant ($P = 0.0076$). I used a parametric test for the correlation analysis.

I used the following equation to measure the repeatability (r) of measurements of the snout index, supraethmoid length, supraethmoid base width, fin lengths, and standard length:

$r = \frac{s_A^2}{s^2 + s_A^2}$, where $s_A^2 = \frac{MS_{Among} - MS_{within}}{n_0}$, and n_0 is the group size (Lessels and Boag 1987).

For each trait, one person measured ten individual mountain whitefish three times. All morphological measurements were highly repeatable. The *r*-value for the snout index was 0.97, for supraethmoid length was 1.0, for supraethmoid base width was 1.0, for pectoral fin length was 0.99, for pelvic fin length was 0.98, for anal fin length was 0.98, and for standard length was 0.99.

Diet Analysis

Stomach and intestine contents were analyzed from individuals with extreme phenotypes to test for diet differences based on pinocchio morphology. The two samples collected in the Bitterroot River (Table 4-1) were each collected from the same location (within approximately 50m) and for each sample all fish were collected at the same time from an electrofishing boat. Fish were kept on ice and stomachs and intestines were dissected as soon as possible after capture and stored in 70% ethanol until analysis. Prey items found in the stomach versus the intestine were not distinguished and below stomachs refer to the whole digestive tract.

For the 2003 sample, I analyzed the diet data in two ways: (1) I split the entire sample into three size classes. The first size class (S1) contained fish less than 180mm. The second size class (S2) contained fish greater than 180mm but less than 230mm. The third size class (S3) contained fish greater than 230mm. (2) I divided the fish from size class three (S3) into two groups of phenotypically extreme individuals, pinocchios and nonpinocchios. For the 2003 sample, there were 14 pinocchios based on values of the snout index and the subjective classification process. I chose 14 nonpinocchio individuals that were similar in body size, to control for differences in diet among size classes of fish (see below). The mean standard length (± SE) of the pinocchio group was 278.57 mm ± 4.22 mm and nonpinocchio mean was 274.57 mm ± 4.21 mm ($t_{26} = -0.561$, $P = 0.580$). The groups varied significantly in snout index values:

pinocchio mean = 3.02 mm^2 ± 0.59 and nonpinocchio mean = -1.86mm^2 ± 0.31 (t_{26} = -7.31, P < 0.0001).

For the 2004 sample, I again formed a pinocchio and a nonpinocchio group with 15 individuals of each type. The mean standard length of pinocchios was 276.67 mm ± 4.58 mm, for nonpinocchios was 269.40 mm ± 3.17 mm (t_{28} = -1.306, P = 0.202). Mean snout index values of pinocchios were 3.848 mm^2 ± 0.524 mm^2 and for nonpinocchios were -1.476 mm^2 ± 0.226 mm^2 (t_{28} = -8.878, P < 0.0001).

Insects in gut samples were sorted to order or family under a dissecting microscope. Two people analyzed the 2003 sample and standardization was achieved through double analysis of a portion of the stomachs. One person analyzed the 2004 sample. The total number of each insect taxon per stomach was counted. One reliable body part was counted per insect taxon, for example the head capsule was used for chironomid larvae. I used prey item counts to calculate the proportion of each food item relative to the total number of food items found in each individual's stomach (proportional contribution by number).

To determine the proportional contribution of different insect taxa to mountain whitefish diets by weight, I determined average wet weights of the relevant insect taxa. Whole insects were collected from the same location in the Bitterroot River and at the same times as the fish used for diet analysis. These insects were collected separately in July 2003 and March 2004 and were stored in 70% ethanol until analysis. I determined wet weights of five to ten individuals to determine an average weight for insects from a given taxon. I allowed ethanol to evaporate for 10 minutes prior to weighing the samples. I used a range of specimens for a given taxon that encompassed the range of sizes I found in stomachs. I multiplied the number of a given taxon by its average weight to determine the total weight of the food items in an individual's stomach. I then determined the average proportion by weight for each taxon category within each fish's stomach. To compare total stomach volumes between the 2003 and 2004 Bitterroot River

samples, I performed a two-way ANOVA using the total weight of the food items in an individual's stomach as the dependent variable and sample (2003 or 2004) and phenotype (pinocchio or nonpinocchio) as the two factors. To quantify the volume of rocks in each stomach, I used a Petri dish with a $1cm^2$ grid and counted the total number of squares occupied. Rocks were not included in the stomach volume calculations.

I used nonparametric tests to analyze prey item counts and proportion of diet by number and by weight. The diet data generally appeared to violate assumptions of normality and equality of variance, even after log or arcsine transformation (of proportions). A Kruskal-Wallis test was used for the analysis of the three size classes from the entire 2003 sample along with a procedure that parallels the Tukey test for post-hoc pairwise comparisons following Zar (1984). I used Mann-Whitney tests for the analyses of phenoptypically extreme groups. *P*-values were not adjusted for multiple tests in Table 4 because Bonferroni corrections tend to be overly conservative (Nakagawa 2004). Instead, Bonferroni adjusted *P*-values appear in the legend of Table 4-4, using both $\alpha = 0.05$ and $\alpha = 0.10$.

Genetic Analysis

I collected genotypic data from the following seven microsatellite loci: *COCL4*, *SSA14*, *SSA456*, *ONE8*, *FGT25*, *SFO8-1*, and *SFO8-2* (Whiteley et al. 2004). DNA was extracted from each fin clip by standard methods. Thermal cycler profiles used for PCR follow Whiteley *et al.* (2004). The general methods used for visualization of subsequent PCR products followed Spruell *et al.* (1999) and Neraas and Spruell (2001). PCR reagent concentrations are available upon request.

I calculated allele frequencies, mean heterozygosities, and mean number of alleles separately for pinocchios and nonpinocchios with the program FSTAT 2.9.2.3 (Goudet 1995; Goudet 2001). To test for a deficit of heterozygotes (Wahlund effect), I tested for deviations from Hardy-Weinberg proportions using a one-tailed test with GENEPOP ver. 3.4 (Raymond and

Rousset 1995). To test for differences in allele frequency distributions, I performed a pseudo-exact test for genic differentiation (Goudet et al. 1996) between groups of phenotypically extreme individuals with GENEPOP ver. 3.4. For both tests, I used Fisher's method to combine probabilities following (Sokal and Rohlf 1995). I presented Bonferroni adjusted *P*-values in the legend to Table 6. I used Principle Components Analysis (PCA) to examine patterns of multilocus genotypes without prior assignment of individuals to phenotypic groups, using the program PCAGEN ver. 1.2.1 written by J. Goudet (downloadable at www.unil.ch/izea/softwares/pcagen.html). This program was used to cluster individuals by multilocus genotypes and generate plots of principle component axes.

I used all of the individuals ($N = 41$) collected from the West Fork Bitterroot River (Table 1) to test for deviations from Hardy-Weinberg proportions. For this sample, I subjectively determined phenotypes (pinocchio, intermediate, nonpinocchio) at the time of capture and snout index measurements were performed after the fish had been frozen. I used these measurements along with the subjective classification to separate individuals with extreme phenotypes into a pinocchio group ($N = 10$) and a nonpinocchio group ($N = 10$) for subsequent genetic analyses, but I did not use these measurements for the overall morphological analysis. The mean snout index ± SE for the pinocchio group was 2.66 mm^2 ± 0.44 and for the nonpinocchios group was -1.73 mm^2 ± 0.23 ($t_{28} = -8.88$, $P < 0.0001$). Mean standard length for pinocchios was 285.10 mm ± 7.52 mm, for nonpinocchios was 247.10 mm ± 8.63 mm ($t_{28} = -3.32$, $P = 0.0038$).

I used individuals from the Bitterroot 2004 sample to replicate the genetic analyses. I used snout index measurements and the subjective classification process to sort individuals into a pinocchio ($N = 20$) and a nonpinocchio ($N = 20$) group, choosing the individuals with the most extreme phenotypes irrespective of body size. I was less concerned about controlling for body size for this comparison (relative to the diet analysis) and instead chose individuals with the most extreme snout morphologies. The mean snout index ± SE for pinocchios was 2.85 mm^2 ± 0.63

mm^2 and for nonpinocchios was -1.44 mm^2 ± 0.22 mm^2 (t_{38} = -6.46, $P < 0.0001$). Mean standard length for pinocchios was 271.70 mm ± 4.70 mm, for nonpinocchios was 222.35 mm ± 6.26 mm (t_{38} = -6.30, $P < 0.0001$).

4.4 RESULTS

Morphology

The snout index developed for this study corresponded closely to phenotypes as determined by the subjective classification procedure. This close correspondence suggests that the snout index performed well in capturing variation in snout size and shape (Figure 4-3). Individuals classified as pinocchios (Figure 4-1a) had the largest mean snout measurements (Figure 4-3a; Table 4-2). Individuals classified as nonpinocchios (Figure 4-1b) had the smallest mean snout measurements (Figure 4-3a; Table 4-2). Individuals classified as intermediate by the subjective classification procedure tended to have a slightly concave forehead and a slightly cylindrical snout that extended out from where the ventral portion of the snout met the upper maxilla but the snout was not excessively large, bulbous, or cylindrical. These individuals also had intermediate values for the snout size measurement (Figure 4-4a; Table 4-2). In addition, both males and females had large pinocchio snouts (Figure 4-3 b and c).

There was little variation in snout index values for individuals below a standard length of approximately 220 mm. Beyond this standard length, variation in snout morphology increased dramatically (Fig. 4-4). The relationship between the snout index and standard length was significantly nonlinear. The coefficient α_2 from equation (1) was highly significant ($P < 0.0001$) for all data combined. For males and females analyzed separately, the α_2 coefficient was also highly significant ($P < 0.0001$, $P = 0.001$ respectively).

The switch point analysis revealed a statistically significant switch point for all of the data combined and for females analyzed separately, but not for males analyzed separately. For all data combined, the value of X^0 that yielded the greatest adjusted R^2 value for equation (2) was

265 mm. The coefficient β_3 was not significant ($P = 0.248$) and therefore the relationship between the snout index and body size was not discontinuous at 265 mm. The coefficient β_2 from equation (3) was highly significant ($P < 0.0001$), indicating a significant change in slope occurred at X^0 = 265mm. The slope (± SE) prior to 265 mm was 0.002 ± 0.004 and after 265 mm was 0.083 ± 0.011. For males analyzed separately (Figure 4-4b), adjusted R^2 values increased for all X^0 values, indicating that there was not a peak in these values and that a switch point did not occur. The lack of switch point in males appears to be due to a greater proportion of nonpinocchio individuals with greater standard lengths (Figure 4-4b). For females analyzed separately (Figure 4-4c), X^0 = 265 mm had the highest adjusted R^2 value. β_3 from equation (2) was not significant ($P = 0.172$), while β_2 from equation (3) was significant ($P = 0.0008$). The slope (± SE) prior to 265 mm was 0.012 ± 0.010 and after 265 mm was 0.69 ± 0.020.

I examined growth patterns of the supraethmoid bone with allometric analysis. If the supraethmoid were growing at a disproportionately greater rate than overall body size, the allometric slope for this trait should be greater than one and be greater than the allometric slope of structures expected to grow in direct proportion with body size. The allometric slope for supraethmoid base width was significantly greater than one and had the highest allometric slope out of all the traits measured for both males and females (Table 4-3). The allometric slope for supraethmoid length was greater than one for males but not females. Of the three fins measured, only the allometric slope for anal fin length in males was significantly greater than one. 95% confidence intervals overlapped for all traits measured for direct comparisons of males and females (Table 4-3).

Variation in supraethmoid base width was not significantly correlated with the snout index for males or females. Pinocchios did not tend to have positive residuals from the regression of supraethmoid base width on standard length (data not shown). The correlation between these residuals and the residuals from the polynomial regression of the snout index on standard length

for males was positive and non-significant ($r = 0.129$, $Z = 1.739$, $P = 0.082$). For females, the correlation between these residuals and residuals from a second order regression of the snout index on standard length was negative and non-significant ($r = -0.073$, $Z = -0.846$, $P = 0.398$). Results were similar if I used a first or second order regression for males or a first order regression for females (data not shown).

For males from Rattlesnake Creek, I found a significant negative correlation between size-adjusted weight and size-adjusted snout index (Figure 4-5). If either a first or second order regression of snout index on body size was used, the correlation between residuals from this regression and residuals from the regression of weight on body size remained negative and significant (data not shown).

Diet Analysis

For the entire Bitterroot 2003 sample, I found significant differences among age classes in diet (Table 4-4). Smaller size classes had significantly greater numbers of Chironomidae larvae, Chironomidae pupae, and small Ephemeroptera nymphs. For average proportion of diet, I found significant variation among age classes for large Ephemeroptera nymphs, Trichoptera larvae, Chironomidae larvae, and Simuliidae larvae (Figure 4-6; Table 4-4). I found significantly more large Ephemeroptera and Trichoptera larvae in stomachs of the fish in the largest size class (S3). Smaller size classes had larger average proportions (by number) of Chironomidae larvae. Proportion of diet by weight results showed similar patterns as total number (Table 4-4).

When I divided individuals from the S3 size class into a pinocchio group and a nonpinocchio group for the Bitterroot 2003 sample, I found significantly more large Ephemeroptera nymphs in pinocchio stomachs (Figure 4-6; Table 4-4). This pattern held for all three measures of diet content. I found significantly more Simuliidae larvae in nonpinocchio stomachs for all three measures (Figure 4-6; Table 4-4).

For the Bitterroot 2004 sample, I again found significantly more large Ephemeroptera nymphs in pinocchio stomachs than in nonpinocchio stomachs, as determined by total number and average proportion by number (Figure 4-6; Table 4-4). The proportion by weight of large mayflies was not significantly greater in pinocchios. I found significantly more Chironomidae pupae in nonpinocchio stomachs (by total number and average proportion by number; Figure 4-6; Table 4-4).

For the analysis of variance performed on weights of food items in the stomachs of both pinocchios and nonpinocchios, mean weights were significantly greater in the 2004 sample than the 2003 sample ($F_{3,53} = 31.485$, $P < 0.0001$). The mean weight of food items did not differ significantly between pinocchios and nonpinocchios within each sample ($F_{3,53} = 0.562$, $P = 0.457$). The interaction term (snout phenotype x sample) was also not significant ($F_{3,53} = 0.573$, $P = 0.453$).

Genetic Analysis

I examined general summary statistics and tested for deviations from Hardy-Weinberg proportions for the West Fork Bitterroot and Bitterroot 2004 samples (Table 4-5; Table 4-6). Allele frequencies were similar for the comparisons of pinocchios and nonpinocchios within each sample, as was the mean expected heterozygosity and average number of alleles (Table 4-5). I did not detect any significant deviations from Hardy-Weinberg proportions in the West Fork Bitterroot sample (Table 4-6). For the Bitterroot 2004 sample, *SSA456* deviated from Hardy-Weinberg proportions with a significant deficit of heterozygotes (Table 4-6). The combined probability for deviations for Hardy-Weinberg proportions based on Fisher's method was not significant for either sample (Table 4-6).

I combined single locus tests for genic differentiation with a multilocus analysis of genotypic distributions using Principle Components Analysis to test for genetic differentiation of pinocchios and nonpinocchios within each sample. None of the exact tests for genic

differentiation was significant for either sample (Table 4-6). In addition, I did not detect any patterns of genotypic differentiation between pinocchios and nonpinocchios within either sample using PCA. For the West Fork Bitterroot sample, PC axes one through four explained 26%, 16%, 14%, and 11% of the variation among multilocus genotypes. There was no tendency for individuals to cluster by phenotype in PCA plots for this sample (Figure 4-7a; axes 3 and 4 not shown). For Bitterroot 2004, PC axes one through four explained 19%, 15%, 13%, and 10% of the variation among multilocus genotypes and again, there was no tendency for individuals to cluster by phenotype in PCA plots (Figure 4-7b; axes 3 and 4 not shown).

4.5 DISCUSSION

Is there discontinuous variation in snout morphology within populations of mountain whitefish?

Enlarged snouts of several other fish species represent either sexually dimorphic characters (e.g. Fernandes et al. 2002) or are putatively related to differential resource acquisition (Kondrashov and Mina 1986; Nagelkerke et al. 1994). While the pinocchio snout of mountain whitefish was not sexually dimorphic, it may be a subtle trophic polymorphism (see below). The continuous snout variation observed does not eliminate this hypothesis. Trophic polymorphisms do not need to be discontinuous as long as phenotypic extremes differ in morphology and/or feeding strategies (Robinson and Schluter 2000).

I found evidence for continuous variation in the snout morphology of mountain whitefish when all fish were considered together (Figure 4-4). However, phenotypic variation in snout size and shape was reduced in smaller individuals and increased dramatically with increasing standard length. Thus, the snout morphology of adults appears to be determined when individuals are approximately 220-240 mm in length, possibly due to a stage-specific ontogenetic switch. Beyond this putative switch point, the pinocchio-related variation might be associated with an alternative growth trajectory. It would be necessary to examine the snout morphology of more

large individuals and to follow growth trajectories of individuals with and without pinocchio snouts to test this hypothesis.

The pinocchio trait could be a genetic polymorphism maintained by frequency-dependent selection or an example of condition-dependent ontogenetic plasticity. Determination of the genetic basis of this trait would require experimental crosses. I performed the necessary crosses but was unable to rear sufficient number of fish to a point where variation in snout morphology could be assessed (data not shown). A genetic polymorphism maintained by frequency-dependent selection, where the more rare morph has less competition for food, is a more mechanistically straightforward hypothesis for this trait. However, it is intriguing that the observed increase in phenotypic variation corresponded approximately to the observed diet shift and in general corresponds to a habitat shift for individuals of this species because this suggests that this trait may be condition-sensitive.

The greater allometric slope of the supraethmoid base width relative to the other traits measured (Table 4-3) suggests that supraethmoid base width does explain some of the variation in snout morphology of mountain whitefish. However, variation in the snout index was not correlated with supraethmoid base width and therefore, it appears that tissue changes beyond underlying bone structure are responsible for pinocchio snout variation. Elucidation of how pinocchios differ from nonpinocchios at the cellular level and exactly what tissue changes occur beyond the supraethmoid in individuals following different growth trajectories would require histological analysis.

The tradeoff between the snout index and size-specific weight in males captured during the spawning season (Figure 4-5) could be due to greater spawning success and therefore reduced gonad weight of pinocchio males at the time of capture. I determined wet-weights of testes from 86 males to explore this hypothesis. I did not find a relationship between males that had receded testes and their body size or their values of the snout index, nor did I find evidence for a relationship between testes weight and body size/snout index (data not shown).

Another explanation for the observed tradeoff is that there may be an energetic cost associated with devoting resources to the snout rather than other body parts and that males that follow the putative pinocchio growth trajectory allocate resources differently than males that do not follow this growth trajectory. Overall body shape differences between pinocchios and nonpinocchios would provide support for this hypothesis. To test for differences in body shape, I performed a preliminary geometric morphometric analysis (Rohlf and Marcus 1993) using 12 landmarks located along the body of individuals with extreme snout phenotypes according to the methods of Langerhans *et al.* (2003) and Langerhans *et al.* (2004). Preliminary results suggested that body shape differences occur between pinocchios and nonpinocchios, where pinocchios tend to be less deep-bodied and nonpinocchios tend to be more deep-bodied with a slight hump along the dorsal margin between the head and dorsal fin (data not shown). However, a more rigorous investigation of this pattern is necessary, as is a direct test of the physiological basis of this potential tradeoff.

Is there a difference in diet between pinocchios and nonpinocchios?

I found subtle but consistent differences in diet between adult pinocchios and nonpinocchios. There were significantly more large Ephemeroptera (Heptageneiidae and Ephemerellidae) nymphs in pinocchio stomachs for both years. The proportion of these prey items by number in pinocchio stomachs were not great in either sample (approximately 16%), but for the Bitterroot 2003 sample, large Ephemeroptera nymphs did comprise a large proportion of diets by weight (35%). For the Bitterroot 2004 sample, pinocchios did not have a higher average proportion of large Ephemeroptera by weight, which was likely due to a masking effect caused by the large proportion of pinocchio and nonpinocchio diets that consisted of large Plecoptera nymphs.

I also observed a large dietary shift between juveniles and adults, which is consistent with previous observations for this species (Pontius and Parker 1973). This dietary shift corresponds

to a habitat shift by older juveniles to deeper faster flowing sections of rivers. Younger juveniles tend to occur in side-water habitat following emergence in spring and then move to either shallow riffles or the tail of pools later their first summer (Northcote and Ennis 1994). Interestingly, the observed diet and habitat shift corresponds approximately to the body size at which I observed increased phenotypic variation in snout morphology.

For adults, greater consumption of large Ephemereoptera nymphs by pinocchios is consistent with the hypothesis that pinocchios feed on the bottom more and use their snouts to probe into cracks and crevices and perhaps to overturn rocks. Ephemerellid and Heptageneiid mayflies nymphs cling to the bottom of the river and feed as scrapers of organic surfaces on and beneath rocks (Merritt and Cummins 1996). Mayfly nymphs do occur suspended in the water column and it is possible that pinocchios were feeding on drifting nymphs. However, to feed on Ephemereoptera in the benthos would generally require that the whitefish probe into crevices between rocks to feed.

Prey items found more often in the stomachs of nonpinocchios are consistent with these individuals feeding in the water column more often than pinocchios. These prey items include simuliids, which occur attached to the surface of rocks (Merritt and Cummins 1996) and chironomid pupae, which occur most commonly suspended in the water column or at the water surface (Merritt and Cummins 1996).

While these diet differences were statistically significant, *P*-values were generally not below Bonferroni adjusted values. However, for Ephemereoptera, the same pattern occurred in both samples. In addition, I observed significant differences when I was least likely to for the following three reasons. First, both samples were collected from the same pool and therefore I was less likely to observe any differences in diet due to potential differences in habitat use by fish with different snout morphologies. Second, all individuals in both samples were collected at the same time and therefore I was less likely to observe any differences in diet due to potential diel differences in feeding behavior. Third, for the 2004 sample, I observed diet differences during

the time of year when I least expected to find a difference. The primary prey items of mountain whitefish are generally the most abundant in the spring and thus mountain whitefish are likely to be the least selective at this time of the year. In the Bitterroot River in March, the large Plecoptera nymphs (especially *Skwala* spp.) that were readily consumed by pinocchios and nonpinocchios are a highly abundant, energy rich, and easily captured food source.

These diet results are consistent with behavioral observations of Troffe (2000). Troffe observed that pinocchios directed feeding attempts towards the substrate significantly more than nonpinocchios for two sites within a tributary to the Fraser River. However, the number of individuals observed was small and it would be necessary to reproduce these behavioral results to confirm behavioral differences between morphs.

Overall, the evidence suggests that the observed phenotypic variation in snout size and shape of mountain whitefish corresponds to subtle differences in diet and perhaps foraging strategies for individuals with extreme phenotypes. The differences in diet I observed are not as great as have been observed in northern lacustrine fishes with trophic polymorphisms (e.g. Skúlason et al. 1989; Snorrason et al. 1994). Instead, my results are more similar to the subtle differences observed in riverine brook trout (McLaughlin and Grant 1994). Whether the differences I observed for mountain whitefish are biologically meaningful remains to be determined, as do behavioral and potential fitness consequences of this trait. For example, it will be important to determine if these differences in diet reduce intraspecific competition between individuals with extreme phenotypes (e.g. Swanson et al. 2003).

Is there evidence of assortative mating by snout morphology?

The genetic results presented here provided no evidence for assortative mating by snout morphology. If assortative mating by phenotype were occurring, I would expect to find more than one locus with a significant deficit of heterozygotes, at least a few loci should have

significant differences in allele frequencies (Table 4-6), and individuals should have tended to cluster by phenotype in the PCA of multilocus genotypes (Figure 4-7).

The genetic results found in this study are not consistent with those of Troffe (2000). These authors proposed secondary contact among distinct evolutionary groups in the Fraser River as a possible mechanism to explain their genetic observations. It is possible that secondary contact among distinct evolutionary groups occurred in the Fraser River but not the Clark Fork River (which is part of the adjacent Columbia River to the south). This would be consistent with a pattern of genetic differentiation between pinocchios and nonpinocchios in one river system but not the other. However, this hypothesis is not consistent with genetic data I have collected for this species (ARW unpublished data). A more likely explanation is that the results of Troffe (2000) are due to drift at a single locus, especially since the locus examined has a small N_e.

Implications for the evolution of trophic polymorphisms

If the pinocchio trait represents a trophic polymorphism, it appears to be at an early stage in the evolutionary trajectory observed for trophic polymorphisms in other fishes. The subtlety in trophic differences and random mating with respect to this trait may be due to the combination of riverine environment and mating system. Comparisons with species that have a similar mating system as mountain whitefish but occur in lakes, as well as comparisons with species that have different mating system but occur in rivers, provide information about the ecological conditions that favor the evolution of trophic polymorphisms.

In several cases where trophic morphs have arisen sympatrically within lakes, it appears that trophic morphs can become highly specialized and reproductively isolated despite mating systems in which assortative mating is unlikely without spatial segregation. For example, two reproductively isolated trophic morphs of lake whitefish (*Coregonus clupeaformis*) occur within northern lakes (Bernatchez et al. 1999). These morphs appear to have arisen sympatrically in at

least some lakes (Bernatchez et al. 1996). The congeneric pygmy whitefish (*Prosopium coulteri*) potentially has two or three morphs that may have evolved within several Alaskan lakes (McCart 1970). Information on the mating system of these two species is limited (Wedekind et al. 2001), but probably is similar to the mountain whitefish (Scott and Crossman 1979). If many males fertilize the eggs of females, as is likely in these species, it should be unlikely for reproductive isolation to occur without spatial segregation of morphs at the time of spawning. Thus, the distinct foraging habitats found in lakes and the subsequent correlation with spawning location apparently supercede the homogenizing effect of the mating system of these species. In addition, if extant morphological differences originated in isolation, the lacustrine environment would be more conducive to the maintenance of these differences if sympatry were re-established. Thus, by providing an example from a less stable environment (rivers) out study highlights the importance of stable environments (lakes) for promoting phenotypic diversification.

The present study also highlights the potential importance of mating system for the origin and maintenance of trophic polymorphisms in rivers. In riverine lenok populations, where two reproductively isolated morphs occur (Osinov et al. 1990), small groups spawn in nests called redds (Baimukanov 1996). Spawning in redds generally provides an opportunity for mate choice in salmonids (Stearley 1992). It is possible that this spawning behavior has allowed assortative mating by phenotype to occur in this species and thus allowed this putative trophic polymorphism with reproductive isolation between morphs to evolve within rivers, despite greater temporal and spatial variability of resources and the likely initial lack of spatial separation of morphs during spawning. Alternatively, if morphological differences in this species arose in isolation, the more derived mating system may have provided conditions that allowed these differences to be maintained. This example highlights the inference that the less derived mating system of the mountain whitefish may contribute to the early evolutionary stage of the pinocchio trait in this species.

Table 4-1. Sample locations for mountain whitefish in the Clark Fork River Basin, Montana.

Sample Location	Date	*N*	Analysis*
West Fork Bitterroot River	October 2000	41	G
Bitterroot River, Stevensville, MT	July 2003	117	M,D
Bitterroot River, Stevensville, MT	March 2004	105	M,G,D
Rattlesnake Creek, Missoula, MT	November 2002	46	M
Rattlesnake Creek, Missoula, MT	November 2003	89	M

*M = morphology; G = genetic; D = diet

Table 4-2 Proportion of individuals subjectively classified as pinocchio, intermediate, or nonpinocchio and mean values of snout index for each category. Number in parentheses is the standard error.

Sample	Proportion Subjectively Classified	Mean Snout Index (mm^2)
All individuals (N = 357)		
Pinocchio	0.185	3.45 (0.33)
Intermediate	0.552	0.10 (0.10)
Nonpinocchio	0.263	-1.53 (0.11)
Immature individuals excluded (N = 338)		
Pinocchio	0.196	3.45 (0.33)
Intermediate	0.555	0.12 (0.10)
Nonpinocchio	0.249	-1.62 (0.12)

Table 4-3. Slopes of allometric relationships between various traits and body size for mountain whitefish. Values are slopes of the regression of the natural log of the given trait on the natural log of standard length (body size). The 95% confidence interval is in parentheses.

Measurement	Males (N = 136)	Females (N = 102)
	b	b
Supraethmoid base width	1.61 (1.47, 1.74)	1.34 (1.20, 1.47)
Supraethmoid length	1.28 (1.15, 1.40)	1.05 (0.90, 1.20)
Pectoral fin length	1.06 (0.99, 1.12)	0.95 (0.88, 1.02)
Pelvic fin length	1.15 (1.08, 1.23)	1.03 (0.94, 1.12)
Anal fin length	1.12 (1.04, 1.19)	1.05 (0.97, 1.12)

Table 4-4. Diet analysis for mountain whitefish from the Bitterroot River. The number of diet items, the average proportion of diet by number of each diet item, and the average proportion of diet by weight are shown. Numbers in parentheses are standard errors. For the Bitterroot 2003 sample, three size classes are shown. *P*-values are from a Kruskal-Wallis test and superscripts reflect post-hoc tests following Zar (1984). For the seven multiple comparisons, corrected *P*-values would be 0.007 for $\alpha = 0.05$ (0.05/7) and 0.014 for $\alpha = 0.10$ (0.10/7). For b) and c) *P*-values are from Mann-Whitney tests.

	Diet Item							
Size Class	Plecoptera Nymphs	Small Ephemeroptera Nymphs	Large Ephemeroptera Nymphs	Trichoptera Larvae	Chironomidae Larvae	Chironomidae Pupae	Simuliidae Larvae	Other
(a) Bitterroot 2003 - all individuals								
number								
S1 (*N* = 17)	0.47 (0.17)	8.47 (1.45)^{a}	2.23 (0.35)	12.06 (4.03)	271.53 (34.63)^{a}	6.24 (1.39)^{a}	30.59 (15.24)^{a}	3.77 (0.98)
S2 (*N* = 25)	0.36 (0.14)	24.04 (12.64)^{b}	2.92 (0.90)	17.52 (4.67)	191.68 (44.43)^{a}	2.68 (0.77)^{a,b}	2.88 (0.93)^{a}	3.84 (1.35)
S3 (*N* = 64)	0.63 (0.14)	2.69 (0.51)^{a}	3.18 (0.53)	16.00 (4.91)	30.45 (10.87)^{b}	1.33 (0.40)^{b}	32.16 (20.17)^{a}	3.25 (1.06)
p	> 0.05	< 0.0001	> 0.05	> 0.05	< 0.0001	< 0.0001	0.0014	> 0.05
mean proportion by number								
S1 (*N* = 17)	0.002 (0.001)	0.028 (0.006)	0.007 (0.001)^{a}	0.046 (0.019)^{a}	0.771 (0.050)^{a}	0.019 (0.005)	0.102 (0.048)^{a}	0.012 (0.003)
S2 (*N* = 25)	0.002 (0.001)	0.098 (0.032)	0.029 (0.010)^{a}	0.169 (0.052)^{a,b}	0.581 (0.072)^{a,b}	0.012 (0.003)	0.014 (0.008)^{a}	0.064 (0.041)
S3 (*N* = 64)	0.020 (0.005)	0.074 (0.016)	0.096 (0.016)^{a}	0.280 (0.037)^{b}	0.237 (0.037)^{a}	0.038 (0.011)	0.077 (0.027)^{a}	0.071 (0.015)
p	> 0.05	> 0.05	0.05	0.0003	< 0.0001		0.032	> 0.05
mean proportion by weight								
S1 (*N* = 17)	0.10 (0.004)	0.003 (0.001)^{a}	0.139 (0.024)	0.073 (0.143)	0.302 (0.040)^{a}	0.013 (0.003)^{a}	0.105 (0.052)^{a}	na
S2 (*N* = 25)	0.007 (0.003)	0.016 (0.009)^{a}	0.178 (0.046)	0.007 (0.002)	0.216 (0.043)^{a,b}	0.008 (0.002)^{a,b}	0.010 (0.003)^{a}	na
S3 (*N* = 64)	0.024 (0.005)	0.003 (0.001)^{a}	0.275 (0.039)	0.049 (0.021)	0.056 (0.018)^{b}	0.008 (0.003)^{b}	0.047 (0.020)^{a}	na
p	> 0.05	0.0014	> 0.05	> 0.05	0.0001	0.0007	0.0034	na
(b) Bitterroot 2003 - subset of adults								
number								
Nonpinocchio (*N* = 14)	0.57 (0.37)	1.86 (0.49)	1.21 ± 0.60	37.86 (20.68)	7.79 (3.80)	1.57 (0.67)	122.29 (89.37)	3.86 (0.80)
Pinocchio (*N* = 13)	0.54 (0.33)	2.39 (1.32)	6.00 ± 1.99	14.31 (5.38)	12.08 (4.88)	0.62 (0.24)	10.69 (10.69)	5.31 (4.09)
p	> 0.05	> 0.05	0.0168	> 0.05	> 0.05	> 0.05	0.0261	> 0.05
mean proportion by number								
Nonpinocchio (*N* = 14)	0.020 (0.014)	0.047 (0.019)	0.029 ± 0.020	0.375 (0.092)	0.132 (0.045)	0.054 (0.034)	0.225 ± 0.103	0.073 (0.021)
Pinocchio (*N* = 13)	0.019 (0.015)	0.071 (0.051)	0.165 ± 0.058	0.263 (0.085)	0.251 (0.099)	0.015 (0.006)	0.051 ± 0.051	0.050 (0.020)
p	> 0.05	> 0.05	0.0138	> 0.05	> 0.05	> 0.05	0.0224	> 0.05
mean proportion by weight								
Nonpinocchio (*N* = 14)	0.021 (0.012)	0.001 (0.001)	0.074 ± 0.038	0.707 (0.090)	0.012 (0.005)	0.011 (0.009)	0.163 (0.087)	na
Pinocchio (*N* = 13)	0.025 (0.014)	0.004 (0.003)	0.352 ± 0.093	0.474 (0.094)	0.096 (0.068)	0.010 (0.009)	0.037 (0.037)	na
p	> 0.05	> 0.05	0.0138	> 0.05	> 0.05	> 0.05	0.0224	na
(c) Bitterroot 2004 - subset of adults								
number								
Nonpinocchio (*N* = 15)	22.60 (7.38)	20.87 (13.89)	19.60 ± 8.53	60.93 (12.75)	156.20 (34.13)	22.33 ± 12.69	0.533 (0.350)	7.60 (1.334)
Pinocchio (*N* = 15)	28.93 (6.98)	15.13 (4.35)	46.67 ± 14.48	76.87 (24.55)	172.73 (48.80)	3.20 ± 0.863	0	7.80 (1.481)
p	> 0.05	> 0.05	0.0379	> 0.05	> 0.05	0.0121	> 0.05	> 0.05
mean proportion by number								
Nonpinocchio (*N* = 15)	0.090 (0.028)	0.083 (0.049)	0.061 ± 0.024	0.230 (0.052)	0.456 (0.061)	0.045 ± 0.018	0.002 (0.001)	0.032 (0.008)
Pinocchio (*N* = 15)	0.134 (0.039)	0.058 (0.018)	0.169 ± 0.043	0.207 (0.038)	0.393 (0.076)	0.010 ± 0.003	0	0.029 (0.005)
p	> 0.05	> 0.05	0.0264	> 0.05	> 0.05	0.0121	> 0.05	> 0.05
mean proportion by weight								
Nonpinocchio (*N* = 15)	0.434 (0.081)	0.001 (0.001)	0.123 (0.032)	0.392 (0.064)	0.040 90.013)	0.011 (0.007)	0.0002 (0.0001)	na
Pinocchio (*N* = 15)	0.519 (0.087)	0.0004 (0.0001)	0.193 (0.051)	0.230 (0.047)	0.058 (0.039)	0.002 (0.002)	0	na
p	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	na

Table 4-5. Microsatellite allele frequencies for groups of pinocchio and normal mountain whitefish. Sample size (*N*), mean expected heterozygosity (H_S), and mean number of alleles (*A*) are shown.

Sample	*SFO8-1*			*SSA456*								
	**158*	**162*	**164*	**138*	**158*	**160*	**162*	**210*	**220*	**222*	**224*	**230*
West Fork Bitterroot River												
Pinocchio	0.300	0.550	0.150	0.450	0.250	0.250	0.000	--	0.050	--	--	--
Nonpinocchio	0.300	0.450	0.250	0.300	0.100	0.500	0.050	--	0.050	--	--	--
Bitterroot River 2004												
Pinocchio	0.400	0.475	0.125	0.175	0.000	0.625	--	0.025	0.050	0.025	0.075	0.025
Nonpinocchio	0.300	0.525	0.175	0.350	0.000	0.475	--	0.000	0.100	0.050	--	0.025

Sample	*COCL4*			*SSA14*					*ONE8*		
	**146*	**150*	**152*	**167*	**169*	**171*	**173*	**175*	**180*	**182*	**184*
West Fork Bitterroot River											
Pinocchio	0.700	0.250	0.050	0.000	0.050	0.550	0.100	0.300	0.100	0.750	0.150
Nonpinocchio	0.650	0.250	0.100	0.050	0.000	0.600	0.100	0.250	0.100	0.650	0.250
Bitterroot River 2004											
Pinocchio	0.658	0.316	0.026	0.075	0.100	0.450	0.150	0.225	0.025	0.700	0.275
Nonpinocchio	0.700	0.225	0.075	0.075	0.100	0.650	0.075	0.100	0.075	0.625	0.300

Sample	*FGT25*			*SFO8-2*		*N*	H_S	*A*
	**170*	**178*	**180*	**195*	**197*			
West Fork Bitterroot River								
Pinocchio	0.200	0.050	0.750	0.900	0.100	10	0.494	3.14
Nonpinocchio	0.000	--	1.000	0.900	0.100	10	0.459	3.00
Bitterroot River 2004								
Pinocchio	0.075	0.050	0.875	0.925	0.075	20	0.461	3.71
Nonpinocchio	0.100	0.025	0.875	0.875	0.125	20	0.468	3.43

Table 4-6. Results of one-tailed tests for heterozygote deficits and exact tests for genic differentiation among pinocchios and nonpinocchios for two independent samples from the Bitterroot River. Numbers listed are p-values. I used Fisher's method to combine *P*-values from each locus. For each chi-square test, there were 14 degrees of freedom. The Bonferroni corrected *p*-value for seven tests is $\alpha = 0.05$ is 0.007 (0.05/7) and for $\alpha = 0.10$ is 0.14 (0.10/7).

Test	Locus							
	COCL4	*SSA14*	*ONE8*	*SSA456*	*SFO8-1*	*SFO8-2*	*FGT25*	Combined Probability
West Fork Bitterroot 2000								
Heterozygote Deficit (N = 41)	0.291	0.785	0.846	0.085	0.101	1.000	0.069	$\chi^2 = 18.14; P = 0.200$
Genic Differentiation (N = 20)	1.000	1.000	0.877	0.297	0.780	1.000	0.050	$\chi^2 = 10.78; P = 0.703$
Bitterroot 2004								
Heterozygote Deficit (N = 40)	0.859	0.893	0.663	0.013	0.660	0.322	0.097	$\chi^2 = 17.86; P = 0.213$
Genic Differentiation (N = 40)	0.448	0.347	0.638	0.188	0.587	0.712	1.000	$\chi^2 = 9.71; P = 0.783$

Figure 4-1. Examples of phenotypically extreme fluvial mountain whitefish: (a) pinocchio; (b) nonpinocchio and the criteria used for the subjective classification of each type.

Figure 4-2. Measurements of (a) snout index and (b) supraethmoid length and width. Details of measurements provided in text.

Figure 4-3. Correspondence between the snout index and subjective classification (described in text) for all individuals (a), males only (b), and females only (c). Snout index is plotted against standard length in each panel. Filled circles represent pinocchios, open circles represent intermediates, and x's represent nonpinocchios.

Figure 4-4. Snout index versus standard length for mountain whitefish from the Bitterroot River and Rattlesnake Creek, Montana ($N = 357$). The snout index was determined with the measurement shown in Figure 4-2a. Histograms show counts for snout index and standard length separately.

Figure 4-5. Correlation analysis for size-adjusted weight and size-adjusted snout index for males from Rattlesnake Creek, Montana.

Figure 4-6. Average proportion of eight prey items in the stomachs of mountain whitefish from the Bitterroot River, Montana. In (a), all individuals from Bitterroot 2003 are shown. In (b), only phenotypically extreme individuals from S3 (Bitterroot 2003) are shown. In (c), phenotypically extreme individuals from Bitterroot 2004 are shown.

Figure 4-7. Plot of principle component scores based on multilocus genotypes of individuals from (a) the West Fork Bitterroot River and (b) the Bitterroot River (2004). Pinocchios are

represented by filled circles, nonpinocchios by open circles. Percentages are the proportion of the total variation among genotypes attributable to each axis.

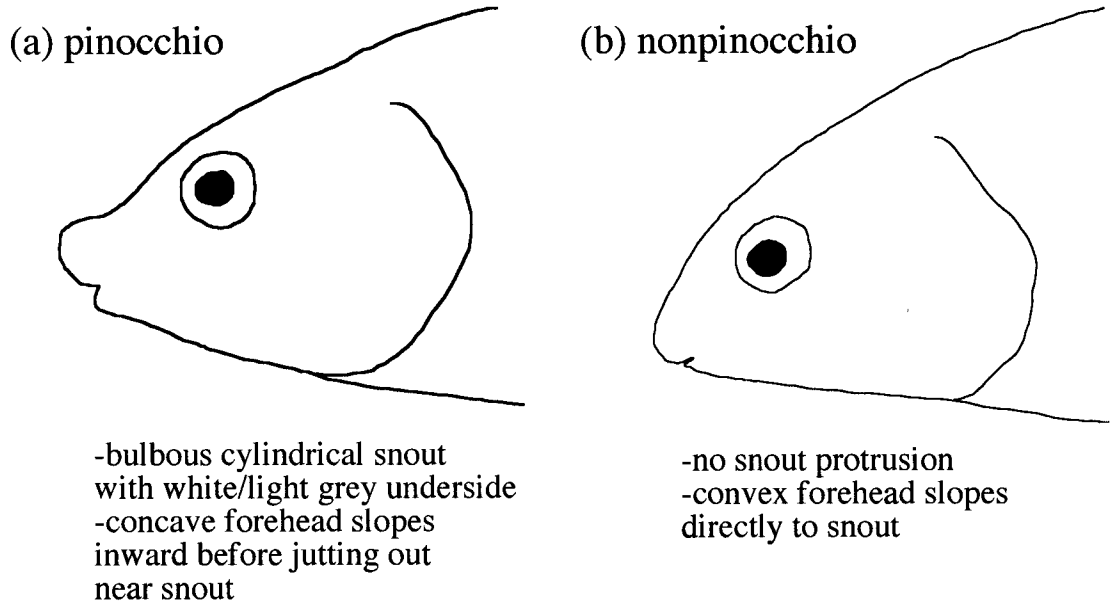


Figure 4-1

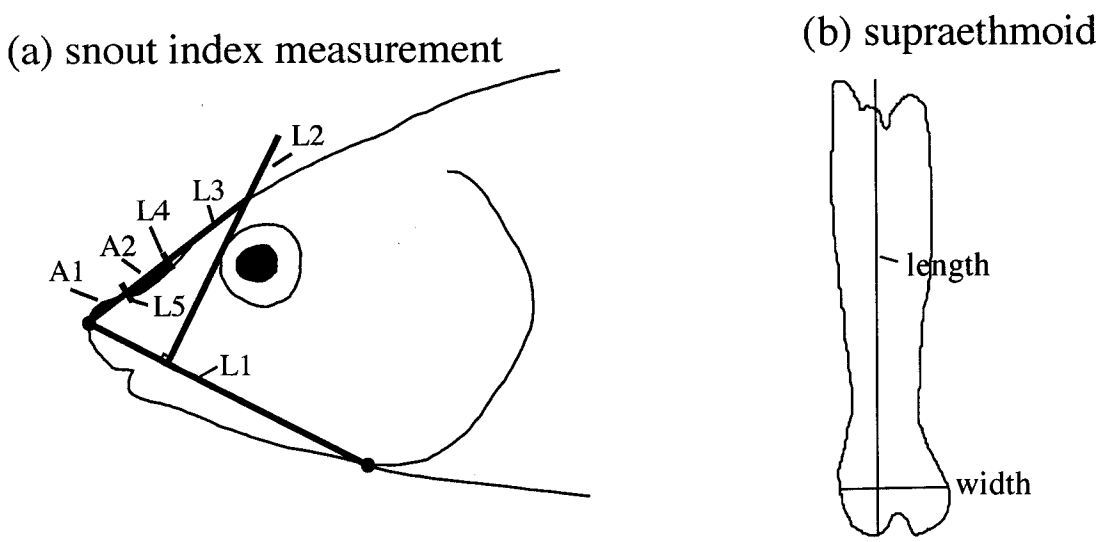


Figure 4-2

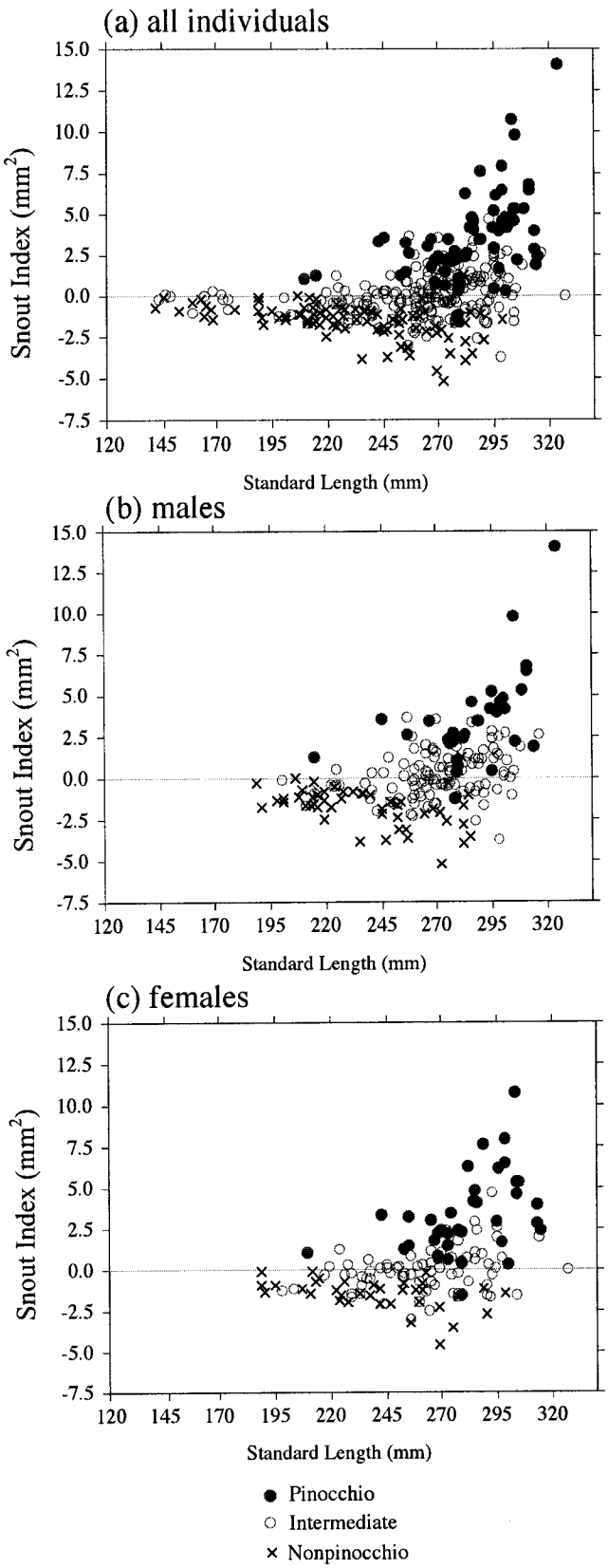


Figure 4-3

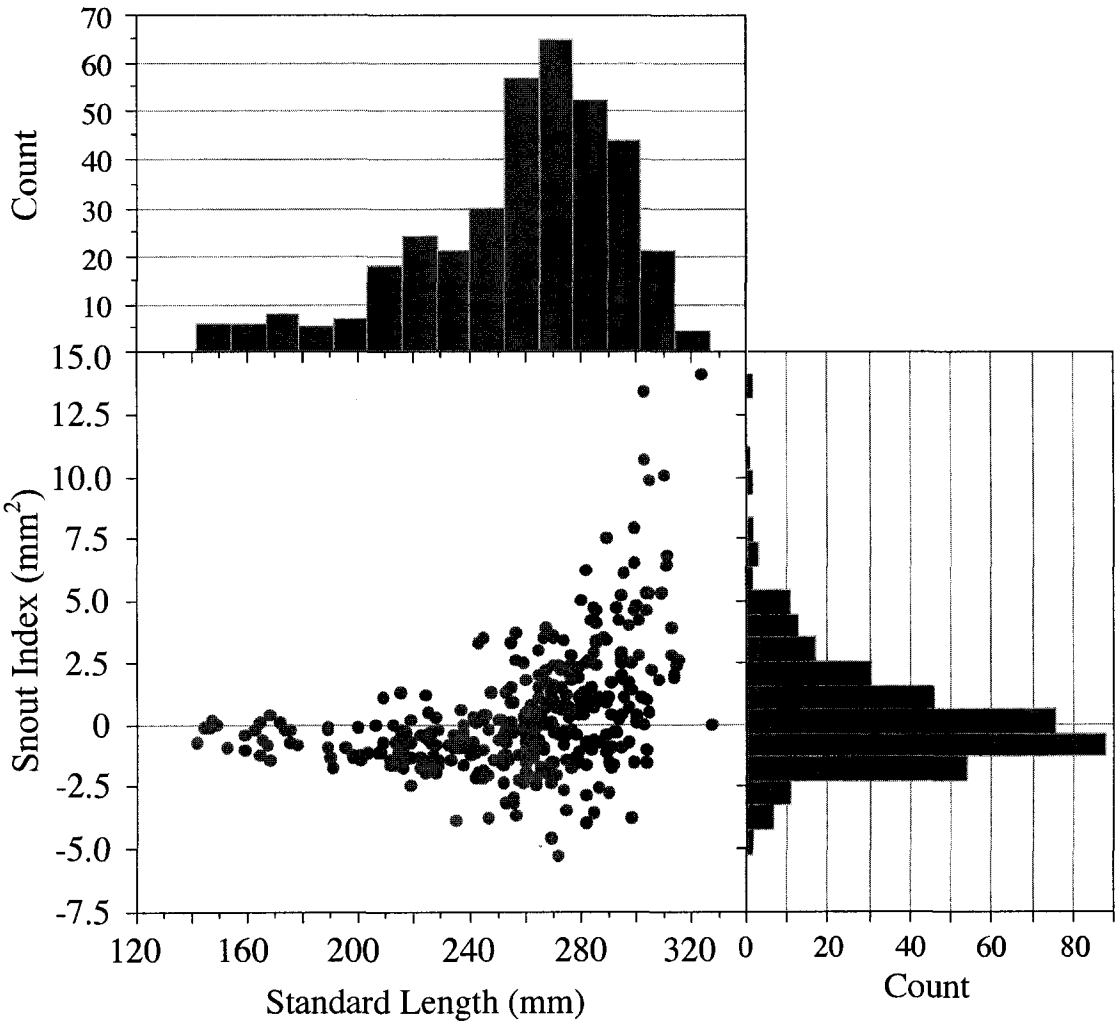


Figure 4-4

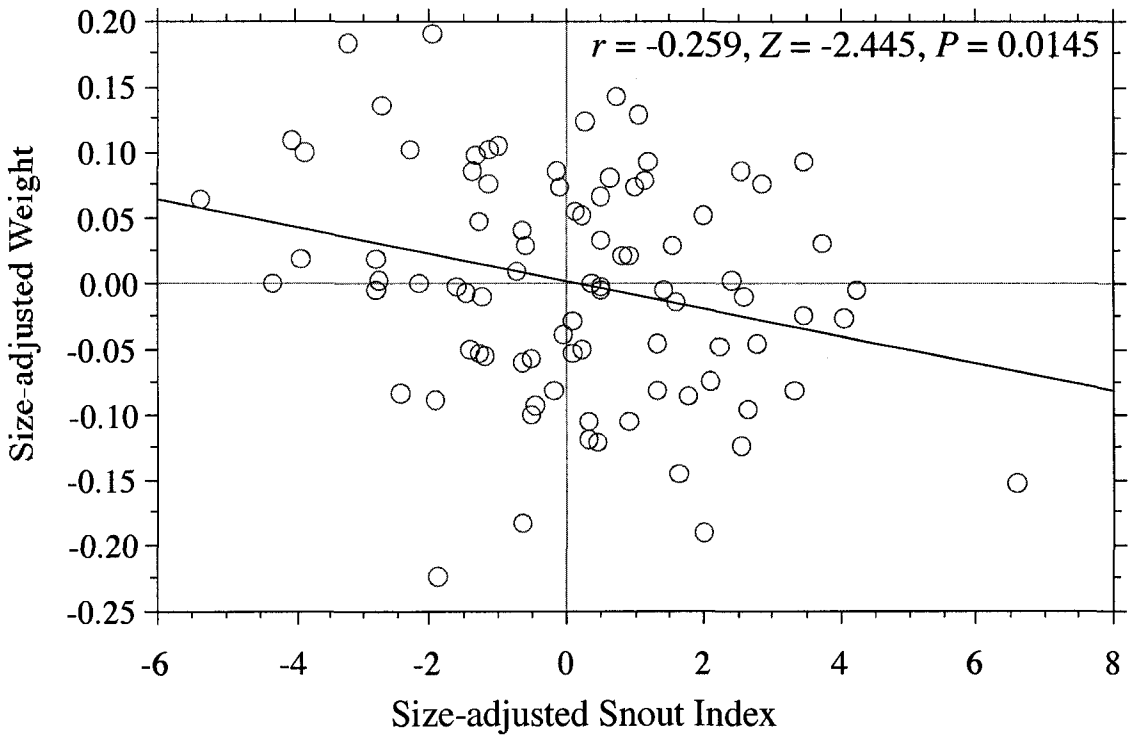


Figure 4-5

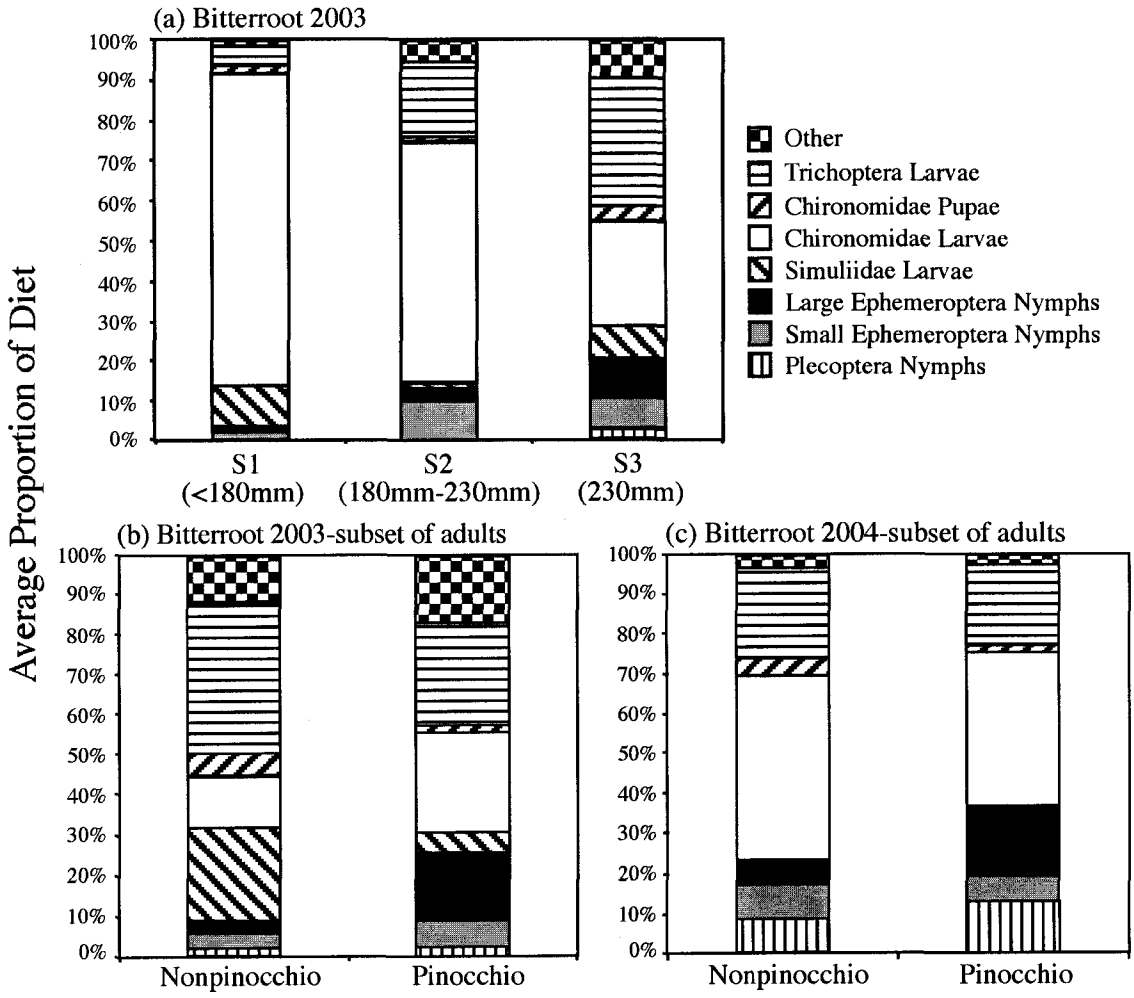


Figure 4-6

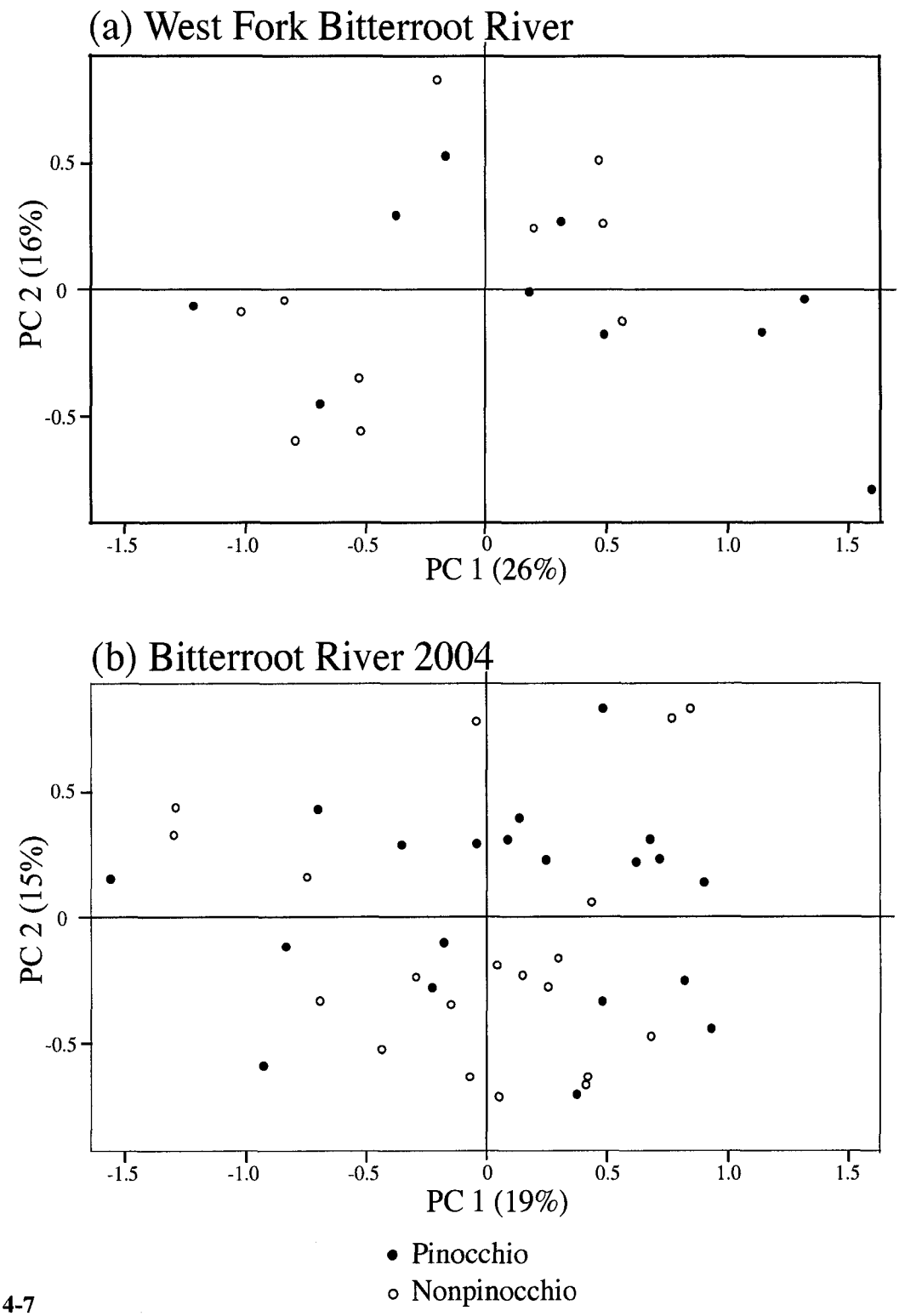


Figure 4-7

CHAPTER 5 - Classroom Mark-Recapture with Crickets

5.1 ABSTRACT

Mark-recapture techniques are commonly used by wildlife biologists and ecologists to estimate abundance of animals in naturally occurring populations and are therefore an important component of curricula that include population ecology. This lab activity teaches mark recapture techniques using crickets in a single 10-gallon aquarium and provides an inexpensive way to teach students about this commonly used technique in a real world context. This alternative teaching method for mark-recapture methods provided highly accurate estimates of cricket abundance and captured student's interest more than other classroom-based strategies for teaching the same material. This lab can easily be done in any classroom and has the advantages of allowing students to handle easily obtained live insects, without the potential drawbacks and uncertainty of teaching mark-recapture in a field setting. We successfully used this technique in a high school general life science course, but it could easily be adapted for use in undergraduate general biology and ecology courses.

5.2 INTRODUCTION

How many deer live in a particular county? How many fish live in the creek that runs near a school? And how have these numbers changed over time in the last 10 years? These are the questions of population ecologists. Population ecology is the study of changes in the abundance of organisms over time and space (Akcakaya et al. 1999). Temporal and spatial trends of animal abundance are commonly used to prioritize conservation and management efforts for various animals. For example, these trends are used to help determine the numbers of hunting permits that will be issued in a given year. Due to its central role for ecology and population biology, many high school and undergraduate biology courses include lessons on population ecology theory.

Curriculum pieces on population ecology theory often include investigations on methods used to estimate animal abundance. One commonly used technique to estimate the size of natural populations is single mark-recapture using the Lincoln-Petersen relative abundance model (Smith and Smith 2001). In this method, animals are captured, given an identifying mark such as a paint spot or a tag with a number, and then released back to their habitat. At a later date, traps are set again in the same places. The ratio of marked to unmarked animals during the second capture event can be used to estimate the size of the population. This method provides a simple means to estimate the population size of animals. The basic form of the Lincoln-Petersen model is mathematically straightforward and appropriate for teaching about mark-recapture methodology. The basic model also provides an ideal mechanism for integrating science and mathematics. More advanced students can explore many extension of this model that address violations of several key assumptions (see below).

Various strategies have been used to teach mark-recapture in high school and undergraduate classrooms. A common teaching strategy uses dried beans or plastic beads as model animals (e.g. Budnitz 1998). In this strategy, a subsample of beans or beads is taken out of

a container, marked, and returned to the container. In a subsequent "recapture" event, another subsample of beans is collected and the proportion of marked beans relative to the number of unmarked beans is used to estimate the total number of beans in the container. While this is relatively simple to do in the classroom, we have found that it often does not work well because too many beans are in the containers to start with and this prevents students from marking a large enough proportion of the beans during the mark and recapture trials. Usually estimates are far from the true number of beans in the container. While this type of investigation illustrates very well one of the issues faced by population biologists that study hard-to-capture animals, we have found that it does not help the students firmly grasp population biology theory and furthermore, the inaccuracy of this method can erode student interest and enthusiasm. More importantly, this exercise does not present mark-recapture as it is used in practice and it is not as captivating for the students to handle beans as surrogates for live organisms.

Another teaching strategy involves the use of live animals, either in schoolyards (Anonymous 2002), or in a more natural field setting (Dussart 1991; Rollinson 2004). Handling live organisms provides a challenge to the students and provides a teaching opportunity about the natural history and biology of the organisms. This is an excellent option if large populations of easily captured organisms are available close to a school. Working with live animals is inherently more interesting to the students. Moreover, students get out of the classroom and into nature. On the other hand, working in a field setting requires significant planning and some uncertainty about the likelihood of successfully capturing enough animals. Furthermore, population estimates can be problematic if the assumptions of mark recapture models are not met.

Given that knowledge of the spatial and temporal distribution of animals is central to understanding important issues in ecology and conservation biology, we developed an activity to teach mark-recapture techniques using live animals in a classroom setting. We chose crickets living in 10-gallon aquaria habitats. Our approach shares the advantage of the bean exercise in that students conduct the investigation in the classroom so there is no uncertainty about finding

enough animals. It shares the advantages of field-based investigations in that students work with live organisms, necessitating an understanding of the biology of the crickets. But most importantly, student's interest is captured by the challenge of handling these animals. We found that using crickets in this investigation captivates students in a similar manner as field-based techniques, but it is much more feasible to use in restricted time periods and classrooms.

5.3 OBJECTIVES OF THIS ACTIVITY

The general goal of this investigation is to complement instruction on population ecology and to teach mark-recapture theory and techniques that are used by population biologists to understand the distribution of animals in space and time. More specifically, students work collaboratively to learn: (1) mark-recapture techniques to estimate population size of naturally occurring organisms; (2) how to calculate a population estimate using equations (i.e. algebraic manipulation of simple ratios and solving equations for one unknown) and data they collect; (3) about the natural history and the handling of a common insect; and (4) to think critically about how wildlife biologists estimate population sizes and about popular press stories that feature abundance estimates of wild animal populations.

This investigation promotes science as inquiry and helps students develop skills in asking questions, collecting and interpreting data, and communicating the results with their peers. It maps easily onto the National Science Education Standards (NRC 1996). This semi-guided inquiry can lead to more open-ended investigations (content standard A) and it emphasizes student collaboration. Moreover, it emphasizes concepts related to population growth and natural resources (content standard F). Finally, students refine their ability to use models and equations to make estimates and predictions (content standard G).

5.4 COUNTING CRICKETS

Materials

Table 5-1 provides a list of the materials needed for this activity. We recommend using the same set of crickets for multiple classes and having students mark crickets multiple times on different body parts and with different colored paint pens. This minimizes set up time and forces the students to be careful with how and where they mark the crickets.

Investigation

This investigation can be completed within a 1 to 1.5 hour class period. There were two short periods of time for direct instruction and two periods where students capture, handle, and mark crickets. We designed an opening interactive lecture focusing on why it is important to estimate the population size of naturally occurring animals. Students then captured, marked, and collected data from crickets. During a second lecture, students learned the theory behind mark-recapture using the Lincoln-Petersen technique. The investigation and the transparencies that can be used for this investigation are available online at www.bioed.org/ecos/.

At the beginning of each class session, students received an investigation sheet that briefly explained the investigation and contained a data sheet for their mark-recapture data (Figure 5-1). Students worked in groups (we recommend three per group) and each group received one data sheet.

The introductory sampling lecture was designed to build on previous population ecology lessons and activities. In this lecture we addressed the following concepts: why it is important to estimate population sizes of naturally occurring animals, the basic idea behind mark-recapture techniques, the importance of understanding the biology and natural history of the animals we study, basic insect anatomy, specifically how to mark crickets, safety and ethical issues with working with live animals (there are minimal safety issues associated with this investigation but students should wash their hands after handling the crickets), and general logistics. More detailed

information on these topics and a PowerPoint file with overhead masters are available at www.bioed.org/ecos. We waited to explain the details of the Lincoln-Petersen model until after the first capture session.

The first step in the investigation was for each group to observe the aquarium setting. We used one 10-gallon aquarium per class. One student from each group removed one "cricket castle", which was a small portion of an egg carton (Table 5-1), and gently shook the crickets from the egg carton into the plastic container. Each group returned to its table with its crickets (5 to 6 worked well). It is important to provide enough pieces of egg carton so that each group can use one and it is also helpful for the instructor to supervise the capture process so that groups overturn only one piece of egg carton. We observed a tendency for the students to overturn many of the pieces of egg carton and to disturb many of the crickets if we left them unsupervised. In addition, the instructor should monitor the approximate number of crickets collected. In general, abundance estimates tend to be close to the true number of animals if at least half of the animals receive marks.

At their table, the groups used a paint pen to mark the crickets. One student held a cricket while another dabbed the specified body part with paint, and a third recorded the number of crickets marked (this is n_1 in the equation described below and in Figure 5-1). We used different colored paint pens for each class and marked either part of the thorax or one of the legs of crickets. Students also recorded data on their data sheet on whether or not the crickets have wings and the gender of each cricket. After all of the groups obtained crickets, the first groups were allowed to gently return crickets to the aquarium.

Once the crickets were back in the aquarium, we presented the Lincoln-Petersen method during another 15-minute lecture. This lecture focused on the variables in the Lincoln-Petersen index of relative abundance, the ratio used to calculate $\hat{N}$ (the estimate of population size), and the assumptions of the model. Overhead masters are available online for you to download at www.bioed.org/ecos. This break in activity allowed the crickets to settle back into their "traps".

The equation for the Lincoln-Petersen model is: $\frac{n_1}{\hat{N}} = \frac{m_2}{n_2}$ (1), where n_1 is the number of animals marked and released during the first session, n_2 is the number of animals captured during the second session, m_2 is the number of animals captured during the second session that are recaptures and were marked during the first session, and $\hat{N}$ is the estimate of population size. This equation can be algebraically manipulated to solve for $\hat{N}$, such that $\hat{N} = \frac{n_1 * n_2}{m_2}$ (2). This model makes the following assumptions: first, the population is closed (no births, deaths, immigration, or emigration). Second, marks are not lost or overlooked by the observer. Third, all animals are equally likely to be captured in each sample and over time. That is, it is assumed that there are no behavioral differences in preference or avoidance of the "trap" between individuals, and also that being trapped once does not make an individual more or less likely to be captured again. It is also assumed that things like weather changes or other environmental factors do not change the probability of trapping animals during the two trapping periods.

In addition to calculating $\hat{N}$, an optional extension for advanced students is to calculate the standard error of the estimate of population size using the following equation:

$S.E. = \hat{N}\sqrt{\frac{(\hat{N}-n_1)(\hat{N}-n_2)}{n_1 n_2(\hat{N}-1)}}$ (3). $\hat{N} \pm 2(S.E.)$ provides the 95% confidence interval about $\hat{N}$ (Smith and Smith 2001).

Finally, we predicted the types of factors that might lead to differences between our estimate of population size and the true population size. Through this discussion, students thought about the equation they had just learned and the consequences of violations of the assumptions of the model. For example, it the crickets lost their marks before the recapture session, this would lead to an upwardly biased estimate of the population size (because m_2 will be biased low and since this is in the denominator of equation 2, $\hat{N}$ will increase).

The recapture event followed this second lecture. Groups repeated the same process of capturing crickets described above. Students recorded m_2 (the number of marked crickets captured during this session) and n_2 (the total number of crickets their group recaptured). Students recorded all marks given during their class period (not just the marks given by their group). Each group reported n_1, n_2, and m_2 in a table made by the instructor on the board. The sum of each variable was used as the class total to calculate one value of $\hat{N}$ per class (Figures 5-1; Figure 5-2).

After students worked through the calculations of $\hat{N}$, a general discussion followed about how close the estimate was to the true value. Reasons why $\hat{N}$ might not be accurate were discussed, along with confidence intervals (optional), and potential violations of assumptions. We referred to the list of model assumptions to discuss each assumption and whether it may have been violated. For example, cricket escapes would violate the closed population assumption. Another possible source of bias could be related to the trapping method used in this investigation. $\hat{N}$ might be biased low because stressed crickets might crawl directly back into the castles to seek cover after the crickets are placed back into the aquarium. Thus, m_2 might be biased high, in turn causing $\hat{N}$ to be biased low. It is helpful to link violations of the assumptions explicitly to the equation to determine how $\hat{N}$ might be affected.

We also had students reflect on their data on the number of males and females that had wings. These data were used to generate hypotheses regarding the observations. For the crickets we used in this investigation, females tended to be wingless while males had wings. One hypothesis is that females might not have wings because of the way they allocate their limited resources to growth versus reproduction. Because they use a lot of energy to make eggs, fewer energy-related resources may be devoted to growing wings during development. Males may need to allocate energy to the production of wings because they might disperse more than females, perhaps to find mates.

We used two follow up exercises to increase student comprehension. First, students were assigned the questions in Figure 5-2. This assignment takes the form of a follow up exercise to increase student comprehension. Second, we provided an extension activity where students estimated the population size of snowshoe hares from a valley in western Montana (Figure 5-3). For the students we worked with, this example was particularly relevant because snowshoe hares are the primary prey of Canada lynx (*Lynx canadensis*). The valley mentioned in this part of the investigation (the Swan Valley) is a stronghold of Canada Lynx in the lower 48 states of the United States (McKelvey et al. 2000). We created a fictitious data set that consisted of a series of n_1, n_2, and m_2 values for six consecutive years. Groups of students were assigned a year for which they calculated $\hat{N}$. Values of $\hat{N}$ for each year were written on the board and students were asked to graph the trend in population size. Once the data were combined, the overall population trend was discussed.

This portion of the exercise provided a link between the mark-recapture method just learned and a real-world use of this method. It also provided an opportunity to discuss potential violations of mark-recapture assumptions, the appropriate time interval between mark and recapture events, the best way to mark different types of animals, and methods of capture (snowshoe hares are trapped with wire Tomahawk live traps baited with alfalfa cubes in the winter or apples in the summer). In addition, because snowshoe hare and Canada lynx populations follow a boom and bust cycle, this portion of the exercise provided an opportunity to link the method the students just learned to relevant and exciting case studies in population ecology.

Extensions

Genetics techniques are now used to estimate the population size of animals. For example, biologists in Glacier National Park, Montana, use hair collected from special hair snagging stations to estimate the number of bears in this park (for more details see:

http://www.nrmsc.usgs.gov/research/glac_beardna.htm). Some of the techniques used to analyze genetic data use extensions of the Lincoln-Petersen model. Thus, after learning the basic theory and technique using the crickets, a further lesson could explore indirect genetic methods to estimate population size.

As another extension, after learning mark-recapture techniques through this investigation, students can design their own research investigation to use mark-recapture with naturally occurring populations of animals, perhaps as part of an independent project. This could be done in the schoolyard with insects using a similar technique described in this paper (e.g. pillbugs; Anonymous 2002). If a pond is nearby, frogs can be marked by clipping toes. Guidelines for toe-clipping can be found at: www.asih.org/pubs/ASIH_HACC_Final.pdf. For a discussion of the ethical aspects of this technique see Funk et al. (2005). If fish can be captured, individuals can be marked by clipping small portions of fins. Note that these more invasive techniques (e.g. clipping tissues) can only be performed with the consent of animal care committees and/or local fish and wildlife departments. We recommend contacting a local university or fish and wildlife department if students are interested in undertaking a project like this. There may be projects underway with opportunities for participation by volunteers.

Did this investigation provide a successful learning experience?

This investigation was tested with high school sophomores in their second year of a biology series. The teachers thought it was a substantial improvement over the bean exercise taught in the past for two reasons: 1) it was more accurate and 2) it better captured student interest. The classes' population estimates were close to the true value of 50 crickets in the aquaria and most estimates were within three of the true value; only one was off by eight from the true value. In contrast, the bean investigation often yielded results that were highly inaccurate, causing students to doubt the efficiency of the technique and, as a consequence, diminished their interest. Interestingly, the challenge of handling and marking live animals was a large part of the

appeal of this exercise. Many students had to confront their fear of insects and most appeared to enjoy handling the crickets.

We also examined how well students performed on the assessment problems and questions (Figures 5-2; Figure 5-3). Overall students answered most of the questions correctly and we conclude that the students gained an overall understanding of mark-recapture theory and technique. In general, students successfully manipulated equations, were able to think critically about assumptions of the Lincoln-Petersen model, and gave thoughtful responses regarding the broader importance of estimating the size of natural populations. We observed that some students had difficulties with using and manipulating the equations (equations 1 and 2, we did not use equation 3 for the standard error) and there was wide variation among answers related to assumptions of the model. We found that these concepts were important to revisit, through additional problems, questions, and class discussion. In summary, this investigation provided a great foundation from which we could increase student understanding of mark-recapture and population ecology concepts.

5.5 CONCLUSIONS

This population ecology investigation provides an inexpensive way to teach students, in a real world context, about a technique commonly used in field biology and ecology. This approach for teaching about mark-recapture methods provided highly accurate estimates of cricket abundance and appeared to capture student's attention more than the typical bean or bead counting strategies for teaching the same material. This investigation is easily done in any classroom setting. Moreover, it has the advantages of allowing students to handle live insects without the drawback, uncertainty, and time necessary to teach mark-recapture in a field setting. Most importantly, students demonstrated they could think critically about how wildlife biologists estimate population sizes and about popular press stories that feature abundance estimates of wild animal populations.

5.6 GLOSSARY

Sampling terminology

Closed population: a population where no births or deaths occur and individuals do not enter (immigrate) or leave (emigrate) during the time of study

Confidence Interval (C.I.): The range in which you expect 95% of all estimates to lie.

Lincoln-Petersen model: a specific mark-recapture technique that requires two sessions where animals are captured. This is a basic technique that forms the basis for more complicated population estimation methods. The equations for the model is: $\frac{n_1}{\hat{N}} = \frac{m_2}{n_2}$, where n_1 is the number animals marked and released during first session, n_2 is the number of animals captured during the second session, m_2 is the number of animals captured during second session that are recaptures from the first session, and $\hat{N}$ is the estimate of population size.

Mark-recapture techniques: a set of techniques used to estimate the population size of animals. All of the techniques involve an initial marking event where animals are captured, marked, and released. Animals are recaptured a second time and the proportion of marked to unmarked animals is used to estimate the population size.

Standard Error (S.E.): a measure of variation about the mean population estimate.

Subsample: a smaller group collected from within a larger population of objects.

Cricket anatomy (Borror et al. 1992)

Head: the anterior body region, which bears the eyes, antennae, and mouthparts

Thorax: the body region behind the head, which bears the legs and wings

Abdomen: the posterior of the three body divisions

Ovipositor: the egg laying apparatus; the external genitalia of the female

Cercus (plural *cerci*): one of a pair of appendages at the posterior end of the abdomen

Table 5-1. Materials for cricket investigation. Numbers of a given item required are in parentheses.

- 10-20 gallon aquarium (1)
- Pet store crickets (~50)
- Cardboard "traps" ("cricket castles"). We used egg cartons and cardboard packing material from an electronic device (~10)
- "Painters" acrylic non-toxic paint pens (Hunt Inc.; number of colors depends on number of participating classes)
- Chopped apple (food and water for crickets)
- Large (32 oz.) plastic containers (1 per group of students)

Handout for Cricket Mark-Recapture Investigation
Name: **Period:**
Date:

For this investigation, we will estimate the population size of crickets in aquariums. You will work in teams of two to catch, mark, release, and recapture crickets. Each team will take a plastic container to the aquarium and capture crickets by scooping crickets out once. This is your first sample. Take the crickets in your container to your desk and mark all of these crickets on their back with a paint pen. Fill in the number of crickets caught during your first sample for n_1 in the data table.

Once everyone has caught and marked crickets, each team will return the marked crickets to the same aquarium. We will wait 15 minutes. Then each team will take a second sample of crickets. Again, take the container of crickets to your desk and record the total number of crickets caught. Also record the total number of crickets with marks. The total number of crickets you caught the second time is n_2. The number of crickets you caught the second time with marks is m_2. Fill these in below. Also record whether each cricket is male or female and whether it has wings or not. Fill in the total number of males and females with and without wings in the table at the bottom of the page.

Your group's totals:
n_1 = _____
n_2 = _____
m_2 = _____
As a class, we will pool our cricket samples to estimate the abundance of crickets in the aquarium.

Class totals:
n_1 = _____
n_2 = _____
m_2 = _____

Fill in the data on sex and wings in this table:

Cricket #	Sex (M/F)	Wings (Y/N)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Fill in the total number of males and females with and without wings in this table:

Winged/wingless	Males	Females
Wings		
Wingless		
Total		

Figure 5-1

Questions for Cricket Mark-Recapture Investigation

1) Use the class data to estimate the population size of crickets in the aquarium ($\hat{N}$). Show your work below and use this equation: $\hat{N} = \frac{n_1 * n_2}{m_2}$.

2) Which assumptions of the Lincoln-Petersen model might we have violated?

3) How would the violations you mentioned effect your estimate of population size ($\hat{N}$)?

4) If the class estimate was close, does this guarantee that we didn't violate any assumptions of the Lincoln Petersen model?

5) What is the value of estimating the size of naturally occurring populations?

Figure 5-2

Extension Activity

Snowshoe hares are important food sources for Canada lynx; so many people are very interested in the population size. We would like to know if a population of snowshoe hares in Seeley Lake is increasing or decreasing. We conducted a 2-day trapping session once a year for 6 years. Each team will estimate the population size for **ONE** of the years. Once we tell your team which year to estimate, circle the year and estimate the population size. Once everyone has estimated the population size for their year, we will put it all together and graph the population size over time.

2004: On day 1, we caught and marked 18 animals. On day 2 we caught 23 animals, of which 12 were marked.
2003: On day 1, we caught and marked 12 animals. On day 2 we caught 15 animals, of which 6 were marked.
2002: On day 1, we caught and marked 16 animals. On day 2 we caught 19 animals, of which 12 were marked.
2001: On day 1, we caught and marked 20 animals. On day 2 we caught 24 animals, of which 19 were marked.
2000: On day 1, we caught and marked 13 animals. On day 2 we caught 15 animals, of which 9 were marked.
1999: On day 1, we caught and marked 14 animals. On day 2 we caught 15 animals, of which 11 were marked.

$n_1 =$ _____
$n_2 =$ _____
$m_2 =$ _____

$\hat{N} =$ _____

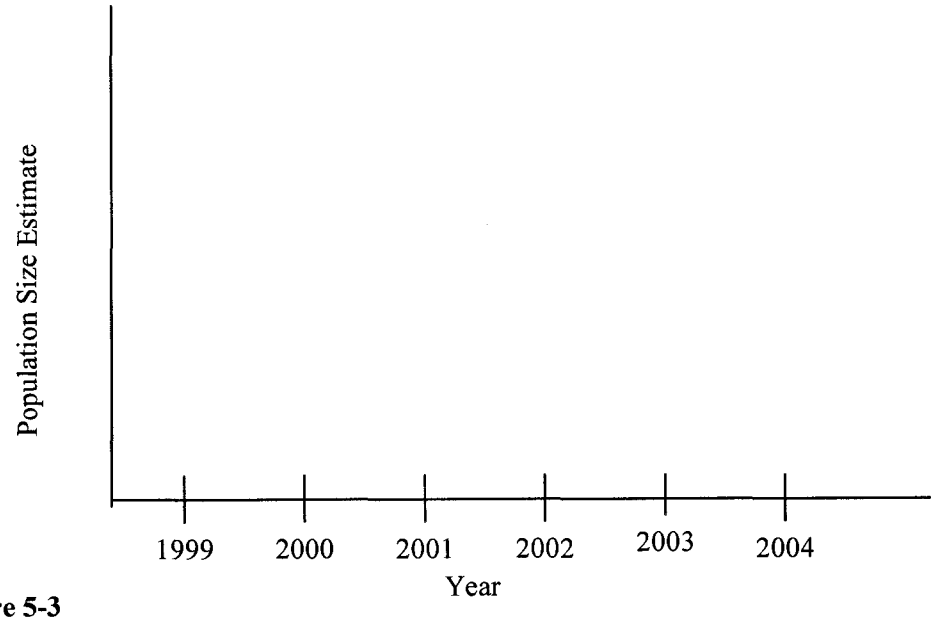


Figure 5-3

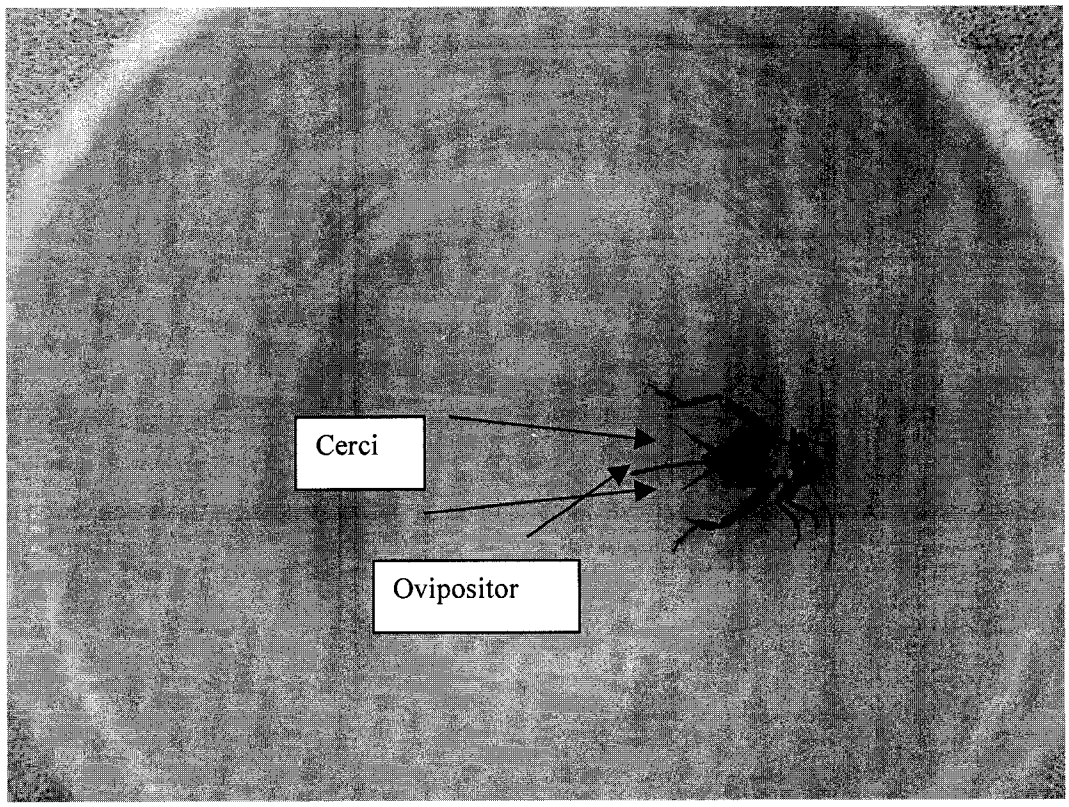


Figure 5-4

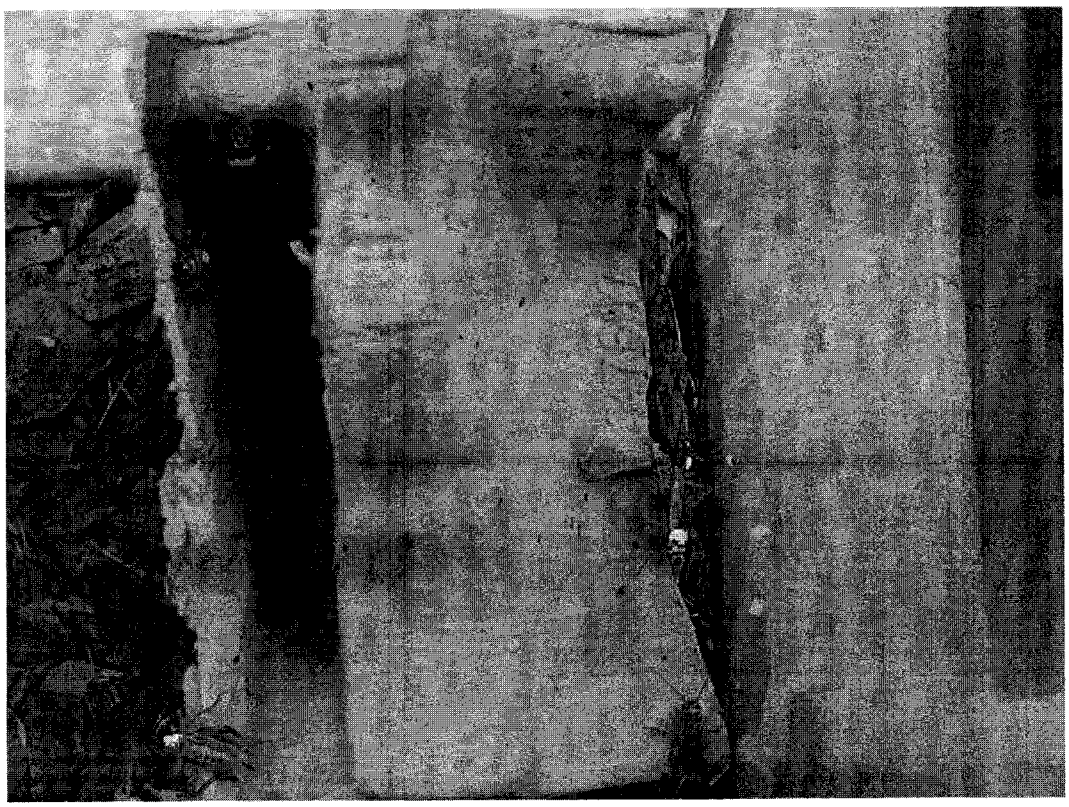


Figure 5-5

APPENDIX A - Allozyme and microsatellite allele frequencies.

Appendix A1. Allozyme allele frequencies for mountain whitefish from northwest North America. Locations correspond to Table 2-1 and Figure 2-1.

Location	*AAT1*		*AAT3*		*AAT4*				*ADH*			*IDH1*				*LDHA1*			*LDHA2*		
	**73*	**85*	**72*	**90*	**55*	**90*	**100*	**103*	**180*	**250*	**360*	**70*	**100*	**117*	**120*	**75*	**100*	**167*	**100*	**146*	**220*
Columbia River																					
K	--	1.000	1.000	--	--	0.407	0.593	--	--	1.000	--	--	1.000	--	--	0.208	0.792	--	1.000	--	--
N2a	0.019	0.981	1.000	--	--	0.741	0.259	--	--	0.944	0.056	0.167	0.833	--	--	--	1.000	--	1.000	--	--
N3b	0.111	0.889	1.000	--	--	0.815	0.185	--	--	1.000	--	--	1.000	--	--	--	1.000	--	1.000	--	--
N3c	0.093	0.907	0.759	0.241	--	0.815	0.167	0.019	--	1.000	--	--	0.907	0.093	--	--	1.000	--	1.000	--	--
N5a	--	1.000	1.000	--	--	0.975	0.025	--	--	1.000	--	0.600	0.400	--	--	--	1.000	--	1.000	--	--
N6	--	1.000	1.000	--	--	0.972	0.028	--	--	1.000	--	--	1.000	--	--	--	1.000	--	1.000	--	--
N7	--	1.000	0.039	0.961	--	1.000	--	--	--	1.000	--	--	1.000	--	--	--	1.000	--	--	--	1.000
N8	--	1.000	0.078	0.922	--	1.000	--	--	0.031	0.969	--	--	1.000	--	--	--	1.000	--	--	--	1.000
N9	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--	1.000	--	--	--	1.000	--	--	--	1.000
N10	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--	1.000	--	--	--	1.000	--	--	--	1.000
O1	--	1.000	1.000	--	--	1.000	--	--	--	1.000	--	--	1.000	--	--	--	1.000	--	--	--	1.000
O2	--	1.000	1.000	--	--	1.000	--	--	--	1.000	--	--	1.000	--	--	--	1.000	--	--	--	1.000
P	0.136	0.864	1.000	--	--	0.886	0.114	--	--	1.000	--	0.159	0.841	--	--	--	1.000	--	1.000	--	--
Q1	--	1.000	1.000	--	--	0.688	0.312	--	--	1.000	--	0.031	0.969	--	--	--	1.000	--	1.000	--	--
Q2a	--	1.000	1.000	--	--	0.845	0.155	--	--	1.000	--	0.125	0.875	--	--	--	1.000	--	1.000	--	--
Q8	--	1.000	1.000	--	--	0.870	0.130	--	--	1.000	--	0.275	0.725	--	--	--	1.000	--	1.000	--	--
S	--	1.000	1.000	--	--	0.750	0.250	--	--	1.000	--	0.180	0.820	--	--	--	1.000	--	1.000	--	--
T1	--	1.000	1.000	--	--	0.881	0.119	--	--	1.000	--	0.238	0.762	--	--	--	1.000	--	1.000	--	--
T2	0.020	0.980	1.000	--	--	1.000	--	--	--	1.000	--	0.333	0.667	--	--	--	1.000	--	1.000	--	--
Saskatchewan River																					
U	--	1.000	1.000	--	--	1.000	--	--	--	1.000	--	0.188	0.812	--	--	--	1.000	--	1.000	--	--
Missouri River																					
V	--	1.000	1.000	--	--	0.976	0.024	--	--	1.000	--	0.988	--	--	0.012	--	1.000	--	--	--	1.000
W2	--	1.000	1.000	--	--	1.000	--	--	--	1.000	--	1.000	--	--	--	--	1.000	--	--	--	1.000
X	--	1.000	1.000	--	0.024	0.976	--	--	0.048	0.952	--	1.000	--	--	--	--	1.000	--	--	--	1.000
Y	--	1.000	1.000	--	--	1.000	--	--	0.034	0.966	--	1.000	--	--	--	--	0.968	0.032	--	--	1.000
Z1	--	1.000	1.000	--	--	1.000	--	--	0.017	0.983	--	1.000	--	--	--	--	1.000	--	--	--	1.000
Bonneville Basin																					
AB	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--	1.000	--	--	--	1.000	--	--	--	1.000
AC	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--	1.000	--	--	--	1.000	--	--	--	1.000
Lahontan Basin																					
AD	--	1.000	1.000	--	--	1.000	--	--	--	1.000	--	0.015	0.985	--	--	--	1.000	--	0.985	0.015	--
AE	0.045	0.955	1.000	--	--	1.000	--	--	--	1.000	--	0.045	0.955	--	--	--	1.000	--	1.000	--	--

Appendix A1. (continued)

Location	*LDHB2*		*MDH2*		*MDH-3,4*		*ME*		*PGM1*			*SOD*				
	**100*	**125*	** 120*	**290*	**80*	**100*	**70*	**80*	**102*	**113*	**124*	**14*	**65*	**90*	**107*	**112*
Columbia River																
K	0.971	0.029	1.000	--	--	1.000	--	1.000	--	0.985	0.015	0.015	--	0.985	--	--
N2a	0.352	0.648	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
N3b	0.643	0.357	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
N3c	0.704	0.296	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
N5a	0.300	0.700	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
N6	1.000	--	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
N7	1.000	--	1.000	--	0.094	0.906	--	1.000	--	1.000	--	--	0.020	0.980	--	--
N8	1.000	--	1.000	--	0.117	0.883	--	1.000	--	1.000	--	--	--	0.969	--	0.031
N9	1.000	--	1.000	--	--	1.000	--	1.000	--	1.000	--	--	0.156	0.844	--	--
N10	1.000	--	1.000	--	0.018	0.982	--	1.000	--	1.000	--	--	--	1.000	--	--
O1	1.000	--	1.000	--	0.292	0.708	--	1.000	--	1.000	--	--	--	1.000	--	--
O2	1.000	--	1.000	--	0.231	0.769	--	1.000	--	1.000	--	--	--	1.000	--	--
P	0.568	0.432	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
Q1	0.688	0.312	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
Q2a	0.155	0.845	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
Q8	0.826	0.174	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
S	0.500	0.500	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
T1	0.381	0.619	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
T2	1.000	--	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
Saskatchewan River																
U	0.562	0.438	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
Missouri River																
V	1.000	--	1.000	--	0.105	0.895	--	1.000	0.122	0.878	--	--	--	1.000	--	--
W2	1.000	--	0.568	0.432	0.193	0.807	0.273	0.727	--	1.000	--	--	--	1.000	--	--
X	1.000	--	1.000	--	0.262	0.738	--	1.000	--	1.000	--	--	--	0.976	0.024	--
Y	1.000	--	1.000	--	0.242	0.758	--	1.000	--	1.000	--	--	--	1.000	--	--
Z1	1.000	--	1.000	--	0.175	0.825	--	1.000	--	1.000	--	--	--	1.000	--	--
Bonneville Basin																
AB	1.000	--	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
AC	1.000	--	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
Lahontan Basin																
AD	0.765	0.235	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--
AE	0.727	0.273	1.000	--	--	1.000	--	1.000	--	1.000	--	--	--	1.000	--	--

Appendix A2. Microsatellite allele frequencies for mountain whitefish from northwest North America.

Locations correspond to Table 2-1 and Figure 2-1.

Location	COCL4											SSA14							ONE8											
	*138	*140	*144	*146	*148	*150	*152	*154	*156	*158	*162	*159	*165	*167	*169	*171	*173	*175	*168	*172	*174	*178	*180	*182	*184	*186	*188	*190	*192
Mackenzie River																														
A1a	--	--	--	0.765	--	0.235	--	--	--	--	--	--	--	0.361	--	0.639	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--
A2	--	--	--	0.587	--	0.413	--	--	--	--	--	--	--	0.667	--	0.333	--	--	--	--	--	--	--	0.955	0.045	--	--	--	--	--
A2a	--	--	--	0.857	--	0.143	--	--	--	--	--	--	--	0.762	--	0.238	--	--	--	--	--	--	--	0.952	0.048	--	--	--	--	--
Peace River																														
B1a	--	--	--	0.880	--	0.060	0.060	--	--	--	--	--	--	0.185	--	0.500	--	0.315	--	--	--	--	--	0.946	0.054	--	--	--	--	--
B1b	--	--	--	0.875	--	0.050	0.075	--	--	--	--	--	--	0.175	--	0.575	--	0.250	--	--	--	--	--	0.975	0.025	--	--	--	--	--
B2a	--	--	--	1.000	--	--	--	--	--	--	--	--	--	0.211	--	0.789	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--
B3	--	--	--	1.000	--	--	--	--	--	--	--	--	--	0.194	0.028	0.750	--	0.028	--	--	--	--	--	0.647	0.353	--	--	--	--	--
Stikine River																														
C	--	--	--	0.800	--	0.200	--	--	--	--	--	--	--	0.571	--	0.429	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--
Fraser River																														
D	--	--	--	0.706	--	0.176	0.118	--	--	--	--	0.062	--	--	0.156	0.656	0.062	0.062	--	--	--	--	--	0.735	0.265	--	--	--	--	--
E	--	--	--	0.750	--	0.150	0.100	--	--	--	--	--	--	0.050	0.250	0.600	--	0.100	--	--	--	--	0.278	0.444	0.278	--	--	--	--	--
F1a	--	--	--	0.750	--	0.200	0.050	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	0.950	0.050	--	--	--	--	--
F2a	--	--	--	0.550	--	0.350	0.100	--	--	--	--	--	--	0.050	0.050	0.700	--	0.200	--	--	--	--	0.300	0.600	0.100	--	--	--	--	--
F3a	--	--	--	0.458	--	0.417	0.125	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	0.833	0.167	--	--	--	--	--
G1	--	--	--	0.800	--	0.140	0.060	--	--	--	--	--	--	0.020	0.020	0.900	--	0.060	--	--	--	--	--	0.780	0.180	0.040	--	--	--	--
Olympic Peninsula																														
H	--	--	--	1.000	--	--	--	--	--	--	--	--	0.022	--	0.978	--	--	--	--	--	--	--	--	0.804	--	0.174	0.022	--	--	--
I	--	--	--	1.000	--	--	--	--	--	--	--	0.600	--	--	0.400	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--
Columbia River																														
J1	--	--	--	0.767	0.017	0.117	0.017	0.033	0.033	0.017	--	--	--	--	0.839	0.161	--	--	--	--	--	--	--	0.661	0.339	--	--	--	--	--
K	--	--	--	0.750	0.029	0.103	--	0.029	0.059	0.029	--	--	--	0.015	0.971	0.015	--	--	--	--	--	--	--	0.603	0.382	0.015	--	--	--	--
L1	--	--	--	0.469	0.188	0.141	0.125	--	--	--	0.078	--	--	0.081	0.048	0.806	--	0.065	--	--	--	--	0.031	0.922	0.047	--	--	--	--	--
M1	--	--	--	0.600	--	0.400	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	0.938	0.062	--	--	--	--	--
N1a	--	--	--	0.095	--	0.595	0.214	--	--	0.095	--	--	--	0.024	--	0.881	0.095	--	--	--	--	--	0.024	0.810	0.167	--	--	--	--	--
N1b	--	--	--	0.130	0.022	0.717	0.109	--	--	0.022	--	--	--	0.022	--	0.935	0.022	0.022	--	--	--	--	0.109	0.609	0.283	--	--	--	--	--
N1c	--	--	--	0.350	0.050	0.525	0.050	0.025	--	--	--	--	--	0.050	0.025	0.850	0.075	--	--	--	--	--	--	0.700	0.225	0.075	--	--	--	--
N2a	--	--	--	0.385	0.038	0.442	0.135	--	--	--	--	--	--	--	0.019	0.870	0.056	0.056	--	--	--	--	0.056	0.907	0.037	--	--	--	--	--
N3a	--	--	--	0.517	0.086	0.207	0.138	--	--	0.052	--	--	--	0.044	0.059	0.691	0.118	0.088	--	--	0.014	--	0.125	0.722	0.125	0.014	--	--	--	--
N3b	--	--	--	0.321	0.161	0.161	0.357	--	--	--	--	--	--	--	0.464	0.357	0.018	0.161	--	--	0.018	--	0.089	0.893	--	--	--	--	--	--
N3c	--	--	--	0.250	0.125	0.438	0.167	--	--	0.021	--	--	--	0.042	0.083	0.667	0.125	0.083	--	--	--	--	0.100	0.840	0.040	0.020	--	--	--	--
N4	--	--	--	0.136	0.182	0.682	--	--	--	--	--	--	--	0.640	--	0.340	0.020	--	--	--	--	--	--	0.975	0.025	--	--	--	--	--
N5a	--	--	--	0.250	0.100	0.600	0.050	--	--	--	--	--	--	0.250	--	0.750	--	--	--	--	--	--	--	0.650	0.025	0.325	--	--	--	--
N6	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--
N7	--	--	0.013	0.050	--	0.675	0.075	0.188	--	--	--	--	--	--	--	1.000	--	--	0.338	0.450	0.050	0.038	--	0.125	--	--	--	--	--	--
N8	--	--	--	0.081	--	0.613	0.113	0.194	--	--	--	--	--	--	--	0.984	0.016	--	0.312	0.484	0.016	0.109	--	0.078	--	--	--	--	--	--
N9	--	--	--	--	--	0.545	--	0.455	--	--	--	--	--	--	--	1.000	--	--	0.667	0.045	--	0.273	--	0.015	--	--	--	--	--	--
N10	--	--	--	--	--	0.695	0.305	--	--	--	--	--	--	--	--	0.878	0.122	--	0.585	0.415	--	--	--	--	--	--	--	--	--	--
O1	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--
O2	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--
P	--	0.023	--	0.250	0.114	0.295	0.227	0.045	--	0.045	--	--	--	0.068	0.045	0.841	0.045	--	--	--	--	--	0.045	0.864	0.091	--	--	--	--	--
Q1	--	--	--	0.667	--	0.300	0.033	--	--	--	--	--	--	--	0.156	0.688	0.094	0.062	--	--	--	--	0.094	0.594	0.312	--	--	--	--	--
Q2a	--	--	--	0.517	--	0.433	0.050	--	--	--	--	--	--	0.050	0.033	0.700	0.083	0.133	--	--	--	--	--	0.700	0.300	--	--	--	--	--
Q2b	--	--	--	0.364	--	0.636	--	--	--	--	--	--	--	--	0.364	0.114	--	0.523	--	--	--	--	--	1.000	--	--	--	--	--	--
Q3	--	--	--	0.817	--	0.150	0.033	--	--	--	--	--	--	--	0.050	0.467	0.217	0.267	--	--	--	--	0.050	0.567	0.383	--	--	--	--	--
Q4	--	--	--	0.644	--	0.272	0.083	--	--	--	--	--	--	0.056	0.039	0.528	0.112	0.264	--	--	--	--	0.144	0.572	0.278	--	--	0.006	--	--
Q5	--	--	--	0.775	--	0.175	0.050	--	--	--	--	--	--	0.100	0.100	0.450	0.175	0.175	--	--	--	--	0.150	0.725	0.125	--	--	--	--	--
Q6	--	--	--	0.720	--	0.220	0.060	--	--	--	--	--	--	0.160	0.080	0.440	0.130	0.190	--	--	--	0.010	0.184	0.643	0.153	0.010	--	--	--	--
Q7	--	--	--	0.679	--	0.190	0.131	--	--	--	--	--	--	0.060	0.036	0.429	0.071	0.405	--	--	--	--	0.155	0.571	0.274	--	--	--	--	--
Q8	0.003	--	--	0.682	--	0.238	0.077	--	--	--	--	--	--	0.035	0.080	0.493	0.140	0.252	--	--	--	--	0.098	0.661	0.241	--	--	--	--	--
R1	--	0.026	--	0.474	--	0.316	0.158	--	--	0.026	--	--	--	--	0.175	0.700	0.050	0.075	--	--	--	--	0.211	0.632	0.158	--	--	--	--	--
S	--	--	--	0.432	--	0.318	0.227	--	--	0.023	--	--	--	0.040	0.080	0.820	--	0.060	--	--	--	--	0.188	0.667	0.146	--	--	--	--	--
T1	--	--	--	0.714	--	0.119	0.095	--	--	0.071	--	--	--	0.024	0.071	0.690	0.048	0.167	--	--	--	--	0.048	0.714	0.238	--	--	--	--	--
T2	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--
Saskatchewan River																														
U	--	--	--	0.979	--	0.021	--	--	--	--	--	--	--	--	0.250	0.062	--	0.688	--	--	--	--	--	0.979	0.021	--	--	--	--	--
Missouri River																														
V	--	--	0.158	0.158	--	0.447	0.184	0.053	--	--	--	--	--	--	0.859	--	0.115	0.026	--	--	--	--	--	0.025	0.850	--	0.112	0.013	--	--
W1	--	--	--	0.237	0.447	0.263	0.053	--	--	--	--	--	--	--	0.750	0.250	--	--	--	--	--	--	--	--	--	--	0.475	0.475	0.050	--
W2	--	--	--	0.182	0.295	0.523	--	--	--	--	--	--	--	--	0.548	0.452	--	--	--	--	--	--	--	0.159	--	--	0.568	0.273	--	--
X	--	--	--	0.095	0.048	0.452	0.333	0.048	0.024	--	--	--	--	--	0.667	0.071	0.238	0.024	--	--	--	--	0.024	0.238	0.381	0.071	0.119	0.071	0.095	--
Y	--	--	0.033	0.250	--	0.433	0.267	0.017	--	--	--	--	--	--	0.650	0.033	0.300	0.017	--	--	--	--	--	0.150	0.583	0.050	0.117	0.017	0.083	--
Z1	--	--	--	0.133	0.067	0.517	0.217	0.067	--	--	--	--	--	--	0.617	0.033	0.350	--	--	--	--	--	--	0.183	0.217	0.083	0.133	0.267	0.117	--
Bonneville Basin																														
AA	--	--	--	--	--	0.779	0.059	0.059	0.103	--	--	--	--	--	--	0.971	--	0.029	0.152	0.606	0.091	0.152	--	--	--	--	--	--	--	--
AB	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	0.083	0.917	--	--	0.242	0.500	0.226	0.032	--	--	--	--	--	--	--	--
AC	--	--	--	--	--	0.806	--	0.194	--	--	--	--	--	--	--	1.000	--	--	0.032	0.387	--	0.581	--	--	--	--	--	--	--	--
Lahontan Basin																														
AD	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	0.471	0.500	0.029	--	--	--	--
AE	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	0.273	0.409	0.318	--	--	--	--

Appendix A2. (continued)

Location	SPDR-1																						SPDR-2												SSA456									
	*143	*152	*156	*158	*160	*161	*162	*164	*166	*168	*170	*172	*174	*176	*178	*180	*182	*186	*188	*190	*192	*194	*189	*193	*195	*197	*198	*199	*201	*203	*138	*154	*156	*158	*160	*162	*164	*166	*170	*174	*182	*184	*186	*185-*326
Mackenzie River																																													
A1a	--	--	--	--	--	--	0.974	--	0.026	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.794	0.206	--	--	--	--	--	--	--	--	--	0.972	--	--	--	--	--	--	--	0.028
A2	--	--	--	0.842	0.062	--	0.875	0.021	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.957	0.043	--	--	--	--	--	--	--	--	--	0.864	--	--	--	--	--	--	--	0.137
A2a	--	--	--	0.071	0.095	--	0.810	0.024	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	0.857	--	--	--	--	--	--	--	0.144
Peace River																																												
B1a	--	--	--	0.357	--	--	0.643	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--
B1b	--	--	--	0.425	--	--	0.575	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--
B2a	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	0.895	--	--	--	--	--	--	--	0.104
B3	--	--	--	0.235	--	--	0.765	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.971	0.029	--	--	--	--	--	0.059	--	--	--	0.676	--	--	0.059	--	--	--	--	0.203
Stikine River																																													
C	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	0.850	--	--	--	--	--	--	--	0.150
Fraser River																																													
D	--	--	--	0.118	0.206	--	0.559	0.088	0.029	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	0.382	--	--	--	0.441	--	--	0.147	--	--	--	--	0.029
E	--	--	--	0.250	--	--	0.700	0.050	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.800	0.200	--	--	--	--	--	0.100	--	--	--	0.800	--	--	--	--	--	--	--	0.100
F1a	--	--	--	0.175	--	--	0.825	--	0.000	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	0.079	--	--	--	0.921	--	--	--	--	--	--	--	--
F2a	--	--	--	0.200	--	--	0.750	0.050	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.950	0.050	--	--	--	--	--	0.300	--	--	--	0.450	--	--	--	--	--	--	--	0.250
F3a	--	--	--	0.042	--	--	0.958	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.583	0.417	--	--	--	--	--	0.083	--	--	--	0.583	--	--	--	--	--	--	--	0.334
G1	--	--	--	0.320	--	--	0.680	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.960	0.040	--	--	--	--	--	--	--	--	--	0.646	--	--	0.062	--	--	--	--	0.293
Olympic Peninsula																																													
H	0.130	--	--	--	0.435	--	--	0.435	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	--	--
I	--	--	--	--	0.212	--	--	0.788	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	--	--
Columbia River																																													
J1	--	--	--	0.047	--	--	0.578	--	0.172	0.047	--	--	0.156	--	--	--	--	--	--	--	--	--	0.016	--	0.047	0.031	--	--	0.906	--	0.117	--	--	--	0.767	0.067	0.017	0.017	--	0.017	--	--	--
K	--	--	--	--	--	--	0.721	0.044	0.015	0.147	--	0.044	0.029	--	--	--	--	--	--	--	--	--	0.029	--	0.015	--	--	0.044	0.882	0.029	0.265	--	--	--	0.735	--	--	--	--	--	--	--	--
L1	--	--	--	0.016	--	--	0.719	0.062	0.203	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.109	0.828	0.062	--	--	--	--	--	--	--	--	0.588	0.031	--	0.047	0.047	--	--	--	0.187
M1	--	--	--	--	--	0.100	0.667	0.067	0.167	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.962	--	--	--	0.038	--	0.462	--	--	--	0.462	--	--	--	--	--	0.077	--	--	--
N1a	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--
N1b	--	--	--	0.283	--	--	0.896	--	0.022	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	0.978	--	--	--	--	--	0.022	--	--
N1c	--	--	--	0.182	--	0.053	0.737	--	0.053	0.026	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	0.950	--	--	--	0.875	--	--	0.025	--	--	0.025	--	0.025
N2a	--	--	--	0.037	--	0.056	0.833	0.019	0.056	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.870	0.130	--	--	--	--	--	--	--	--	--	0.889	--	--	0.093	0.019	--	--	--	--
N3a	--	--	--	0.016	--	0.016	0.969	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	0.042	--	0.069	--	0.861	--	--	--	0.028	--	--	--	--
N3b	--	--	--	0.018	--	0.089	0.821	0.054	0.018	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.946	0.054	--	--	--	--	--	0.054	--	--	--	0.946	--	--	--	--	--	--	--	--
N3c	--	--	--	0.160	--	--	0.680	0.060	0.100	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.920	0.080	--	--	--	--	--	--	--	0.080	0.920	--	--	--	--	--	--	0.040	0.040
N4	--	--	--	0.564	--	--	0.432	0.068	0.136	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	0.720	--	--	--	0.160	--	--	0.100	0.020
N5a	--	--	--	0.125	--	0.250	0.425	0.300	0.100	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	0.600	--	--	0.025	0.275	--	--	0.075	0.025
N6	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--
N7	--	--	--	0.026	--	--	--	0.090	0.013	0.064	0.449	0.051	0.179	0.013	0.051	0.013	--	--	--	--	0.038	0.013	--	--	0.788	0.162	--	0.050	--	--	--	--	--	--	--	0.962	0.013	0.026	--	--	--	--	--	--
N8	--	--	--	0.016	--	--	--	0.078	--	0.172	0.312	0.109	0.188	0.078	0.016	--	--	0.031	--	--	--	--	--	--	0.859	0.078	--	0.062	--	--	--	--	--	--	--	0.969	0.016	0.016	--	--	--	--	--	--
N9	--	--	--	--	--	--	--	--	0.015	--	0.470	--	0.273	--	0.076	0.061	--	0.106	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	0.970	--	0.030	--	--	--	--	--	--
N10	--	--	--	--	--	--	--	--	--	--	--	--	0.598	--	--	--	--	0.195	0.024	0.146	--	0.037	--	--	1.000	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--
O1	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--
O2	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--
P	--	--	--	0.136	--	--	0.750	0.023	0.091	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.955	--	--	--	0.045	--	0.023	--	--	--	0.864	--	--	0.068	--	--	--	--	0.046
Q1	--	--	--	0.219	--	--	0.625	0.125	0.031	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	0.438	--	--	0.062	0.406	--	--	--	--	--	--	--	0.094
Q2a	--	--	--	0.700	--	--	0.133	0.167	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.567	0.350	--	--	--	--	--	0.367	--	--	0.017	0.517	--	--	0.100	--	--	--	--	0.093
Q2b	--	--	--	0.136	--	--	0.045	0.818	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.773	0.227	--	--	--	--	--	0.182	--	--	--	0.818	--	--	--	--	--	--	--	--
Q3	--	--	--	0.517	--	--	0.517	0.167	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.917	0.083	--	--	--	--	--	0.283	--	--	0.083	0.483	--	--	0.017	--	--	0.017	--	--
Q4	--	--	--	0.379	--	--	0.451	0.170	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.896	0.104	--	--	--	--	--	0.333	--	--	0.056	0.544	--	--	0.011	--	--	--	--	0.112
Q5	--	--	--	0.275	--	--	0.400	0.325	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.816	0.184	--	--	--	--	--	0.500	--	--	0.050	0.425	--	--	0.025	--	--	--	--	0.057
Q6	--	--	--	0.350	--	--	0.510	0.140	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.860	0.140	--	--	--	--	--	0.390	--	--	0.090	0.400	--	--	--	--	--	--	--	--
Q7	--	--	--	0.464	--	--	0.152	0.083	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.866	0.134	--	--	--	--	--	0.155	--	--	0.095	0.867	--	--	--	--	--	--	--	0.120
Q8	--	--	--	0.313	--	--	0.518	0.169	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.839	0.161	--	--	--	--	--	0.346	--	--	0.111	0.471	0.004	--	--	--	--	--	0.004	0.084
R1	--	--	--	0.275	--	--	0.900	0.075	0.050	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	0.175	--	--	--	0.825	--	--	--	--	--	0.025	--	0.065
S	--	--	--	0.260	--	--	0.540	0.100	0.100	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	0.060	--	--	--	0.820	--	--	0.040	--	--	--	--	0.080
T1	--	--	--	0.286	--	--	0.643	0.048	0.024	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.929	0.071	--	--	--	--	--	0.095	--	--	--	0.833	--	--	--	--	--	--	--	0.072
T2	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	0.425	--	--	--	0.575	--	--	--	--	--	--	--	--
Saskatchewan River																																													
U	--	--	--	0.958	--	--	0.042	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.875	0.125	--	--	--	--	--	0.292	--	--	--	0.708	--	--	--	--	--	--	--	--
Missouri River																																													
V	--	--	--	0.410	--	--	--	0.551	0.038	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.026	0.974	--	--	--	--	--	--	--	--	--	0.925	0.100	--	0.075	--	--	--	--	--
W1	--	--	--	0.750	--	--	0.050	0.125	0.075	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	0.725	0.275	--	--	--	--	--	--	--
W2	--	--	--	0.595	--	--	--	0.205	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	0.500	0.500	--	--	--	--	--	--	--
X	--	0.048	0.024	0.405	--	--	--	0.452	0.071	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	0.810	0.190	--	--	--	--	--	--	--
Y	--	--	--	0.417	--	--	--	0.517	0.067	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	0.733	0.233	--	--	--	--	--	--	--
Z1	--	0.017	0.017	0.533	--	--	--	0.350	0.033	0.050	--	--	--	--	--	--	--	--	--	--	--	--	--	--	0.967	0.033	--	--	--	--	--	--	--	--	0.034	--	0.759	0.207	--	--	--	--	--	--
Bonneville Basin																																													
AA	--	--	--	--	--	--	--	--	--	0.074	0.779	0.059	0.074	--	0.015	--	--	--	--	--	--	--	--	--	0.956	--	--	0.044	--	--	--	0.015	0.029	--	0.441	0.015	--	--	--	--	--	--	--	--
AB	--	--	--	--	--	--	--	--	--	0.065	0.145	0.097	0.097	0.048	0.484	0.016	0.048	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	0.145	--	0.403	0.435	0.016	--	--	--	--	--	--
AC	--	--	--	--	--	--	--	--	--	0.242	0.419	--	0.339	--	--	--	--	--	--	--	--	--	--	--	0.710	--	--	0.290	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--
Lahontan Basin																																													
AD	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--
AE	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--	1.000	--	--	--	--	--	--	--	--	--

§ For presentation purposes, alleles from SSA456 between 156 and 326 base pairs are shown in summary.

APPENDIX B - Observations of mountain whitefish spawning behavior

An important aspect of the biology of mountain whitefish that I have not fully elaborated on elsewhere is the mating system and mating behavior of this species. Here, I will briefly described observations made during the course of my research. I have based conjectures for several parts of subsequent chapters on snorkeling observations I made of fish in Rattlesnake Creek, Missoula, Montana. Very little prior knowledge is available on this topic (but see Brown 1952; Stalnaker et al. 1974). In Rattlesnake Creek, mountain whitefish spawn from the Mountain View Bridge behind Rattlesnake School to the mouth of Rattlesnake Creek. Spawning occurs at dusk, and perhaps into the night, as Stalnaker et al. (1974) suggested. During the spawning season, which in Rattlesnake Creek lasts from mid-October through late November, the sex ratio appears to be biased towards males. In several electrofishing samples I collected during the spawning season, the sex ratio was close to 10:1 males to females. Males have well developed spawning tubercles and a more pronounced red lateral stripe than females. These secondary sexual traits allowed me to distinguish between males and females while snorkeling. I also inserted colored floy tags into 22 individuals prior to snorkeling observations (N = 17 males, N = 5 females). I often observed large groups of males in pools and I observed many examples of courtship, where females swam near groups of males and several (2-3) males would follow the female, nudging her with their heads and bodies.

I observed the spawning event on November 10, 2001. At dusk, a putative female backed into a group of approximately 15-20 individuals. This putative female was not tagged but had a distended abdomen and did not have pronounced spawning tubercles. Within the larger group of fish, two individuals had male-colored tags. In addition, I observed spawning tubercles on the majority of the remainder of the individuals. Thus, it is likely that the group into which the putative female swam consisted of mostly, if not all, males. I observed a massive writhing of

bodies. The sex of the putative female was confirmed as she laid eggs close to the substrate surface. At least five to ten males in the immediate vicinity of the female released milt that spread in a cloud. This spawning event occurred in shallow water (< approximately 60 cm) immediately downstream from a pool.

Several inferences about the mating system of mountain whitefish can be made from these observations. It appears that males aggregate, possibly where females prefer to spawn. It is possible that females choose either among spawning sites or among the groups of males. Thus, this mating system may be lek-like, where females choose among groups of males in different pools. This has been suggested for *Coregonus alpinus* (Wedekind et al. 2001), a whitefish in a different genus than the mountain whitefish. However, this hypothesis is not based on empirical data (Wedekind et al. 2001). While the mountain whitefish mating system may or may not be lek-like, it is possible that female choice occurs. However, it is likely that many males fertilize the eggs of a single female and female choice might not be very strong. Future studies are needed to determine the evolutionary implications of this mating system. In the work presented here, I assume that many males fertilize the eggs of single females, and that choice is less precise than other redd-digging salmonids.

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