Historical and anthropogenic influences on genetic variation in bull trout (*Salvelinus confluentus*) in the Arrow Lakes, British Columbia

by

Stephen J. Latham
Bachelor of Science (Honours), University of British Columbia, 1995

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE
DEGREE OF MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES
(Department of Zoology)

We accept this thesis as conforming to
the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

January 2002

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Zoology

The University of British Columbia
Vancouver, Canada

Date January 31, 2002

DE-6 (2/88)

Abstract

This work investigates, within a watershed, (i) the distribution of an organism and its genetic variation and (ii) the mechanisms and consequences of anthropogenic influences on those distributions. I collected genetic data (at mitochondrial DNA and five nuclear microsatellite loci) for bull trout, *Salvelinus confluentus*, sampled from sites spanning approximately 260 km of the Columbia River, in Canada. Bull trout are recognized as a species of special conservation concern throughout almost all of their declining range. I performed analyses focussed on the conservation implications of management initiatives on resident and migratory life history forms.

Large proportions of genetic variation were distributed among geographic locations. Populations were strongly genetically divided by waterfalls. Another obvious division occurred among mutually accessible habitats. I concluded that postglacial geological history was largely responsible for observed genetic divisions in both cases. Analyses further suggested that migratory behaviour (i.e., homing) and local selection against dispersers sustain this differentiation. Genetic diversity among bull trout populations is vulnerable to habitat degradation and to attempts to compensate for that degradation. Invasibility of exogenous allelic variation may be higher in resident populations than in migratory ones, but activities that reduce densities of bull trout (e.g., poaching, collection of broodstock) may promote recruitment of exogenous allelic variation in migratory populations.

Despite anthropogenic impacts, substantial genetic variation still exists in the study area. Prioritizing populations and their habitats for conservation is difficult because they generally represent distinct evolutionary histories, as interpreted from allelic diversity and identity. Further, while allelic diversity in resident populations correlated positively with the presence of other fish species, rare alleles were more common in genetically depauperate populations. Consequently, intrapopulation diversity and uniqueness are traded-off intraspecifically, and habitats containing representative bull trout populations are unlikely to represent other biological diversity. Conservation of representative migratory populations is difficult also, as they are harvested in a mixed-population fishery. The fishery likely poses a greater risk to some populations than to others. Migratory bull trout are not panmictic in their feeding areas, however, and judicious use of no-harvest zones could protect susceptible populations with high value for conservation.

Table of contents

List of tables

List of figures

Acknowledgements

I was nervous at my first committee meeting and don't recall it perfectly. I do remember that Rick Taylor (my supervisor) urged me to focus on a few questions and ***act*** to answer them; Mike Whitlock forced me to consider details; Don McPhail discussed broad questions with me about my study and the philosophy of science; and Bob Devlin challenged me to explain the boilermaker (if mixing was so wrong). Little did I know that I would rely so much on them to act in those same capacities. Don would permit and listen to my flights of fancy; Rick would make me focus only on the ones that had a chance of being worthwhile; Mike helped me to make those ideas testable; and Bob asked me questions that helped me frame the answers. As Rick was my supervisor, I relied on him to do more than I've described above. And he did it. He would find simple solutions from shore when I was nearly drowning, looking too deep to see them in the shallows. I feared that I appeared ridiculous to him. Many friends came to my thesis defense on Robbie Burns Day, 2002, and I thank Drs. Martin Adamson, Tony Pitcher, and Don McPhail for examining me. When Rick examined me he told everyone there that he appreciated how I immersed myself and thought deeply about my topic. My confidence grew from there and my defense was very successful. My respect for him can be described by the positive influence those few words have had on how I feel toward my own work and experience at U.B.C.

Many other people helped with this project, and most did so in more than one capacity. Despite brevity in the description below, I will not forget their contributions. Their efforts made the work better, more enjoyable, possible.... Thanks to all; thanks for everything.

Megan McCusker, Jen McLean, Mike Stamford, Patrick Tamkee, Dave O'Brien, Allan Costello, Zoe Redenbach, Derek Louie, and Drew Hoysak helped me with lab work and were wonderful entertainment while I did it. Jason Ladell, Cory Suski, and Laura Bannister gave similar help in the field. I obtained samples and information on where to sample bull trout from Gord Haas, James Baxter, Fred Salekin, Karen Bray, Larry Ingham, and anonymous anglers. Motivation, perspective, research experience, and advice on presenting were generously given by Ernest Keeley and Andrew Hendry. I discussed various analytical issues with John Nason, Ranajit Chakraborty, Mark Miller, Phillippe Casgrain, John Brzustowski, Don Jackson, Cort Griswold, and Lisa Manne. Bob Fulton, John Clague, and Nelson Rocha informed me regarding geological interpretations. I had other helpful discussions with Josh Taylor, John Hagen, Dolph Schluter, Cort Griswold, Paul Spruell, Eric Parkinson, Cole Burton, Dawn Cooper, Peter Troffe, Josh Lepawsky, Gord Latham, Alistair Blachford, Andrew Knowles, Andrew Lotto, Leanna Warman, Liz Gillis, Shannon Arnold, and Jeff and Katie Thomson. I also received support from many other friends and faculty in the Department of Zoology at U.B.C and from my family in Alberta. Rick Taylor, Allan Costello, and Karen Bray helped me to extend the scope of my study by sharing their data.

Institutions provided various forms of technical assistance via access to their staff: Ministry of Environment, Lands and Parks: Bob Millar, Grant Thorp, Diane Koller, Bob Lindsay, and Colin Spence; B.C. Hydro: Gary Birch and Ric Olmsted; Columbia Basin Fish and Wildlife Compensation Program: Karen Bray, Steve Arndt, and Harald Manson. Access to the libraries of each institution was also valuable. The University of British Columbia and the Columbia Basin Fish and Wildlife Compensation Program provided funds to travel and present my work at workshops in Canmore, Alberta, and Revelstoke, B.C., respectively. Funding was provided by the Columbia Basin Fish and Wildlife Compensation Program. When I thought I could answer more than was expected, the program offered additional funding and the time required.

Dedication

Since I began this project, Don McPhail retired and survived a heart attack, Ric Olmsted passed away, and others mentioned above have changed jobs and locations. I am unsure how to describe the time in a more ecocentric way. Over that period, in completion of my study I disturbed organisms and perturbed ecosystems. I hope the costs inflicted upon them are compensated by conservation benefits derived from this study's findings. I am sorry it took so long.

Chapter 1. On distinguishing ancient and recent effects on population genetic structure

Errors based on inadequate data are much less than those based on no data at all.
Charles Babbage (1791 – 1871)

Molecular genetic data are often applied to questions regarding behaviour and demography and can aid conservation management decisions (Ryman 1991, Shaklee *et al.* 1999). Equilibrium conditions are often assumed when researchers interpret these data, although such assumptions are warranted rarely, if ever (Whitlock and McCauley 1999). Historical contingencies often play a dominant role in population genetic variation, and must be acknowledged to avoid erroneous conclusions regarding contemporary demography (Angers and Bernatchez 1998 and references therein, Slade *et al.* 1998, Angers *et al.* 1999). Baseline data, or data in time-series are essential to determine whether a given pattern reflects historical influences, equilibrium biological conditions, or recent anthropogenic perturbations (Brown *et al.* 1992, Templeton *et al.* 1995).

My thesis explores the geographic distribution of genetic variation in bull trout (*Salvelinus confluentus*) in the Columbia River drainage of British Columbia's West Kootenay region (henceforth CRDWK, Figure 1.1). This region was shaped by glacial processes during the Pleistocene Epoch and entirely covered by ice 19 000 - 10 000 years ago (Fulton 1968, Fulton and Smith 1978), so genetic variation in the area's species was presumably influenced by geological history. The extent of glaciation has changed relatively little over the past 9 000 years (Ryder 1981, Fulton and Archard 1985), and this relative glacial stasis has potentially allowed signatures of early postglacial colonization (geological evidence, species and genetic distributions) to fade. Recently, anthropogenic impacts have dominated changes in landscape. These recent changes in landscape have also, in all probability, influenced genetic variation. For example, on Columbia River's mainstem and tributaries, hydroelectric developments influence migration patterns of native fishes (e.g., Martin 1976). These dams have also flooded spawning and rearing habitats for several fish species (Lindsay 1986, 1987) and have reduced nutrient throughput to habitats downstream (Sebastian *et al.* 2000).

In addition to potential influences on genetic variation within and among fish populations, these hydroelectric initiatives have negatively influenced sport fishing opportunities in CRDWK (Lindsay 1986, 1987). Attempts to ameliorate these negative effects have included further habitat alteration (reviewed by Sebastian *et al.* 2000). For example, one waterfall (on Halfway River) was dynamited in 1990, and potential barriers to migrations of fish were mechanically removed from another tributary (Slewiskin Creek). A dam on Illecillewaet River (a large tributary to Columbia River) was removed in 1977 in partial compensation for larger hydroelectric developments on the Columbia mainstem. Further compensation has been provided at Hill Creek in the form of a spawning channel and hatchery propagation to increase population sizes of sport fish species. This compensation requires appropriation of water from nearby Mackenzie Creek which is further de-watered by a private hydroelectric development. To evaluate feasibility and assist development of hatchery protocols, McPhail and Murray (1979) studied life history of bull trout in a relatively pristine Mackenzie Creek, and recommended collection of genetic data prior to commencement of hatchery operations. After more than fifteen years of relatively unmonitored hatchery supplementation, the first comprehensive study – this study – of genetic variation in the area's bull trout began.

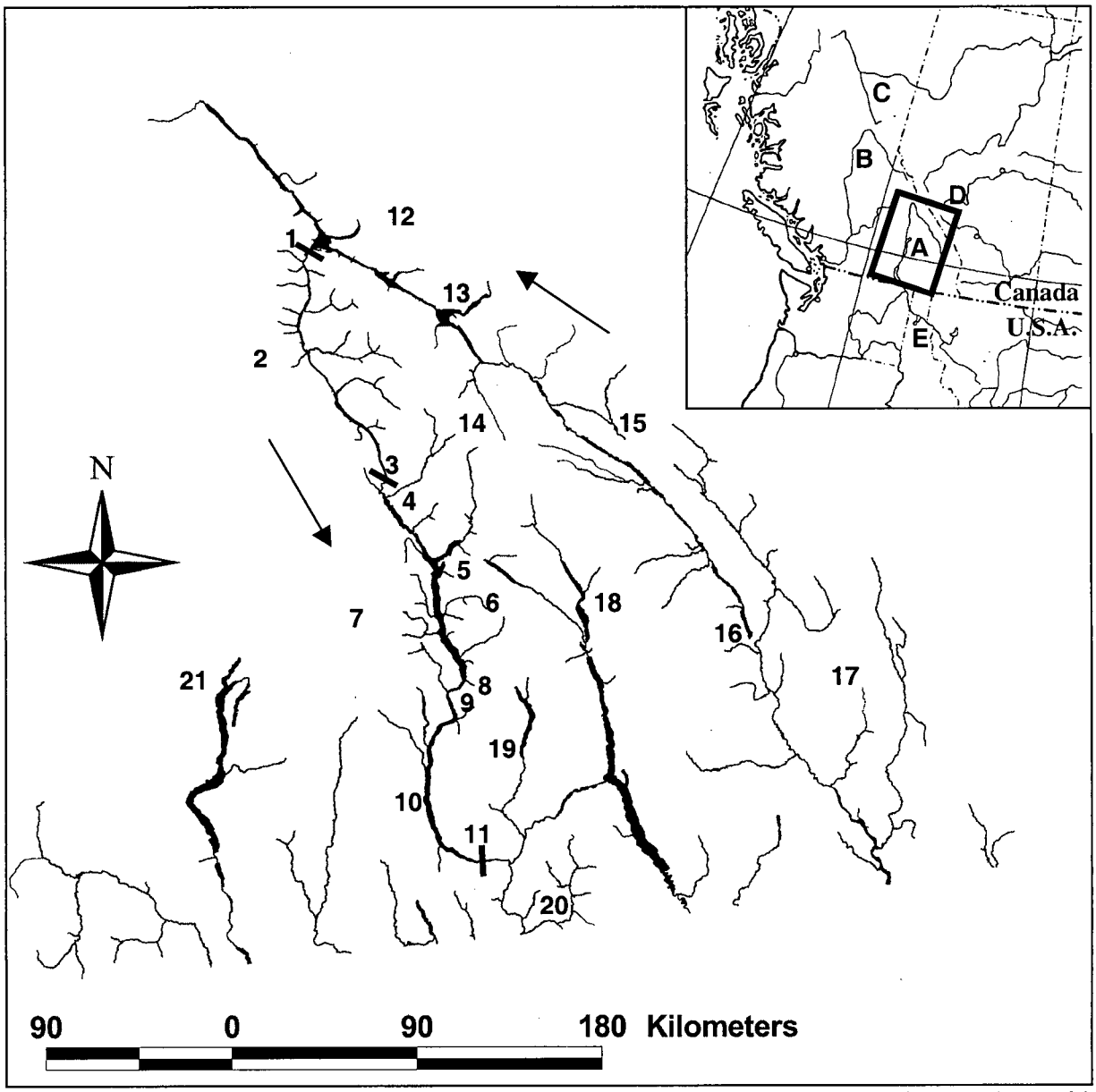

Figure 1.1. Columbia River drainage (in Canada) and surrounding watersheds. Numbers 1-11 are within the study area, CRDWK: 1 Mica Dam, 2 Revelstoke Reservoir, 3 Revelstoke Dam, 4 Illecillewaet River, 5 Hill and Mackenzie creeks, 6 Halfway River, 7 Upper Arrow Reservoir, 8 Slewiskin Creek, 9 The Narrows, 10 Lower Arrow Reservoir, and 11 Hugh Keenleyside Dam. Numbers 12-17 are within the Rocky Mountain Trench: 12 Kinbasket Reservoir, 13 Sullivan Arm, 14 Glacier National Park, 15 upper Columbia River drainage (Yoho National Park), 16 Canal Flats, and 17 upper Kootenay River drainage. Other locations noted in this thesis: 18 Duncan River drainage, 19 Slocan River drainage, 20 Salmo River, 21 Okanagan River drainage. Arrows indicate Columbia River's direction of flow. (More detail of CRDWK can be obtained from Figure 2.2.) Inset: broad view of watersheds in the Pacific Northwest; A West Kootenays including CRDWK, B Fraser River drainage, C Peace River drainage, D Saskatchewan River drainage, E Clark Fork River drainage; dotted lines represent state, provincial, and national borders.

The goal of this thesis is to help inform conservation and management efforts. To achieve this, I try to not only describe the distribution of genetic variation of bull trout in CRDWK, but I also try to explain that distribution. Knowledge about the relative roles of glacial history, the biology

of bull trout, and anthropogenic influences in shaping that distribution is central to development of an effective management plan (c.f., Brown *et al.* 1992).

Unfortunately, as this is the first comprehensive genetic study of bull trout in CRDWK, no baseline genetic data exist. That is, each anthropogenic perturbance represents an uncontrolled experiment. Worse, demographic observations from those experiments have been lacking or confounded – even basic information (e.g., changes in population size) is lacking and not retrievable. For example, life history and demographic comparisons of bull trout in Mackenzie Creek, before and after closure of Revelstoke Dam, are invalidated by the de-watering of that site (see above). Finally, the list of "experiments" is not complete. Many other perturbances of unknown magnitude have been non-randomly applied to stream populations. For example, whereas legitimate angling for bull trout occurs mostly in Upper Arrow Reservoir (Lindsay 1986), targeted poaching of tributary populations may occur mostly in tributaries of Lower Arrow Reservoir (J. Beck, Penticton Conservation Office, personal communication).

Luckily, perhaps, tributaries in CRDWK typically run through hanging valleys (Ryder 1981), and bull trout commonly occupy habitats above the resulting waterfalls. In lieu of baseline data, if these isolated populations were founded thousands of years ago, early in deglaciation, genetic variation among them should reflect the genetic variation present in the founding populations. That is, use of populations above waterfalls to estimate historical population structure may allow inferences to be drawn regarding recent influences on population structure.

I compared molecular genetic variation detected in CRDWK to that detected elsewhere and evaluated the assumption that populations above waterfalls are temporal reference points for population genetic analyses. I found that contemporary population structure above and below waterfalls generally reflects postglacial colonization, and I estimated genetic influences of upstream populations on those below to be small whereas influences from downstream populations on those upstream are potentially large (Chapter 2). Discussion also includes mechanisms of founding in these populations with reference to geological phenomena. I then focussed more on lacustrine and adfluvial samples (Chapter 3). I examined the mechanisms that have maintained historical population structure as well as mechanisms of anthropogenic change to that structure. I concluded that while homing and selection maintain historical structure, activities that reduce population density help to obscure it. I related these findings to the study area's bull trout fishery and to management initiatives regarding compensation and conservation.

The National Marine Fisheries Service (NMFS) of the United States recently released guidelines for conservation of evolutionarily significant units ("distinct population segments" under the Endangered Species Act), directed toward identifying more basic units within species of the genus *Oncorhynchus*: viable salmonid populations (VSPs). Such populations have a low probability of extinction over 100 years that is not significantly affected by immigration from other such populations (McElhany *et al.* 2000). Isolation that confers demographic independence has a side effect of making populations smaller and more likely to become extinct. The value of demographic independence and disdain for small populations expressed in the VSP guidelines is mirrored in conservation genetics by the antagonism of concerns regarding inbreeding and outbreeding depression. Genetic differentiation among populations implies evolutionary differentiation that may promote loss of fitness when populations interbreed (i.e., outbreeding depression; Leberg 1993, Waser and Price 1994). Genetic differentiation often accrues more quickly among small populations, however, within which loss of genetic variation, less efficient natural selection, and accumulation of deleterious alleles (i.e., inbreeding

depression) are often greater concerns (Frankham 1995a). My thesis reports molecular marker data almost exclusively and was not designed to rigorously evaluate hypotheses regarding natural selection or demographic processes in bull trout populations. Yet this thesis is underlain by these themes; they motivated many analyses in Chapters 2 and 3 and largely constituted interpretations therein. As is common in conservation genetics and other fields in conservation science (where research is initiated after anthropogenic effects are expected; e.g., Weiss *et al.* 2001), proper controls and baseline data are lacking in this study, particularly with respect to its underlying themes and their implications. I end with an evaluation of my work and its implications and with an exploration of complimentary opportunities for future research (Chapter 4).

Chapter 2. Historical patterns of genetic diversity: causes and implications

Introduction

Importance of populations above waterfalls

The importance of peripheral populations to the conservation of species is a matter of debate. In some circumstances they may be sinks, unable to sustain themselves without immigration. Such populations have little conservation value in the long term, relative to more productive source populations (Buechner 1987). Peripheral or recently founded populations may also be characterized by low genetic variation (Merila *et al.* 1996, 1997, Hewitt 2000, Edmands 2001), suggesting that they are not adaptable and that they represent little of a species' evolutionary legacy. In contrast they may be shaped by different selective regimes, and they may provide a source of genetic variation that will permit the species to successfully adapt to changing environmental conditions (Scudder 1989, Lesica and Allendorf 1995). These opposing perspectives can be distinguished by their evaluations of habitat quality and gene flow. The first view associates geographical marginality with ecological marginality (Scudder 1989) such that immigration is required to save peripheral populations from extirpation or to recolonize the habitat following extirpation. Therefore, maintenance of connectivity, corridors and gene flow is a priority for conservation in the first view (e.g., Melnick et al. 2000). The second view implies that gene flow is counter productive – that local adaptive evolution can be swamped by immigrating maladaptive genetic variation (e.g., Storfer 1999, Hendry *et al.* 2001). This second perspective values the peripheral populations themselves and maintenance of their isolation.

Populations of stream-dwelling organisms peripherally isolated above waterfalls provide a special case. The waterfalls restrict gene flow from more 'central' downstream populations but may promote gene flow from the peripheral populations to the central ones. Thus, some authors (e.g., Northcote 1992) have suggested that populations isolated above barrier falls are repositories of evolutionary potential for species, consistent with the conservation value imputed by the second view described above. Consistent with the first view, genetic variation is often lower in individual peripheral populations (above waterfalls) than in downstream populations. Under these special circumstances, though, high genetic variation in the central populations may result from gene flow from differentiated peripheral populations above waterfalls (Shaw *et al.* 1991, 1994).

The importance of interactions across waterfalls for conservation depends upon (i) the degree of differentiation between upstream and downstream populations, (ii) the amount of gene flow that occurs, and (iii) the fitness consequences of that gene flow. (For example, the consequences for conservation of a population isolated above an historic falls are likely to differ from those for a population recently isolated above a hydroelectric dam.) These three factors are not entirely independent: the fitness of immigrant alleles will influence gene flow, which will in turn affect the level of differentiation (see Ingvarsson and Whitlock 2000). Likewise, the degree of differentiation may influence the fitness of immigrants and thus the amount of gene flow (Leberg 1993, but see Utter 2001). Beginning with an investigation of historical causes of differentiation, some resolution of these interactions may be achieved.

Phylogeographic information, information about genetic lineages in space and time, is useful in this context. In addition, it can help define unique populations or geographical areas (e.g., evolutionarily significant units; Moritz 1994), and it can aid interpretations of the evolutionary consequences of population genetic variation (Bernatchez and Wilson 1998, Hutchison and Templeton 1999) or life history data (e.g., comparative analyses; Partridge and Harvey 1988). It can also elucidate relationships between organisms and their habitats that have allowed them to

persist through periods of change (e.g., identify refugial sources and corridors and factors involved in colonization; Taberlet *et al.* 1998, McCusker *et al.* 2000). These issues are important for effective management and conservation of threatened biodiversity (Bernatchez and Wilson 1998, Utter 2001).

Stream dwelling organisms, because they are confined and constrained by historical drainage patterns, are useful for elucidating biogeographical history (Sivasundar *et al.* 2001). Study of populations above barrier falls may be particularly informative, especially when combined with geological information. With respect to colonization, populations above waterfalls can act as temporal guideposts for colonization and the roles played by geological phenomena such as proglacial lakes and changed drainage connections. Postglacial geological history and the relative timing of availability of colonization routes for stream-dwelling species can be inferred from both intra- and interspecific comparisons above and below waterfalls (e.g., Hughes *et al.* 1996, Remple and Smith 1998). Stronger inferences are possible when historical faunal distinctions in intraspecific (morphological and genetic) and interspecific data are preserved by waterfalls and are congruent with geological data (e.g., Currens *et al.* 1990). But even data at a single genetic locus within a single species can be informative. For example, an ancestral allozyme allele dominates in high elevation brown trout populations above migration barriers in northwestern Europe, but it is rare in downstream populations, suggesting two distinct waves of postglacial colonization (Hamilton *et al.* 1989). Following development of such thorough descriptions of phylogeographic signals in allelic data, deviations from expected patterns can reveal information on special geographical circumstances or on anthropogenic impacts (e.g., Hamilton *et al.* 1989, Weiss *et al.* 2001).

Above, I provided two arguments for studying populations peripherally isolated above waterfalls and their relationships to other populations. First, such study may discover an evolutionarily significant component of variation within a given species. Second, populations above waterfalls can help elucidate phylogeographic history, which itself has consequences for conservation. In this chapter, I analyse genetic variation above and below waterfalls and try to begin extraction of relevant, small-scale phylogeographic information on bull trout (*Salvelinus confluentus*), a species considered endangered or of special conservation concern throughout its range (Anon 1999).

Study system

Bull trout spawn in cold stream habitats and exhibit four life histories defined by feeding migrations (McPhail and Baxter 1996): anadromous (oceanic feeding), adfluvial (lacustrine feeding), fluvial (riverine feeding), and resident (no feeding migrations). The fluvial and adfluvial forms are long-lived (> 20 years), iteroparous, and may rear in natal habitats for more than 4 years (McPhail and Baxter 1996). The average generation time in these migratory populations is generally greater than five years (Pratt 1992), although precocious males have been observed in several migratory populations (McPhail and Murray 1979, Baxter 1997). Compared to fluvial and adfluvial bull trout, anadromous and resident bull trout have received relatively little study (McPhail and Baxter 1996). Resident populations in other char species are characterised by their members' slower growth rates, smaller maximum sizes, and earlier maturation compared to migratory populations (e.g., *S. malma*, Maekawa *et al.* 1993; *S. leucomanis*, Maekawa *et al.* 1994). Resident individuals tend to spawn more frequently (annually versus every one, two, or three years), and resident females produce smaller and fewer eggs (< 200 versus > 2 000) than migratory char (Maekawa *et al.* 1993). Preliminary study

supports similar differences between migratory and resident bull trout (J.J. Ladell, UBC Dept. of Zoology, personal communication).

I studied resident and adfluvial bull trout populations in tributaries of impounded portions of the Columbia River in British Columbia. This region, CRDWK (see Figure 1.1), is characterized by steep and rugged terrain. Here the Columbia River flows south and is fed by tributaries that run through hanging valleys in the Monashee (to the west) and Selkirk (to the east) mountains into Revelstoke (in the north) and Arrow (to the south) reservoirs. The area was completely covered by ice between 19 000 - 10 000 years ago and periodically over the previous two million years (Fulton 1968, Fulton and Smith 1978, Ryder 1981). Hanging valleys result from glacial erosion and are evidenced by waterfalls that are present in most tributaries in CRDWK. Tributaries with southerly confluences tend to be short and have small waterfalls very close to their mouths. Fish communities above these waterfalls are mostly introduced (T.G. Northcote, Summerland, B.C., personal communication) and are composed almost exclusively of rainbow trout (*Oncorhynchus mykiss*) or eastern brook trout (*S. fontinalis*). More northern tributaries (e.g., Downie and Kuskanax creeks, Jordan, Illecillewaet, Incomappleux, and Halfway rivers) are longer, and their waterfalls are generally farther upstream. There are few records of stocking above waterfalls in these more northerly tributaries (http://www.pisces.env.gov.bc.ca/FishWizard.asp), but I commonly found resident bull trout and sculpin (*Cottus cognatus*) populations there.

Arrow Reservoir was historically divided into an upper and a lower lake by a shallow, winding constriction called The Narrows. Transition between longer, more northern tributaries with resident bull trout populations and shorter, southern tributaries without resident bull trout populations occurs near the latitude of The Narrows. In addition, a fault detected between granitic rock in the south and late Mesozoic-Paleozoic rock in the north (Reesor and Moore 1971, but see Nasmith 1972) is congruent with the distribution of stream morphologies and the presence of resident fish populations. Surficial geology also suggests that a crustal "hinge" or flexion point operated near Nakusp (a short distance north of The Narrows) and possibly contributed to dissimilar postglacial histories for northern and southern CRDWK (R.J. Fulton, Geological Survey of Canada, personal communication).

If waterfalls in CRDWK are barriers to upstream migrations, there are several explanations for how these populations were colonized. Human-mediated colonization is one possibility, and there are several geological alternatives. Habitat above barriers could be colonized if the relevant streams followed a different course or direction in the past, owing to evulsion or headwater capture. Evulsion, forcible displacement of a stream channel to new terrain, is unlikely in CRDWK because of the confined nature of the valleys there (J. Clague, Geological Survey of Canada, personal communication). Alternatively, flooding from rebound and formation of proglacial lakes could have inundated present-day barriers (see Northcote *et al.* 1970), allowing easy upstream passage. These possible histories, and relationships between mechanisms of colonization and coincident geological and biotic distributions, can be investigated using molecular genetic data.

At a larger scale, flooding and changing drainage connections that resulted from glacial recession were largely responsible for the colonization of aquatic habitats throughout British Columbia by several freshwater fish from glacial refugia within the Columbia River basin. Drainage connections between the Okanagan and Fraser watersheds (Fulton 1969) facilitated most of this colonization (McPhail and Lindsey 1986). Within several salmonid species and white sturgeon (*Acipenser transmontanus*), these connections are reflected by greater genetic similarities

between upper Fraser and upper Columbia populations relative to similarities between these populations and those from the lower rivers (Wehrhahn and Powell 1987, Brown *et al.* 1992, Utter *et al.* 1984, McCusker *et al.* 2000).

Analyses of bull trout mtDNA suggest that they, too, colonized British Columbia's interior from a refuge (or several poorly differentiated refugia) in the Columbia drainage, into the Fraser, and then into the Peace and other northern watersheds (Taylor *et al.* 1999). In other phylogeographic analyses, mtDNA diversity was highest south and east of CRDWK, near Clark Fork River, Idaho (Williams *et al.* 1997a), and allozyme diversity was highest at Clark Fork River, Kootenay Lake, and southern CRDWK (Leary *et al.* 1993). In each of the above studies, most genetic diversity was detected among – rather than within – populations, and even the most diverse populations were less diverse than populations of other salmonids (see Leary *et al.* 1993). Nevertheless, this geographic pattern of diversity suggests that an important glacial refuge for bull trout existed near (and perhaps to the southeast of) my study area.

At the level of species, the respective distributions in the North and South Thompson rivers of the torrent sculpin (*Cottus rhotheus*) and westslope cutthroat trout (*Oncorhynchus clarki lewisi*) suggest Columbia-Fraser connections north of Arrow Lakes (McPhail and Lindsey 1986). This area is east of the Okanagan drainage, and it also experienced geologically significant flooding. Kame terraces (indicating minimum elevations of standing glacial meltwaters) have been found at some northern sites in CRDWK at elevations more than 215 m above the present water level (Fulton and Archard 1985). Flooding of this magnitude could certainly provide access across drainages and to habitats above some waterfalls and thus explain the presence of fish populations there, but only if the founders could utilize the corridors during the dynamic, tumultuous time period when they were available.

After nearly 9 000 years of geological calm, the landscape of CRDWK has been affected by anthropogenic disturbances in the form of hydroelectric developments, as well as attempts to ameliorate those disturbances. Waterfalls already limited spawning habitat for adfluvial bull trout and their primary prey (kokanee, *O. nerka*). Flooding of much of the remaining spawning habitat (Lindsay 1986, 1987) for sport fish in the area began with completion of Hugh Keenleyside Dam (1967) in the south, followed by Mica Dam (1973) in the north, and finally Revelstoke Dam (1980-1984; see Figure 1.1). The Arrow Reservoir sport fishery was negatively affected (Lindsay 1986, 1987, but see Sebastian *et al.* 2000). Mitigation efforts included reduction of a waterfall (via dynamite) on the Halfway River to make upstream habitat available to adfluvial bull trout. To this end, the local public supports similar blasting activity of barriers in other tributaries (K. Bray, Columbia Basin Fish and Wildlife Compensation Program, personal communication). As was the case with Halfway River, the relevance to conservation and sometimes even the existence of fish populations above these barriers is unknown.

Research approach

Evidence from previous studies (see above) suggests that populations affected by Columbia basin impoundments were colonized from a Columbia refuge; but in designating and prioritizing conservation units (areas, populations, or groups of populations with distinct conservation value) or developing appropriate compensation plans within CRDWK, that coarse level of phylogeographic information is not adequate. Tools used to delineate phylogeographic history at a broad scale (allozymes, mtDNA) would not alone be sufficient (owing to a lack of diversity) to describe genetic history at this fine scale. Congruent with less variable markers, microsatellites have identified evolutionary units at broad scales but have also demonstrated utility at

subdividing those units (e.g., Spruell and Allendorf 1997). I used microsatellite DNA and mtDNA to investigate the phylogeographic history of bull trout populations in CRDWK and to begin an informed evaluation of their significance for conservation.

Biodiversity hotspots – habitats with large numbers of species – may warrant special consideration in conservation (Myers *et al.* 2000). Value or priority in conservation, however, is generally ascribed to things that are least replaceable (Magurran 1988). For this reason, endemic species weigh heavily in the prioritization of habitats for conservation. Approaches that give special consideration to rare or endemic species (reviewed by Margules and Pressey 2000) often prioritize habitats differently than those that consider species richness alone. Also, an arrangement of habitat preserves with nested species compliments (i.e., low *beta* diversity) is inferior to one with unique combinations of species (Wright and Reeves 1992). That is, habitats with many species, with unique species, and with unique combinations of species are perceived to have high value for conservation. Priorities are analogous at the intraspecific level, where populations are evaluated based on their genetic variation.

Under some circumstances intra- and interspecific phylogeography may be congruent (Moritz and Faith 1998, Bernatchez and Wilson 1998) – biogeographic correlations may exist between species and genetic diversity, and between endemism and private alleles. Such congruence could simplify priorities for conservation and has been sought at various levels of taxonomic classification. For example, Balmford *et al.* (1996) found that total species diversity in potential reserves was predicted reasonably well by diversity at higher taxa and also by the number of species within a single taxonomic group. I compared allelic variation among populations above different waterfalls and found an apparent association between genetic variation and the number of other fish species above those waterfalls. I examined this putatively historical pattern, compared genetic variation in CRDWK bull trout populations and populations elsewhere in British Columbia, and examined distributions of genetic diversity within populations to describe the postglacial colonization of CRDWK. I also evaluated more recent influences on the distribution of genetic variation in CRDWK: anthropogenic upstream influences on populations above waterfalls and downstream influences of these peripheral populations on the 'central' (mainstem) populations below.

Methods

Sampling

Fish were captured from lacustrine environments (reservoirs) and from fluvial environments both above and below putative migration barriers (waterfalls or cascades) in tributaries to the reservoirs. Samples from reservoir-dwelling bull trout were obtained from recreational anglers, primarily at fishing derbies and creel stations, and from a bull trout telemetry project operating within the study area. Sampling of live fish was non-lethal. Most stream-dwelling bull trout were captured using Gee traps baited with dog food or fish, although electrofishing was also heavily employed. Encounters with other fish species were noted. To reduce the possibility of pseudoreplication, I avoided use of samples from fry, which may be poorly dispersed (Hansen *et al.* 1997). Exceptions were made below waterfalls from Jordan River, St. Leon Creek, and Taite Creek, from which fry supplemented sample sizes. Samples included fish from Hill Creek Hatchery's broodstock collection program, and snorkelling in streams provided access to additional samples of adfluvial adult bull trout (either spawning fish or carcasses). Snorkelling proved inefficient for capture of stream-dwelling juveniles and yielded only a few samples. Sampling at most sites was replicated spatially, temporally, or both and occurred between 1996 and 1999 (see Table 2.1).

Table 2.1. Sample locations, sizes, and constitutions. Samples are identified as taken from above putative barriers to migration (A), from stream sites not above putative barriers (B), and from lacustrine environments (L). Spatial (s) and temporal (t) replication is also indicated. Numbers of hatchery-clipped adults are given by *. Samples marked ^ were combined for analysis; ^^ indicates a sample ignored in most analyses because of low sample size.

Site	Site type	Replication	Locus mtDNA	*Sco*1	*Sco*19	*Sco*23	*Sfo*18	*Ssa*197	adfluvial adults
Kinbasket	L	s	43	5	30	37	37	43	43
Revelstoke	L		8	8	8	8	8	8	8
Arrow	L	st	125	70	70	96	96	124	125**
^Whatshan	L		7	7	7	7	7	7	7
^Fife	A		14	11	11	11	11	11	
Bigmouth	B	s	31	31	30	31	31	31	
Downie	B	t	20	21	20	20	20	20	1
Downie	A		10	10	10	10	10	10	
Carnes	B	st	25	25	25	25	25	26	
Jordan	B		10	10	10	10	10	10	
Jordan	A		11	11	11	11	11	11	
Illecillewaet	B	st	45	45	45	45	45	45	22***
Incomappleux	B	st	40	32	32	39	39	40	
Hill	B		21	21	21	21	21	21	21*
Mackenzie	B	st	28	28	28	28	28	28	1
Payne	A		14	11	11	11	11	11	
Halfway	B	t	33	33	33	33	33	33	10*
Halfway	A	st	45	29	29	45	45	45	
St. Leon	B	t	36	39	39	39	39	39	4*
St. Leon	A	st	42	11	11	23	23	23	
^^Kuskanax	B		3	3	3	3	3	3	3
Kuskanax	A	s	18	18	18	18	18	27	
Slewiskin	B	st	44	40	40	44	44	44	2
Caribou	B		20	20	20	20	20	20	12
Snow	B	t	22	22	22	22	22	22	5
Woden	A	st	32	11	11	34	34	34	
Taite	B		20	20	20	20	20	20	
telemetry	L		47	40	40	46	47	47	47*
total			814	632	655	757	758	803	311

DNA extraction, PCR, and identification of variants

Various tissues were collected from dead bull trout, while non-lethal sampling involved only fin tissue. All tissues were stored in 95% ethanol until analysis. Genomic DNA was extracted following the protocol of Taggart *et al.* (1992) and was diluted to approximately 1 ug/uL. Two mitochondrial DNA (mtDNA) fragments were amplified using 30 to 35 cycles of the polymerase chain reaction (PCR). One fragment, nictinamide adenine dinucleotide dehydrogenase (NADH) subunits 5 + 6, was amplified using the primers C-Glu and C-Leu3 (Park *et al.* 1993), and the other, cytochrome *b* + control region, was amplified using HN20 (Bernatchez and Osinov 1995), and the reverse complement of C-Glu. Annealing temperatures were 54 and 50°C, respectively. Three uL of each PCR product were combined and digested with restriction enzymes that resolved polymorphisms in other bull trout studies (Kanda *et al.* 1997, Williams *et al.* 1997a, Taylor *et al.* 1999): *Alu* I, *Hae* III, *Hinf* I, *Msp* I, and *Rsa* I. Restriction fragment length polymorphism (RFLP) patterns were visualised using ethidium bromide stain and ultraviolet

light, following electrophoresis on 2% agarose gels. I then used these patterns to define composite haplotypes as in Taylor *et al.* (1999). The term "haplotypes" is used in place of "composite RFLP haplotypes" throughout this thesis.

Five microsatellite loci were also sampled: *Sfo*18 (Angers *et al.* 1995), and *Ssa*197 (O'Reilly *et al.* 1996), *Sco*1 (E.B. Taylor, UBC Dept. of Zoology, unpublished data), *Sco*19 and *Sco*23 (Taylor *et al.* 2001). Annealing temperatures were 55, 51, 63, 65, and 58°C, respectively, and between 30 and 35 cycles were employed for PCR. For *Ssa*197 and *Sfo*18, the forward primer was radiolabeled with ^{32}P, and PCR products were combined and electrophoresed on 6% polyacrylamide gels with an M-13 size standard. The reverse primer was labelled for *Sco*19 and *Sco*23, and analysis of *Sco*23 was similar to that of the heterologous microsatellite loci – with allele visualisation on autoradiographic film. The *Sco*19 primer was flourescently labelled with tetrachloroflourescein, while the forward primer for *Sco*1 was labelled with flourescein. I combined and diluted PCR products for these latter two loci, and they were examined with an ABI automated sequencer. Alleles were sized using GENESCAN 672 (Applied Biosystems).

*Sco*1

Products from *Sco*1 reactions commonly displayed three or four allelic variants per individual. Such occurrences could result via homoplasy for *Sco*1 priming regions or a locus duplication. Homoplasy seemed unlikely, and the tetraploid history of Salmonidae (Allendorf and Waples 1996) made locus duplication the favoured explanation for observed banding patterns. Both allozyme and microsatellite isoloci are common in Salmonidae (e.g., Allendorf and Seeb 2000).

Larger alleles amplified less effectively than shorter ones, but did so in a repeatable manner, in accordance with the number of copies, or "dosage," of each allele. It was therefore possible to distinguish between, for example, various two-allele genotypes (e.g., 173 231 231 231 versus 173 173 173 231) by intensity of fluorescence, which was digitally provided by GENESCAN (see Slade *et al.* 1998). In some cases, unfortunately, relative intensities were most easily explained by a lack of one allelic product, and the possible presence of null alleles made determinations among one- and some two-allele genotypes impossible (e.g., 173 null null null, 173 173 null null, and 173 173 173 null). Because distinguishing dosage of allelic variants was difficult in some cases, Hardy-Weinberg equilibria were not directly tested. Instead, the proportion of fish in which a given allele was present or absent (not number of copies) was used to predict that allele's frequency in each population sampled, and this number was compared to my counts. Prediction was accomplished using the Hardy-Weinberg formula expanded for tetraploids, $p^4 + 4p^3q + 6p^2q^2 + 4pq^3 + q^4 = 1$, and assigning p as the allele frequency of interest (with q representing the sum of all other allelic frequencies, including presumed null alleles). Because $p = 1 - q$, $p = 1 - (\text{frequency of absences})^{1/4}$. For example, if allele 173 was not detected in 40% of fish from a particular sample, its estimated frequency in the population would be $1 - (0.4)^{1/4} = 0.205$. Mitochondrial haplotypic frequencies and allelic frequencies at the four other microsatellite loci were simply counted.

Justification of dichotomising samples

Isolation of headwater populations was evaluated by the presence of physical barriers to upstream movement, observation of precocious males and females, and significant deviations from Hardy-Weinberg and linkage equilibria (when combined with samples from immediately downstream of the suspected barrier). I did not consider sites to be isolated if none of the above characteristics applied or if adfluvial adults were observed there. (Fish were identified as adfluvial adults based on behaviour, seasonal abundance, and primarily size.) Observations and

results suggested that streams sampled at sites on either side of putative barriers should be analysed as two distinct populations – one adfluvial population accessible to other populations below barriers and one isolated population. Stream samples were accordingly divided and analysed as "data set 1."

Waterfalls could act as one way valves, allowing only downstream fish movement and gene flow. Some analyses would be biased by such an influence, so a second data set was constructed. In this "data set 2," samples immediately downstream of population isolates were 'corrected' for possible genetic influences from upstream. To identify fish in the downstream sample with origins above waterfalls (henceforth referred to as "fallers") and estimate a "falling" rate, the probabilities of a genotype arising below and above a waterfall were compared using DOH (http://www.biology.ualberta.ca/jbrzusto/Doh.html). In a population directly below a waterfall, individuals with genotypes less than twice as likely to arise in the population below the waterfall (versus above) were not included in data set 2. Furthermore, because F_1 progeny of faller x adfluvial crosses would have a high assignment probability below falls if fallers were included, the procedure was applied a second time with the first fallers removed, and the same exclusion criterion was applied. That is, neither putative fallers from immediately upstream nor the putative offspring of fallers were included in data set 2. This process is described graphically in Figure 2.1. (Because populations above waterfalls in some streams were not found or not sampled, I may have failed to remove some undetected downstream influences from populations isolated above barriers.)

DOH permitted analysis of all six loci in a single data set and was used for several analyses of assignment probabilities as follows. Individuals missing data at some loci were excluded from analyses facilitated by DOH. Data set 2 included only individuals with six-locus genotypes. All data were entered as tetraploid loci, with missing alleles ignored. That is, individual fish were "missing" two alleles at diploid microsatellite loci (e.g., 01 03 - -), and three for mtDNA (e.g., 02 - - -). Assignment probabilities of zero would result if some alleles were not sampled within populations, so allele frequencies of zero were replaced using two methods: i) one copy of the absent allele was added to the population and its frequency was recalculated (the "add-one-in" option); ii) alternatively, an allele frequency of 0.01 was assumed instead of zero. Sampling error can promote differences between results generated by the two options (e.g., addition of one allele to a sample size of 10 generates a different allele frequency than addition of that allele to a sample size of 40). Results were robust however, yielding the same qualitative information, and I present results from data set 1 using the "add-one-in" option unless otherwise stated.

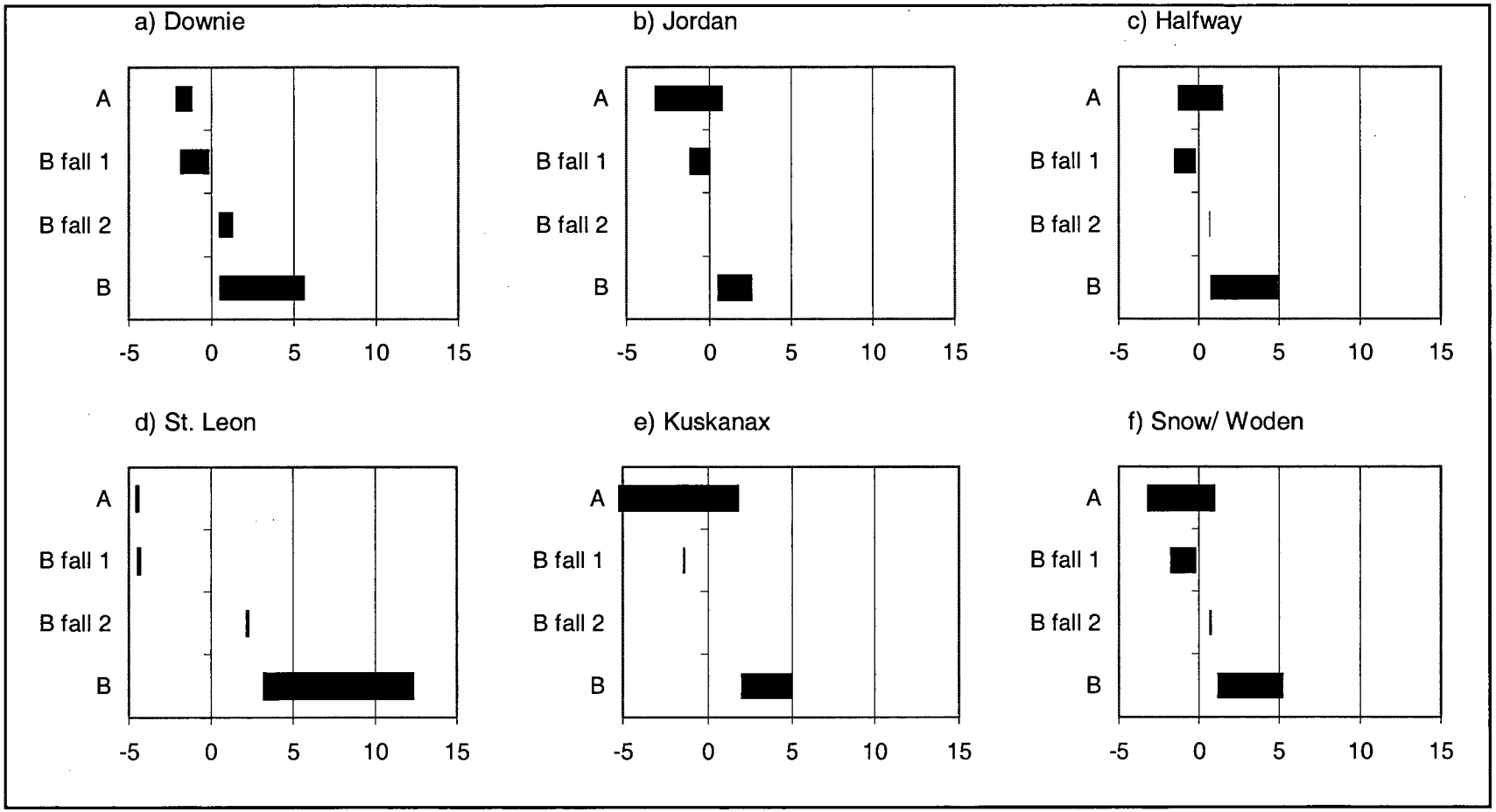

Figure 2.1. Relative certainty of "faller" designation. For each individual sampled from each A/B stream pair, assignment indices to the population below the putative barrier were divided by assignment to the population above. Ranges of the log of this value (horizontal axis) are presented for different kinds of individuals: A = fish sampled above barriers, B = fish considered not to have recent ancestry from above the barrier, B fall 1 = identified "fallers" from the first application of the assignment criterion, B fall 2 = identified "fallers" (or hybrid offspring) from the second application of the assignment criterion. Assignment ratios shown are from the first application only. Arithmetic means of results from the two methods for eliminating allele frequencies of zero are shown.

Statistical analyses of data sets 1 and 2

To evaluate assumptions of independence within samples and among loci, and to test for possible Wahlund effects, data from *Sco*19, *Sco*23, *Sfo*18, and *Ssa*197 were analysed with respect to Hardy-Weinberg and linkage equilibria. As in examination of the combinations of population pairs upstream and downstream of supposed barriers, I employed exact tests (Louis and Dempster 1987, Guo and Thompson 1992) available in GENEPOP (Raymond and Rousset 1995) for this purpose. Unbiased heterozygosity (H_e, Nei 1978) was calculated for diploid loci in each sample with TFPGA (Miller 1997). Costello and Taylor (in preparation) analysed these same four microsatellite loci from two other regions in British Columbia – the Pine watershed (Peace River drainage) and populations near the southern Rocky Mountain Trench in the East Kootenay region. Like bull trout populations in CRDWK, bull trout in these other regions were sampled from above and below putative migratory barriers (although barriers in these other regions were not examined as extensively; A.B. Costello, UBC Dept. of Zoology, personal communication). Six populations above barriers were sampled in the East Kootenays (three within the upper Kootenay drainage, two in the upper Columbia drainage in Yoho National Park, and one in a tributary to Red Deer River in Banff National Park), and five samples above waterfalls were scattered throughout the Pine system. A t-test compared migratory and resident populations within CRDWK, and comparisons were made to each type of population in other drainages.

Provincial biogeography of sample groups

Testing for nested subsets is a method of evaluating biogeographical hypotheses regarding species compositions of isolated communities (e.g., Cook and Quinn 1995, Wright *et al.* 1998), and of evaluating conservation values of potential wildlife reserves (Wright and Reeves 1992, but see Boecklen 1997). Unfortunately, available statistical analyses are statistically flawed owing to difficulties in modelling null hypotheses – null distributions from randomisation are insufficiently nested because they cannot account for differential commonness of species (Cook and Quinn 1998). I *qualitatively* assessed the nestedness of *alleles* among CRDWK, Pine, and upper Kootenay samples. Perfectly nested structure occurs when less diverse populations contain only alleles that are also found in every more diverse population. Occurrences of rare alleles in depauperate populations and absences of common alleles from diverse populations disrupt nested structure. In a nested matrix, alleles are listed left to right from most to least common, and samples are arranged top to bottom from most to least diverse. Disruptions to nestedness that occur in the lower right corner (unexpected presences of rare alleles in depauperate populations) or in the upper right corner of nested matrices (unexpected absences of common alleles in diverse populations) are most notable. Idiosyncratic distributions among populations may signal autecological properties or environmental noise in nested matrices of species, but, in nested matrices of neutral molecular markers, idiosyncrasies more likely reflect colonization from differentiated sources or new mutations. Total allelic diversity was compared among the watersheds, and the cumulative heterozygosity option of DOH was used to reduce the effect of sample size on these comparisons. Individuals were resampled from their populations, and numbers of alleles at the four loci were estimated for samples of equal size.

Biogeographical inferences can be aided by partitioning genetic variation geographically at a scale above the sample level. Hierarchical analysis of molecular variance components, AMOVA (Excoffier *et al.* 1992), is supported by the ARLEQUIN program (Schneider *et al.* 1997) and was performed while imposing a variety of hypothetical genetic structures on data from CRDWK. The four diploid microsatellite loci were analysed concurrently whereas, by necessity, *Sco*1 and mtDNA were analysed individually. I examined the amount of variation partitioned among populations above and below waterfalls to the amount partitioned among northern and southern

populations in CRDWK. Also, I measured the amount partitioned among A/B sample pairs (samples up- and downstream of a waterfall) for comparison.

Biogeographical patterns were also evaluated by generating dendrograms representing genetic relationships among samples from each of the watersheds. For this analysis, data were grouped to a lesser degree than in the nestedness analysis above and subdivided into potential phylogeographic groups (as determined above). Dendrograms were made with the TFPGA software program (Miller 1997), applying the unweighted pair group method using arithmetic averages (UPGMA, Sneath and Sokal 1973) to various genetic distances: Nei's standard distance, D_S (biased and unbiased; 1972, 1978), Roger's (1972) distance, and Reynolds *et al.*'s (1983) coancestry coefficient.

Analysis of individual population isolates

Within CRDWK, I qualitatively examined nestedness of alleles among populations above putative barriers using all six loci. Total allelic diversity of isolated populations was evaluated. Again, the cumulative heterozygosity option of DOH was used to reduce sample size effects, but as this option accepts only diploid data, alleles at *Sco*1 were divided into pairs and analysed as two diploid loci. This increased variance in estimates but did not bias comparisons. Differentiation among population isolates was measured, as well as differentiation between them and the adfluvial population. One distance measure used was D_{LR} (Paetkau *et al.* 1997), an assignment-based distance calculated using all six loci (with the program DOH). More common methods were also used. For the four diploid microsatellite loci, Weir and Cockerham's (1984) F_{ST} statistic, θ, and exact tests of differentiation among populations were calculated using GENEPOP. Ignoring mutational differences among mtDNA haplotypes and defining no genetic structure, the F_{ST} analog, Φ_{ST}, computed by ARLEQUIN is equivalent to θ (Baker *et al.* 1998), and thus F_{ST} estimates for mtDNA and microsatellite loci could be compared. Allelic frequencies at *Sco*1 were similarly analysed with ARLEQUIN. For convenience, all statistics estimating F_{ST} are henceforth reported as F_{ST}.

Consideration of mutational distances among alleles can provide misleading results (Angers and Bernatchez 1997, Orti *et al.* 1997), and all F_{ST} and analogous statistics presented herein were calculated ignoring quantitative distances between haplotypic and allelic variants. Taylor *et al.* (1999, 2001) found that including mutational information for the same mtDNA regions and diploid microsatellite loci examined here tended to blur structure among bull trout populations in British Columbia's interior, despite more geographically extensive sampling. Preliminary R_{ST} analyses (with GENEPOP) of my microsatellite data also indicated that no additional information would be provided by that approach.

Regionally-defined index of genotypic affinity: RDIA

Given multiple multi-allelic loci, genetic differentiation among samples can occur in several dimensions or along multiple axes. A biogeographic pattern may exist undetected within the differentiation measured by traditional F-statistics. To extract biogeographic information on populations isolated above waterfalls I used a north-south pattern discovered in analyses described above to determine whether given genotypes had greater affinity to northern or southern samples. I examined assignment of genotypes in populations above putative barriers and in populations immediately below them. I divided the geometric mean of each genotype's predicted frequency of generation in northern tributaries by the sum of the geometric means of its predicted frequency of generation in northern and southern tributaries. The quotient was thus a number between 0 and 1 (northerly genotypes closer to 1, southerly genotypes to 0). I then

logit-transformed this number. The resulting value served as a regionally-defined index of genotypic affinity (RDIA, hereafter), with positive values indicating northern affinities and negative numbers suggesting southern ones. I used assignments to only migratory (below waterfall) populations to define the north-south axis, and I included neither self-assignment (unlike calculation of D_{LR}) nor assignment to other populations directly downstream of populations isolated above barriers. To further reduce potential biases, I ignored genotypes of identified fallers in calculation of RDIA. That is, I examined only data set 2.

To examine the possibility that a population above a barrier was phylogenetically subdivided, RDIA scores were compared (by linear regression) among sites within population isolates that were subsampled spatially. Calculation of RDIA here was identical to the calculation described above. Comparison among the spatially-subsampled populations was performed via analysis of covariance (ANCOVA).

Results

Identified allelic variation

More than 600 bull trout collected from 26 locations in the study area yielded six mtDNA haplotypes, 11 *Sco*19 alleles, and 20 *Sco*1 alleles (including a presumed null allele). By contrast, *Sco*23, *Sfo*18, and *Ssa*197 were diallelic. Thus, the total number of molecular variants detected was 43 (see Appendix A). For restriction enzymes shared between the two studies, mtDNA variants resolved here displayed restriction fragment patterns consistent with RFLP haplotypes found in populations throughout the interior range of bull trout (Taylor *et al.* 1999). I used the nomenclature of Taylor *et al.* (1999) to name these shared haplotypes when possible, and I examined their geographic distribution within CRDWK (Figure 2.2). A common restriction pattern from CRDWK's southern tributaries was consistent with that of RFLP Haplotype 1 from Taylor *et al.* (1999). Haplotype 4 was found only in the Whatshan A sample (where it was the only haplotype detected), and Haplotype 19 was observed at low frequencies in reservoir samples from the northern part of the study area and above the Jordan River waterfall. A single individual from Kinbasket Reservoir had a restriction pattern consistent with Composite Haplotype 8, a "coastal" variant (confirmation was impossible as I did not use enzymes diagnostic for distinguishing the coastal clade of Taylor *et al.* 1999). One haplotype with a novel *Rsa*I restriction pattern was called Haplotype 13a because it differed by one restriction site from Taylor *et al.*'s (1999) Haplotype 13, which was the most common haplotype in my study and dominated samples from northern locales. Relationships among these haplotypes and estimates of their sequence divergence from one another (range: approximately 0.3-0.8%) are given in Taylor *et al.* (1999).

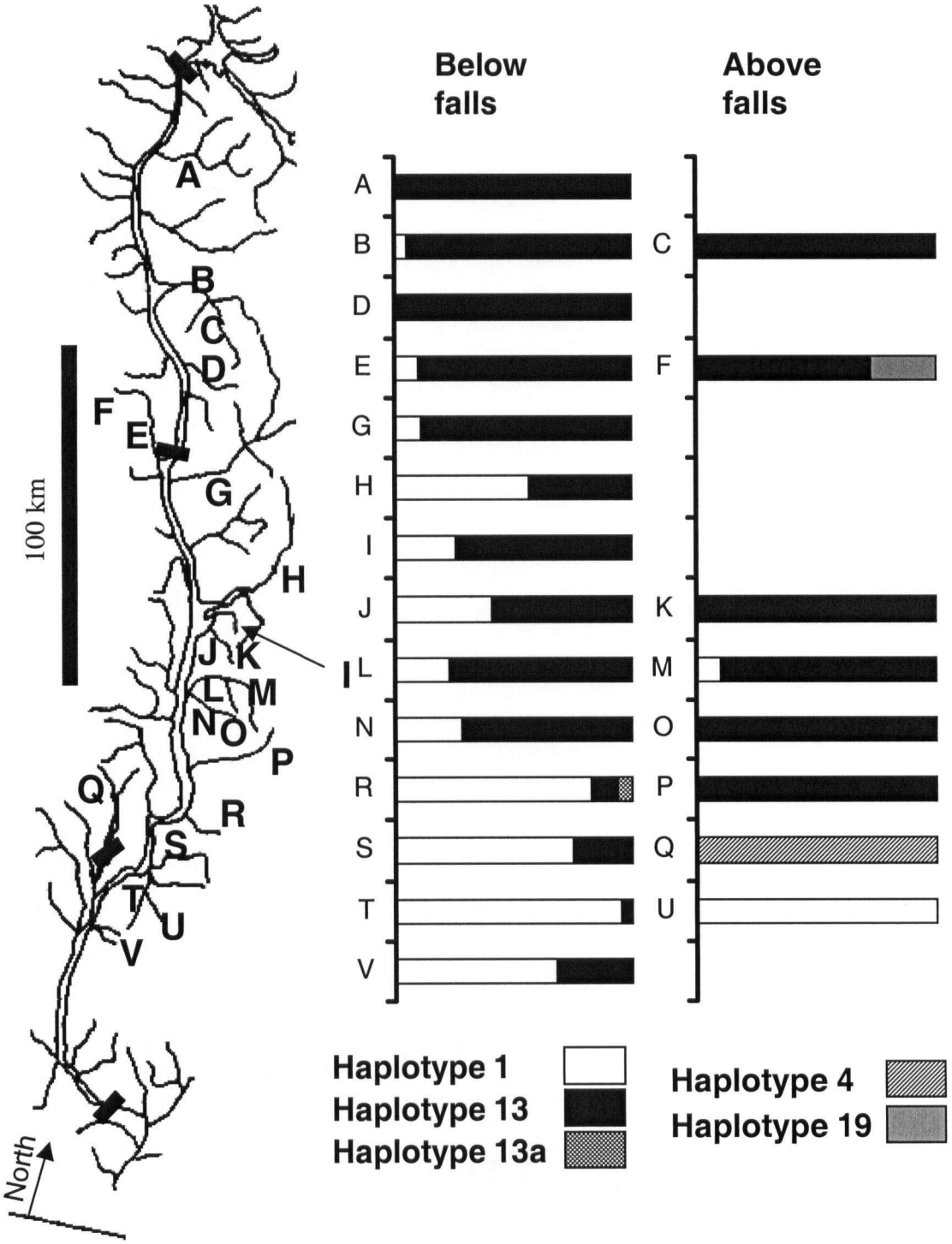

Figure 2.2. Distribution of mtDNA haplotypes above and below barriers among sites within the CRDWK study area. Sites are: Bigmouth A, Downie B & C, Carnes D, Jordan E & F, Illecillewaet G, Incomappleux H, Hill I, Mackenzie J, Payne K, Halfway L & M, St. Leon N & O, Kuskanax P, Whatshan Q, Slewiskin R, Caribou S, Snow T, Woden U, Taite V. Dark bars on the map represent dams, from north to south: Mica, Revelstoke, Whatshan, and Hugh Keenleyside.

Microsatellite variants at diploid loci also tended to be the same as those sampled elsewhere in B.C. Fourteen alleles were shared by Pine, Kootenay, and CRDWK groups of samples, whereas ten private alleles were sampled (only 4 of which were sampled at more than one site within a group of samples). When pooled, samples from CRDWK contained only one private allele.

This allele (208 at *Sco*19) was widespread in the study area, though at a low frequency. Bull trout from Pine River (Peace River drainage) displayed private alleles (*Sco*19: 183, 185, 187) that occurred at a low frequency and were near to one another in size and distribution. In contrast, upper Kootenay River samples contained several private alleles that occurred at relatively high frequencies with more extensive distributions (Costello and Taylor, UBC Dept. of Zoology, unpublished data). Alleles detected at multiple sites demonstrated an essentially nested distribution among the three watersheds (Figure 2.3). That is, alleles detected within Pine River samples were generally found within at least one CRDWK sample, and alleles found within CRDWK were generally found within at least one Kootenay River sample. A nested pattern is consistent with colonization of each site from a common source.

Samples	Alleles at *Sco*23, *Sfo*18, *Ssa*197, and Sco*19*																										Total
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	z	alleles
Kootenay	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1	1	1	^{1}	^{1}	^{1}				22 (18.0)
CRDWK	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1										17 (16.1)
Pine	1	1	1	1	1	1	1	1	1	1	1	1	1	1										^{1}	^{1}	^{1}	17 (14.9)

1 indicates that the allele was detected in the samples
^{1} indicates that the allele was detected at only one site
(#) average number of alleles in resamples of 200 individuals

Figure 2.3. Nesting of allelic variation at four microsatellite loci among samples from three British Columbia watersheds. Samples are arranged from most to least diverse, and alleles are presented from most to least commonly sampled. Alleles are represented by columns: a through f are *Ssa*197 (alleles 119, 123), *Sfo*18 (150, 156), and *Sco*23 (92, 94). Alleles g through z are *Sco*19 (g = 190, h = 200, i = 204, j = 206, k = 174, l = 198, m = 202, n = 210, o = 194, p = 196, q = 208, r = 192, s = 214, t = 216, u = 212, v = 158, w = 170, x = 183, y = 185, z = 187).

Waterfalls as barriers

I observed traits reflective of the stream resident life history in samples collected above putative barriers to upstream migration within CRDWK tributaries: precocial males (running milt) were sampled more commonly above putative barriers than below them, precocial females were only sampled above putative barriers, and individuals sampled in previous years were recaptured only above putative barriers. With the exception of Carnes Creek, adfluvial (large adult migrants from a reservoir) bull trout were observed at all sites thought not to be above a barrier to migration; adfluvial adults were never observed above putative barriers except at Mackenzie Creek. Tests of Hardy-Weinberg and linkage equilibria also suggested that samples upstream and downstream of putative barriers represented isolated populations. More frequent than expected by chance alone, 8 of 42 (19% versus 5% expected) tests of disequilibrium in diploid microsatellite data were significant for samples combined across putative barriers. This suggested subdivision between each A population (above a barrier) and the adjacent B sample (below a barrier). When probabilities were combined across loci via Fisher's method (Sokal and Rohlf 1995), samples from three stream pairs (Downie A/B, St. Leon A/B, and Woden A/Snow B) demonstrated significant departures from equilibrium expectations (see Table 2.2).

When A/B pairs were split and putative fallers were removed from the B samples (i.e., after construction of data set 2), departures from equilibrium were about as frequent as expected by chance alone (9/163 = 5.5%) and were associated with potential Wahlund effects (indicating admixture of populations). Three of the deviations were found in lacustrine samples, and three

others occurred within samples above barriers on Halfway River and Kuskanax Creek. In this data set, no departures from equilibrium remained significant after combining probabilities across loci within populations or applying the sequential Bonferoni procedure of Rice (1989). Using these corrections for multiple tests with data set 1 (with fallers), only the St. Leon B sample departed significantly from Hardy-Weinberg and linkage equilibria, though nonsignificant deviations in other samples downstream of waterfalls were greater in data set 1 than in data set 2 (except in Halfway B). At *Sco*1, allelic frequencies estimated from counts and predicted from absences agreed quite well, again with the greatest discrepancies found in potential admixtures of populations associated with waterfalls (not shown). These results justified analysis of populations above and below identified barriers as distinct units in comparisons among stream populations.

Table 2.2. Evaluation of physical isolation (migratory versus resident fish) and genetic isolation (linkage disequilibrium, LD, and deviations from Hardy-Weinberg proportions, HWE, statistically corrected for multiple comparisons) within stream samples interrupted by putative barriers to upstream migration. NA indicates that sufficient adfluvial samples weren't available; spaces indicate that confirmation eluded observation. All stream sites not isolated from a reservoir by a putative barrier are included in "all other stream samples."

Sample site	Barrier to migration present	Observed precocial males	Observed precocial females	Deviation from equilibrium expectations	Recapture of individuals among years	Observed adfluvial adults
Downie Cr.	Reported	Yes		LD		
Jordan River	Yes					
Payne Cr.	Yes	Yes	Yes	NA	Yes	
Halfway River	Yes	Yes	Yes		Yes	see text
St. Leon Cr.	Yes	Yes	Yes	HWE, LD	Yes	
Kuskanax Cr.	Yes	Yes		NA		
Whatshan	Yes	Yes		NA		
Woden Cr.	Reported	Yes	Yes	HWE	Yes	
Mackenzie Cr.	Yes	Yes			Yes	Yes
All other in-stream samples		In some larger streams				Yes (except Carnes Creek)

Differences between resident and adfluvial populations

Heterozygosities observed in this study ranged from 0.00 (above St. Leon Creek's waterfall, all loci) to 1.00 (*Sco*1, several samples). Compared to measures with the same diploid microsatellite loci elsewhere in British Columbia, H_e (unbiased) in CRDWK samples from below and above putative barriers to migration was not unique. Heterozygosities were lower in samples from populations designated as isolates than in samples from below putative migration barriers ($P = 0.005$), and similar trends occurred in other regions (Figure 2.4).

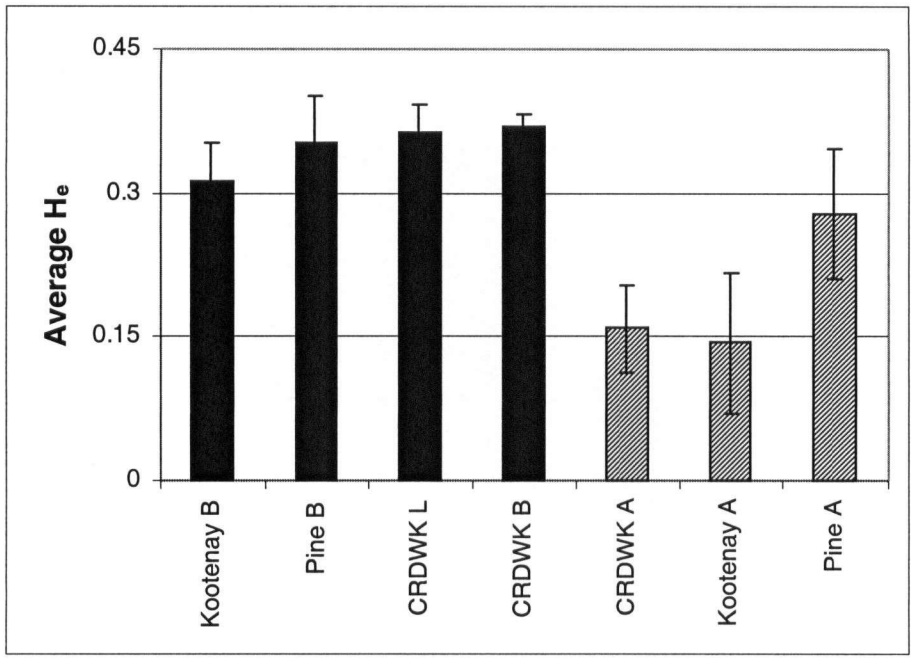

Figure 2.4. Expected heterozygosity (unbiased) from samples from migratory populations (solid columns) and from populations resident above natural barriers (hatched) in three British Columbia watersheds. Data for Kootenay and Pine watersheds are from Costello and Taylor (unpublished data). Bars represent standard errors.

Although reduced genetic variation was found within populations above waterfalls, this variation included mtDNA variants not detected in adfluvial populations and high frequencies of microsatellite alleles that were rare below falls (see Appendix A) – populations above falls demonstrated genetic uniqueness. Population isolates were also strongly differentiated from populations downstream of them (Table 2.3). (This differentiation was emphasized to a greater extent by analysis of data set 2; not shown.) Combined analyses from the four diploid microsatellite loci revealed the largest and most statistically significant differences, and every A/B pair was differentiated by at least one locus (uncorrected for multiple comparisons). Still, in each B sample, some proportion of individuals was assigned to the A population immediately upstream. In St. Leon Creek, where the difference between A and B samples was greatest, the estimated proportion of fallers was low (22% of the B sample). None of the fish identified as fallers in St. Leon Creek was an adfluvial adult. Cross-assignment (assignment of individuals from B samples to A populations) was more frequent in other streams. Migratory adults were not, on the whole, less likely than were juveniles to be genotypically similar to fish above barriers. That is, ratios of assignment to A and B samples did not differ among adults and juveniles within streams ($P > 0.5$, combined probabilities of Wilcoxen signed-rank tests, despite $P = 0.08$ for St. Leon Creek). A proportion of adfluvial adults, both from B samples (see Table 2.3) and lacustrine samples, was assigned to populations above falls, suggesting that fallers may adopt a migratory life history. Adfluvial adults captured in streams or reservoirs were never assigned to St. Leon A or Payne A, however, contradicting this possibility for these streams. Cross-assignment in the other direction (individuals from A samples to B populations) was relatively rare. That is, genotypes of fish sampled above barriers were generally unlikely to arise in populations below them, but Jordan and Halfway rivers were exceptions. In Halfway A, 12 of 18 bull trout sampled downstream of the most upstream site were assigned to below the waterfall (not shown). Figure 2.1 illustrates the relatively high likelihood of cross-assignment for individuals in Jordan A and Halfway A samples.

Table 2.3. Estimates of differentiation across waterfalls for diploid microsatellite loci, *Sco*1, and mtDNA for data set 1 (* $P < 0.05$, ** $P < 0.005$). Proportions of identified fallers in B samples (all individuals and migratory adults only) are shown. ^ indicates that only data for the most upstream site of the A population were included. No adfluvial adults were sampled at Jordan River and no juveniles were sampled at Kuskanax B.

Stream system	Differentiation (Pairwise Fst)			Cross-assignment of "B" individuals to "A" samples (%)	
A/B pair	mtDNA	4 usat	Sco1	total	adults
Downie	0	0.14 **	0.35 **	25	100
Jordan	0	0.14 **	0.02	50	NA
^Halfway	0.13 *	0.17 **	0.03 *	34	22
St. Leon	0.18 **	0.61 **	0.28 **	22	0
Kuskanax	0.58	0	0.06 *	33	33
Woden	0.03	0.31 **	0.05 **	32	20

Comparison of populations above barriers

Within CRDWK, populations above barriers were highly differentiated from one another. Estimates of F_{ST} yielded by mtDNA, diploid microsatellite data, and *Sco*1 were 0.94, 0.54, and 0.39, respectively. Allelic variants displayed some nesting among isolated populations, which suggested that differences were generally weak among the source populations for the founding of isolated populations. Notable exceptions to nesting did occur, however, and some of these exceptions seemed coherent (rather than random; Figure 2.5). Samples from St. Leon A and Payne A, for example, had low genetic diversity and shared an allele at *Sfo*18 that was not found in samples from other isolated populations. They also lacked the alternate allele at *Sfo*18 for which all other isolated populations appeared fixed. Allele 200, the most common *Sco*19 allele throughout CRDWK was not found in St. Leon A and was represented by only a single copy in Payne A. St. Leon A and Payne A therefore seem to be the remnants of a colonizing group different from that represented by the other isolated populations. To a lesser extent, however, rare allelic variation at *Sco*19 was also shared among St. Leon A and Whatshan A samples, and between Payne A and Kuskanax A.

Among isolated populations, private alleles occurred in each of the three most diverse populations: one was detected in Halfway A, two in Jordan A, and three in Kuskanax A (see Figure 2.5). The only other private allele detected above a barrier was Whatshan A's Haplotype 4, which was the only molecular variant detected above falls that was not also detected in any B or L (lacustrine) samples in this study.

Sample location	Alleles at mtDNA, *Sco*1, *Sco*19, *Sco*23, *Sfo*18, and *Ssa*197 a b c d e f g h I j k l mn o p q r s t u v w x y z a' b' c' d' e' f' g' h'	Total alleles
Halfway A	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	25 (23.1)
Kuskanax A	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	19 (19.1)
Jordan A	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	18 (20.7)
Woden A	1 1 1 1 1 1 1 1 1 1 1 1 1	10 (14.3)
Downie A	1 1 1 1 1 1 1 1 1	9 (10)
Whatshan A	1 1 1 1 1 1 1 1*	8 (8)
Payne A	1 1 1 1 1 1 1	7 (7)
St. Leon A	1 1 1 1 1 1	6 (6)

1 indicates that the allele was detected in the sample
* indicates an allele that was not detected below waterfalls
(#) average number of alleles in resamples of 10 individuals

Figure 2.5. Nesting of molecular variation detected at six loci among eight populations isolated above barriers in CRDWK. Matrix is constructed as in Figure 2.3. Because *Sco*1 was analyzed as a diploid locus (see Methods), resamples sometimes contained more 'alleles' than occurred in the raw data. Columns represent alleles: a and h are alleles 123 and 119, respectively, at *Ssa*197; b and l are alleles 94 and 92 at *Sco*23; e and x are alleles 150 and 156 at *Sfo*18; f, s, d', and e' are mtDNA haplotypes 13, 1, 19, and 4; d, l, u, v, w, b', c', and h' are alleles 200, 202, 206, 174, 194, 196, 204, and 190 at *Sco*19; c, g, j, k, m, n, o, p, q, r, t, y, z, a', f', and g' are *Sco*1 alleles 173, 235, 237, 179, 231, 233, 181, 222, 229, 241, 243, 177, 206, 225, 227, and 239. The suspected null allele at *Sco*1 is not represented.

Colonization of habitats by alleles in bull trout seemed related to colonization by other salmonid fish species. Populations with the most allelic variation were sympatric with both *Cottus cognatus* and *Oncorhynchus* species and were also least diverged from lacustrine samples (Figure 2.6). Unlike allelic variation, detection of fish genera was perfectly nested among population isolates (e.g., I detected *Oncorhynchus* species in habitats above barriers only if those habitats were also home to sculpins). This relationship between number of species and number of allelic variants, however, was not congruent with the nested pattern of genetic variation. St. Leon A and Payne A (the samples described above as sharing the most rare allelic variation) were collected from habitats with different species compliments. Bull trout were sympatric with sculpins above the Payne Creek barrier, but were allopatric with all other fish species in St. Leon Creek. Likewise, shared exceptions to nesting in Kuskanax and Payne creeks occurred across species diversities. Also, Woden A and Halfway A were the only samples above barriers that contained Haplotype 1, but whereas rainbow trout and sculpins occur above Halfway River's waterfall, only bull trout were found above Woden Creek's barrier. Thus, colonization (or persistence) of other trout species above barriers was clearly positively associated with the *number* of allelic variants in bull trout populations but only poorly associated with the *identity* of those allelic variants. The same was true for genetic distance. Diverse isolated populations were least diverged from adfluvial populations (Spearman rank correlation, $r = -0.95$, $P < 0.005$) and contained the greatest number of private alleles among isolated populations. Whatshan A appeared fixed for a variant that was not detected elsewhere in CRDWK, but Whatshan A was less distinct from adfluvial populations than were less diverse populations (Payne and St. Leon creeks) that had no unique genetic variation (see Figure 2.6).

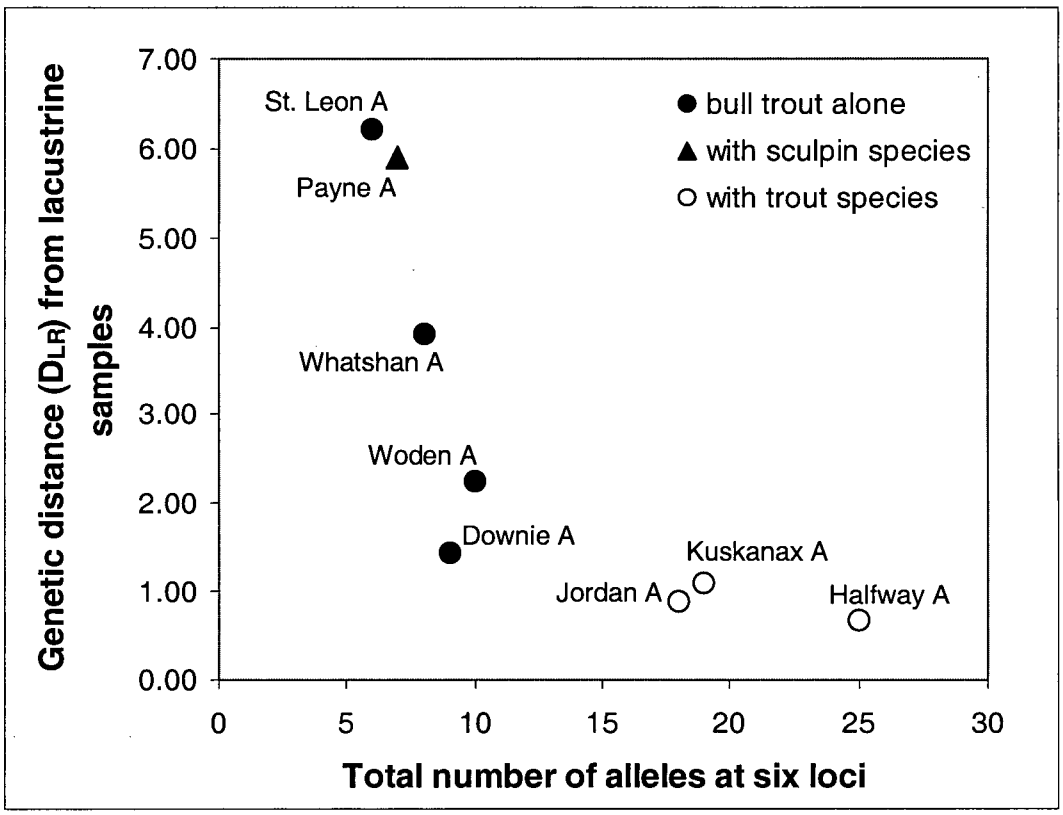

Figure 2.6. Allelic diversity and differentiation from lacustrine samples of bull trout populations found alone, sympatric with sculpins, and sympatric with both sculpins and *Oncorhynchus* species – rainbow or cutthroat trout (open symbols).

Phylogeographic groups within CRDWK

Populations isolated above waterfalls do not form a genetically cohesive group (see above), and distributions of other species do not facilitate resolution into multiple phylogeographical groups (see above). Other patterns gave stronger phylogeographic signals. Stream samples north and south of Slewiskin Creek tended to differ in frequencies of mitochondrial haplotypes (see Figure 2.2), and concordant differences were found at microsatellite loci (particularly *Ssa*197, see Appendix A). Samples from both adfluvial populations and populations above waterfalls reflected these general differences. Phylogeographically, AMOVA analyses suggested that geographic distributions of alleles most strongly reflected an historical discordance between northern and southern CRDWK. Some variation (at *Sco*1) was partitioned above versus below waterfalls and, alternatively, among pairs of populations above and below waterfalls (mtDNA), but F_{CT} (regional differentiation) was generally greatest when samples were compared as northern and southern groups (Table 2.4). Strong differentiation at mtDNA among pairs of A and B populations was coincident with northern and southern groupings that I imposed.

Alternate north-south groupings were examined using adfluvial populations, but none accounted for a greater proportion of genetic variance than defining (as suggested by Figure 2.2) Slewiskin, Caribou, Snow, and Taite creeks as "southern" and other creeks as "northern" (see Figure 2.7). These definitions were used in subsequent analyses.

Table 2.4. Total spatial differentiation (F_{ST}), amount partitioned among groups (F_{CT}), and proportion (%) of spatial differentiation partitioned among groups for mtDNA, *Sco*1, and diploid microsatellite loci. Imposed groupings compared samples north versus south of Slewiskin Creek, samples above versus below waterfalls, and pairs of samples separated by barriers. Differentiation was partitioned into northern and southern groups using all data and using data only from streams with samples above and below barriers. Statistical significance, uncorrected for multiple tests, is indicated by * ($P < 0.05$) and ** ($P < 0.005$).

Comparison	Estimate	mtDNA	4 usat	*Sco*1
North versus south	F_{CT}	0.47 **	0.08 **	0.00
(2 groups)	F_{ST}	0.56 **	0.15 **	0.02 **
	%	84	51	14
North versus south	F_{CT}	0.59 **	0.12 **	0.00
(2 groups, but includes	F_{ST}	0.73 **	0.33 **	0.14 **
only streams with both A and B samples)	%	80	36	0
Above versus below	F_{CT}	0	0.06 *	0.01 *
barriers	F_{ST}	0.58 **	0.31 **	0.11 **
(2 groups)	%	0	18	11
Downie A/B versus	F_{CT}	0.68	0.00	0.00
Jordan A/B versus...	F_{ST}	0.73 **	0.33 **	0.14 **
(5 groups)	%	94	0	0

On the basis of Nei's (1972) D_S, UPGMA grouped "southern" samples in the study area together with migratory populations in the upper Kootenay watershed (70% of 5000 bootstrap permutations), rather than with other CRDWK samples (Figure 2.8). The assumed relationship between northern tributary samples designated as adfluvial and lacustrine samples within CRDWK was strongly supported (92%). Samples from above and below barriers within the Pine watershed were grouped together (66%) relative to either kind of population in the upper Kootenay and CRDWK regions. Bootstrap support over the remaining topology was generally low. Nevertheless, samples above barriers in CRDWK grouped together with the area's northern and lacustrine samples, and, notably, together with samples above barriers in the East Kootenays, as opposed to the adfluvial upper Kootenay-southern CRDWK grouping (Figure 2.8). This branch-order was indicated regardless of which measure of genetic distance was used. Using Reynold's coancestry coefficient, upper Kootenay-southern CRDWK samples were most anciently diverged from all other samples (the positions of the upper Kootenay-southern CRDWK samples and Pine samples were reversed in comparison to the topology shown in Figure 2.8), but no other variance in topology was observed. (The apparent affinity between northern and above-barrier samples in CRDWK was examined in subsequent analyses.)

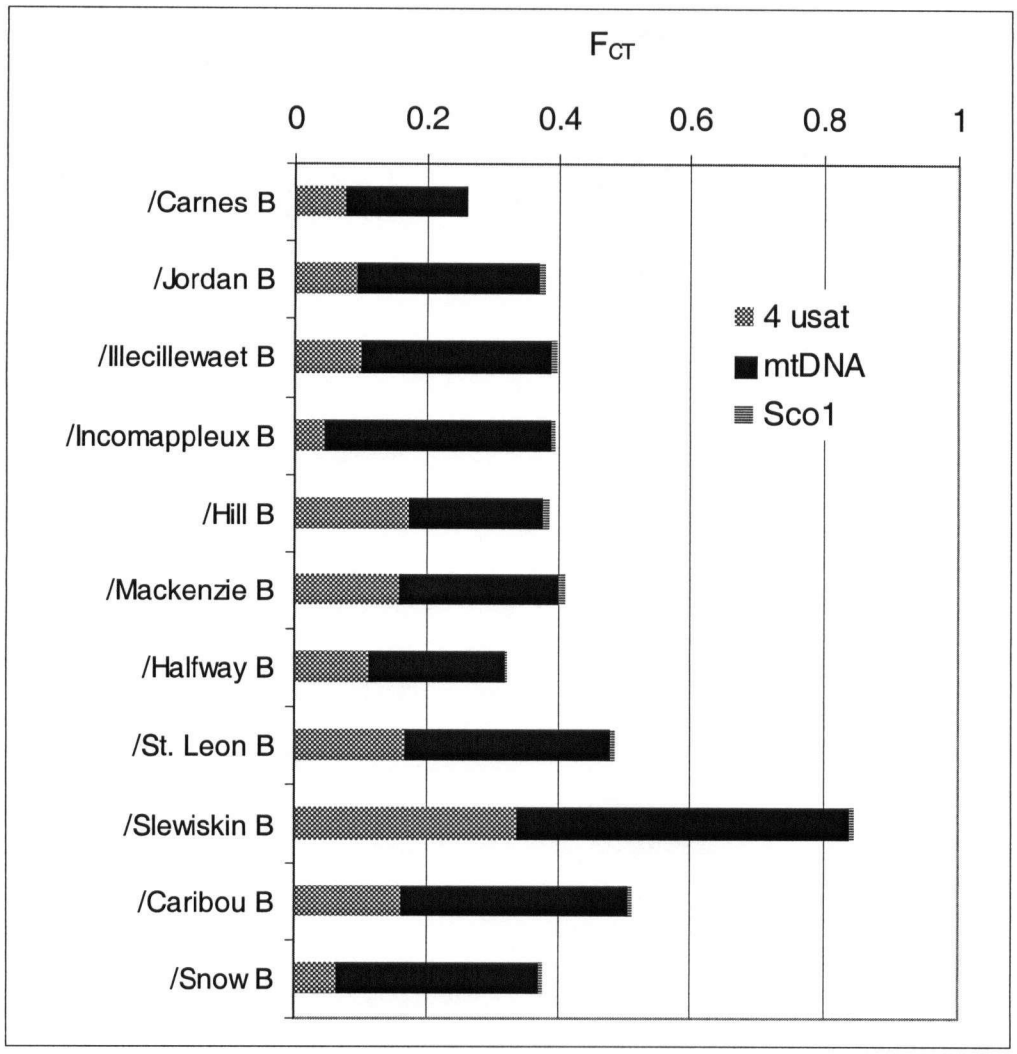

Figure 2.7. F_{CT} estimates from AMOVA for groups of northern and southern samples. The imposed division between north and south is indicated by "/". For example, /Carnes indicates that samples from Carnes and more southerly locations were combined in a group versus a group of samples collected from north of Carnes Creek. For proportional representation among loci, estimates for "4 usat" were multiplied by four, and estimates for different loci were stacked to facilitate comparisons among groupings.

Provincial relationships among phylogeographic groups

I pooled data to reflect and evaluate the validity of putative phylogeographic groupings, described above, via comparison with other watersheds in British Columbia. This comparison was limited to the shared diploid microsatellite loci (as in Figure 2.3) and made use of frequencies of alleles rather than simply their presence or absence.

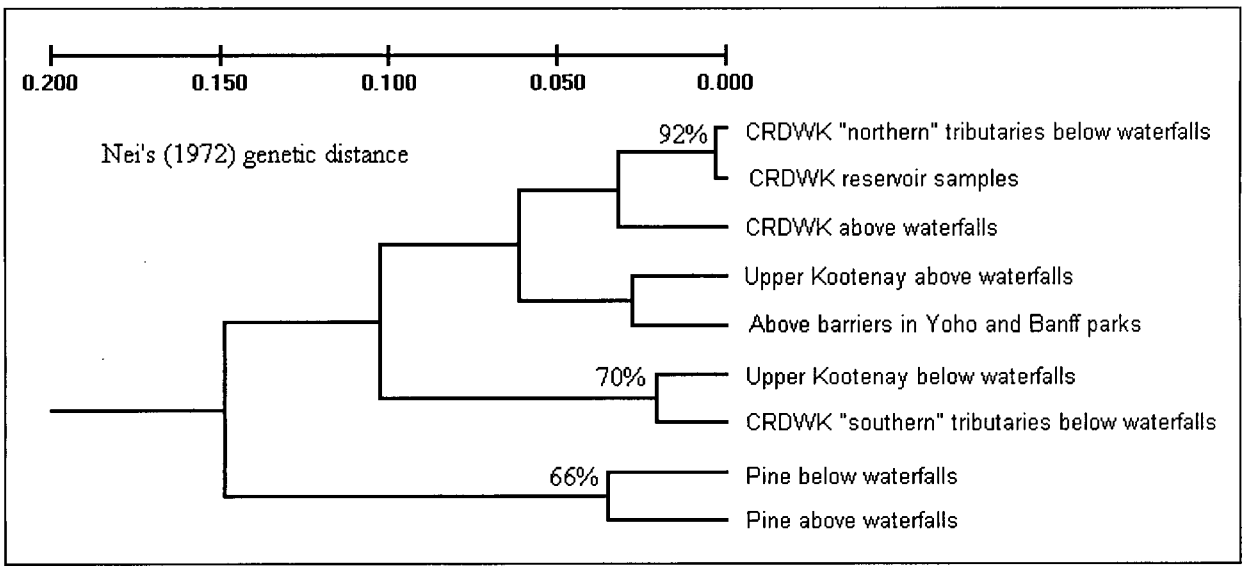

Figure 2.8. UPGMA dendrogram of groups of samples from three regions of British Columbia. Data are from this study (CRDWK) and Costello and Taylor (in preparation; East Kootenay and Pine). Within each region, populations above and below barriers were grouped separately, and CRDWK populations below barriers were further divided into "northern" (from tributaries north of Slewiskin Creek) and "southern" (Slewiskin, Caribou, Snow, and Taite creeks) groups. Bootstrap support greater than 50% and Nei's (1972) standard distance are shown.

Isolated populations and the north-south phylogeographic pattern

Genotypes in the Whatshan A sample and above barriers in Downie, Payne, St. Leon, and Kuskanax creeks had stronger northern than southern affinities, as estimated with RDIA (Figure 2.9). In accordance with its proximity to southern tributary samples, the bull trout population in Woden Creek was much more southerly in RDIA. Samples from above potentially breached barriers on Halfway and Jordan rivers also had relatively strong southern affinities, despite their geographic locations (see Figure 2.9). The northerly genotypes of other populations isolated above waterfalls support a possible phylogeographic relationship with populations above. waterfalls in the Rocky Mountain Trench (see Figure 2.8). Of isolated populations paired with adfluvial samples in the same tributary, however, only the resident population above St. Leon Creek's waterfall was convincingly more northern than the population downstream of it (see Figure 2.9).

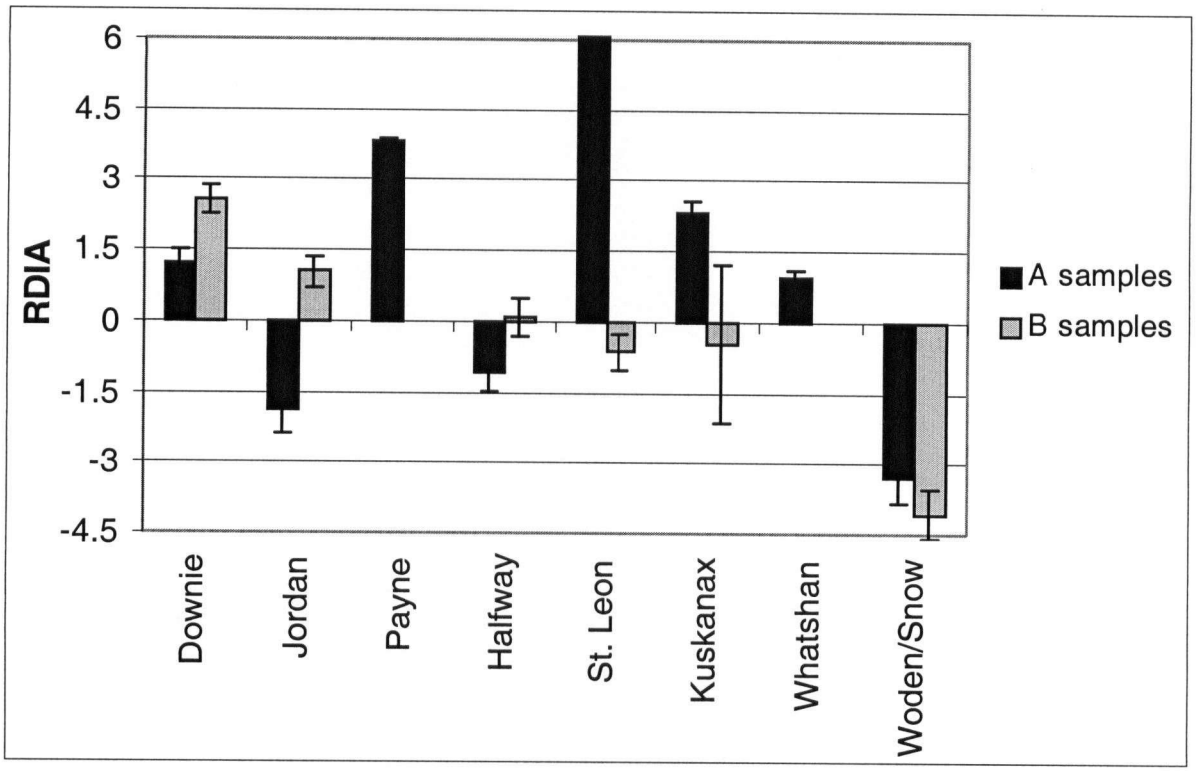

Figure 2.9. Mean RDIA estimates (calculated using data set 2) for populations upstream (A samples) and downstream (B samples) of barriers. Positive values indicate 'northern' genotypes. Bars represent standard errors. No bull trout were found downstream of either Whatshan A or Payne A.

Differentiation within population isolates

Within the populations above barriers with enough molecular variation to facilitate analysis, genetic subdivision was generally indicated. Of the four spatially subsampled A populations, more than one mitochondrial haplotype was observed among sites within only Halfway A, which was also spatially subdivided at other loci. Sites were less obviously distinct within Kuskanax A and Woden A (Table 2.5; no allelic variation was found within St. Leon A). From AMOVA results, the four diploid microsatellite loci suggested that genetic variation was significantly partitioned among sites within these streams. Thus, each population isolated above waterfalls may be subdivided into differentiated population segments that represent different historical or phylogeographic groups. There was little indication that upstream samples within these populations were genotypically more "northern" than downstream samples (see Figure 2.10), and ANCOVA detected significant differences among streams only (intercepts, $P < 0.0001$), with no significant relationship within streams (slopes, $P = 0.29$, one-tailed). That is, differentiation observed within isolated populations was not predictably distributed along the phylogeographic north-south axis described by other populations in CRDWK.

Table 2.5. Genetic differentiation among sample sites within populations resident above waterfalls. Individually, F_{ST} estimates differentiation (* $P < 0.05$, ** $P < 0.005$) within populations. F_{CT} estimates differentiation among the populations, as calculated by AMOVA, and F_{SC} estimates differentiation within them.

Comparison		mtDNA	4 usat	*Sco*1
Individually	Halfway A	0.09 *	0.12 **	0.02 *
	Kuskanax A	NA	0.09 *	0
	Woden A	NA	0	0
AMOVA	F_{CT}	NA	0.04	0.06 **
	F_{SC}	NA	0.08 *	0.01

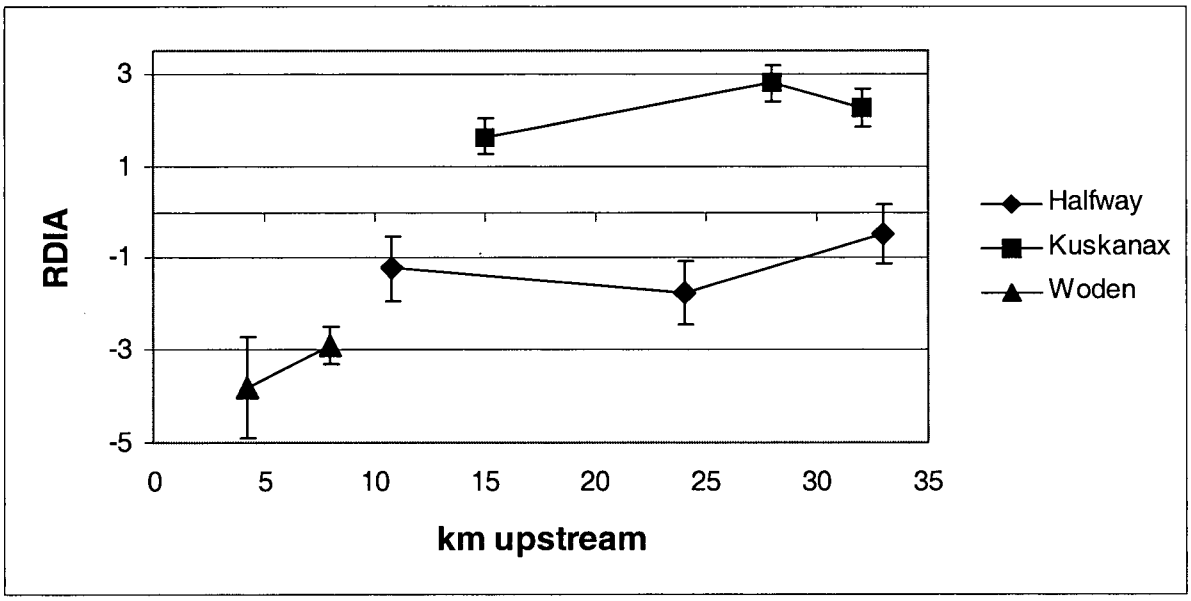

Figure 2.10. RDIA as a function of a sample's distance from the reservoir. Results using the "add-one-in" option of DOH are shown. Positive RDIA values indicate 'northern' genotypes. Bars represent standard errors.

Discussion

Independence of populations above and below waterfalls within CRDWK

Generally, the morphology and reproductive status of bull trout rearing above putative barriers to upstream migration suggested a stream resident life history (see Table 2.2). Bull trout too large to live permanently in Mackenzie Creek were sampled above the putative barrier there and must have migrated from Arrow Reservoir. Subsequently, I considered bull trout from Mackenzie Creek to be adfluvial samples, but I presumed that bull trout samples above other putative barriers represent stream resident populations isolated by one-way barriers to migration. Genetic analyses generally supported those presumptions. Heterozygote deficiencies (violations of Hardy-Weinberg equilibrium that may indicate Wahlund effects) were not apparent in all streams interrupted by waterfalls, but these tests generally have low power (Jin and Chakraborty 1995). Strong differentiation of samples above and below putative barriers (see Table 2.3) offered stronger support for the isolation of A populations. Although some genetic differentiation was detected among samples within isolated populations (see Table 2.5), estimates of F_{ST} were much greater across the barriers.

As demonstrated by AMOVA analyses (see Table 2.4), populations above waterfalls within CRDWK did not form a cohesive group when compared to migratory populations, nor did they group with their downstream counterparts (see also Appendix B). That is, much of the genetic variation detected above different waterfalls was unique. Mitochondrial haplotypes 4 and 19 were sampled from above barriers at Whatshan A and Jordan A, respectively, and were not observed in samples from adfluvial bull trout in CRDWK (see Figure 2.2). Also, some allelic variants at microsatellite loci were found almost exclusively above waterfalls (see Appendix A). Recent anthropogenic colonization cannot be ruled out for all resident bull trout populations and is in fact supported by some analyses (see below). In contrast, the presence of genetic variation that is rare or absent in – and not representative of – nearby populations argues against recent anthropogenic colonization. The nestedness of fish species in these habitats, with bull trout and especially sculpins more frequent than *Oncorhynchus* species, further attests that angling enthusiasts were not responsible for the presence of bull trout above barriers.

Barriers that prevented upstream migration from adfluvial populations did not act to prevent movement of fish in the downstream direction. Cross-assignment within A/B sample-pairs suggests downstream movement (see Table 2.3). Because I was being conservative (erring on the side of removing too many individuals) in the creation of data set 2, some fish were probably mistakenly identified as fallers by the assignment process. Only the relative assignments for the A/B pair were considered, and some "faller" genotypes had assignment probabilities to other B samples that were higher than to the relevant A population. That is, some "fallers" may have been strays (or progeny of strays) from other streams. The probability of generation of some genotypes by *any* sampled adfluvial populations is exceedingly low, however, and a high proportion of them could only be explained by immigration from above waterfalls. For example, the only genotype observed above the St. Leon waterfall is predicted (by allelic frequencies below falls) to occur in migratory populations at a frequency of four in 100 million. Yet, in this study seven such individuals were detected in a sample of 39 and all were found in St. Leon Creek below the waterfall. This indicates a high immigration rate, yet St. Leon A and B were highly differentiated.

How are strong and significant differences between A/B population pairs (Table 2.3) maintained in the face of such high migration rates? The St. Leon Creek population pair is the best one with which to answer this question. The low diversity and rare alleles of the isolated population make fish that fall from above the St. Leon waterfall and their progeny easy to detect (see Figure 2.1). Also, the population above the falls is very dense, and both the habitat and bull trout population below the falls are small, so the ratio of "fallers" in the sample below the falls should be high. (That a higher proportion of fallers was estimated for other A/B pairs suggests that falling was overestimated for them. Increased genetic variance observed in data set 2 among some populations also suggests this; see Appendix C.) Populations at Payne A and Whatshan A also possessed limited and rare genetic variation, but habitat downstream of barriers there yielded no bull trout samples.

Assuming that the frequency of fallers in the sample below St. Leon Creek's waterfall reflects genotypic frequencies in the spawning population, F_1 hybrid genotypes in the next generation would make up 29.5% of the next generation, with random mating and no natural selection. I detected only one putative F_1 hybrid genotype, or 2.6% of the sample, which is a highly significant deficit (Fisher's exact test, $P < 0.001$). Thus, within St. Leon Creek, interbreeding between adfluvial bull trout and bull trout who fall over the barrier appears to be severely

limited. Alternatively, interbreeding could be common, but survival of the offspring may be very low, so that few F_1 genotypes would be observed. Neither the genotype of the St. Leon A population nor those of F_1 hybrids were ever detected elsewhere. Allele 174 at *Sco*19, which appears fixed in St. Leon A, was rarely detected in nearby samples (see Appendix A). Thus few, if any, fallen bull trout appear to leave St. Leon Creek and have success elsewhere.

Because mtDNA is maternally inherited and only Haplotype 13 was detected in St. Leon A, it was possible to identify the female parent of the lone 'hybrid' individual as an adfluvial fish because the hybrid had Haplotype 1. Mating of life history forms may be positively assortative, and perhaps the 'hybrid' individual was the result of sneaking behaviour on the part of a fallen, precocious male. Sneaking by small, precocious males is common in salmonid species (e.g., McGowan and Davidson 1992, Maekawa *et al.* 1994, Koseki and Maekawa 2000) and other fish (e.g., Chan and Ribbink 1990, Uglem *et al.* 2000, Munehara and Takenaka 2000, Jones *et al.* 2001) and is a life history strategy that can yield high fitness (e.g., more than half of all offspring in some Atlantic salmon populations may be sired by reproductive parr; Taggart *et al.* 2001). Little genetic distinction is generally found between cohabiting resident and migratory life history forms of brown trout, in contrast to the strong distinctions found between sympatric populations with these life histories and resident populations isolated upstream of them by waterfalls (Cross *et al.* 1992, Pettersson *et al.* 2001). Although sneaking facilitates gene flow among migratory and resident life history forms of bull trout (Baxter 1997) and between resident Dolly Varden and adfluvial bull trout (Redenbach 2000), evolutionary differences between migrants (fallers) from populations resident above waterfalls and indigenous resident individuals within largely migratory populations may explain limitations to gene flow observed in my study. Furthermore, I can not conclude that sneaking is responsible for even the lone hybrid individual in St Leon Creek. Adfluvial females may have 100 times more eggs than resident females (J.J. Ladell, UBC Dept. of Zoology, personal communication), whereas sperm may not be limiting (D.J. Hoysak, UBC Dept. of Zoology, personal communication), so adfluvial mtDNA would be much more common among hybrids even if hybridization was symmetric between males and females.

Whatever the mechanism, whereas there is evidence for very restricted male-mediated downstream gene flow from A to B populations, there is no evidence that females assume this role. If female fallers did commonly and successfully interbreed with adfluvial populations, some mtDNA haplotypes would not occur only above waterfalls, as appears to be the case with Whatshan Lake and Jordan River populations. Reassuringly, supportive data were collected in other bull trout studies – some mtDNA haplotypes were frequent in bull trout above waterfalls in the Duncan River drainage, but were not detected in the migratory population below (O'Brien 1999). I could not determine whether behaviour, other evolutionary explanations, or demographics of falling accounted for the inability of bull trout from above waterfalls in CRDWK to contribute to the effective population size and genetic variance of migratory populations. Some studies of other species have suggested that populations resident above falls contribute little to the productivity of migratory populations because falling is rare (Michael 1983, Parkinson 1984, Skaala and Naevdal 1989, Pettersson *et al.* 2001) as a result of strong selection against downstream migratory behaviour (Northcote 1981, Jonsson 1982). My results suggest that even when fallers make up a substantial proportion of fish below a waterfall, their contributions to migratory populations are minimal.

As predicted in Table 2.2, waterfalls have utility in predicting one-way barriers to migration. In fact, because gene flow is limited in both the upstream and downstream direction, waterfalls

predict a robust two-way evolutionary independence of bull trout populations above and below them. In phylogeographic analyses, populations above waterfalls were therefore used as temporal guideposts to historical population structure.

Phylogeographic comparisons among watersheds

Alleles and haplotypes detected within CRDWK were mostly indistinguishable from those sampled elsewhere in British Columbia. Of samples in British Columbia, only one private allele was found in CRDWK, and the closeness in size and geographic distribution of private alleles in the Pine system (*Sco*19: 183, 185, 187) suggests that they arose *in situ*. That is, genetic variation at diploid microsatellite loci in the Pine and CRDWK samples was generally nested within the diversity detected in upper Kootenay River samples (see Figure 2.3), thus suggesting colonization of each region from a common refuge or from relatively undifferentiated or amalgamated refugia. Differential allelic diversity within major watersheds did not appear to be an artefact of sampling. Samples from the three major watersheds showed similar levels of differentiation, given comparable life history attributes (see Table 2.6).

Table 2.6. Differentiation (F_{ST}) within three different watersheds in B.C. Estimates are from samples above putative barriers (A), samples from adfluvial populations (B), and both sample types combined. All estimates are significantly greater than zero. Values are for data set 1.

Site	A samples	B samples	A and B
Kootenay	0.62	0.15	0.26
Pine	0.38	0.12	0.23
CRDWK	0.57	0.11	0.24

On the basis of mtDNA analyses across the range of bull trout, Taylor *et al.* (1999) proposed a single source for the colonization of British Columbia's interior, and they suggested that this colonization occurred from the south. Data on diversity at microsatellite loci support their assertions. As the greatest number of alleles was detected in the upper Kootenay River system and the fewest in the Pine system, the pattern of allelic diversity suggests that the source for colonization was relatively close to southern British Columbia and relatively far from the Pine system. As the Pine River is part of the Peace River drainage, founding involved more distance and at least two drainage divides (from a southern refuge in the Columbia into the Fraser and finally into the Peace), and it was not surprising to find less diversity there.

Why less diversity was detected within CRDWK than in the upper Kootenay drainage is not so obvious. The upper Kootenay drainage is a greater distance from Columbia River's mainstem (south of the historical ice sheet) than is CRDWK, and that distance includes an historic barrier falls (Bonnington Falls) near Kootenay River's confluence with the Columbia River. Extant geomorphology includes no such barrier between CRDWK and a potential refuge in the Columbia mainstem (south of the ice sheet). The result may be attributable to sequential founder effects (inferred for postglacial colonization of northern habitats in many species; see Hewitt 2000 for a review) that promoted loss of molecular variation as bull trout colonized British Columbia. Because greater genetic variation in the upper Kootenay area suggests that colonization of the upper Kootenay watershed involved fewer or weaker founder effects than did colonization of CRDWK, an important and relatively diverse refugial population may have existed east of the Columbia mainstem. This agrees with relatively high diversity detected in

other studies at mtDNA (Williams *et al.* 1997a) and allozyme loci (Leary *et al.* 1993) in samples from the Clark Fork River drainage in Idaho.

Taylor *et al.* (1999) found that no mtDNA variation was partitioned among watersheds in British Columbia's interior (F_{CT} not significantly different from zero). Also demonstrating that postglacial recolonization by bull trout was largely unrestricted by present-day watershed boundaries, my study suggested small founder effects among major watersheds in British Columbia, and I found closer relationships of some samples among watersheds rather than within them. I propose that the results of my study reflect patterns of deglaciation and colonization in British Columbia's Kootenay region as follows. Populations in large refugial regions, such as a Columbia refuge, were probably subdivided in the same manner that large populations are subdivided today (Angers and Bernatchez 1998). One group had access to, and subsequently colonized habitats above barriers in the upper Kootenay drainage and across Canal Flats into northern CRDWK via the upper Columbia. A second source group, not strongly differentiated from the first, may have followed a more westerly route into British Columbia and colonized southern CRDWK and only B (below barrier) habitats to the east (refer to Figure 1.1). This hypothesis is consistent with the amount of allelic diversity found in British Columbia watersheds. It is also consistent with other genetic analyses and geological evidence. (Figure 1.1 shows many of the locations discussed in the next three sections.)

Support for an east-north corridor

Populations above waterfalls and adfluvial populations in the north of CRDWK were grouped together by UPGMA (though with low bootstrap support) with populations above waterfalls in more eastern drainages (see Figure 2.8). That is, northern populations in my study area and populations above waterfalls in both CRDWK and in the East Kootenays (in the upper Kootenay River and in the upper Columbia and Red Deer rivers) appear to have a shared heritage. Tributary valleys in the Kootenay region's southern Rocky Mountain Trench were free of ice before the trunk glacier in the Trench valley wasted away (Clague 1980, Ryder 1981, Fulton and Archard 1985), and tributary meltwaters were therefore dammed and formed lakes in these tributaries. Overflow ran beside the trunk glacier, elevated at the margins of the valley (Ryder 1981). These lakes and meltwater channels provided early access to habitats presently above barriers in the East Kootenays, and across Canal Flats into the Columbia River. A random sampling effect from a nearby source for this colonization would occur, and via sequential founder effects among available habitats, loss of allelic variation would be observed during northward colonization and colonization into northern CRDWK. Interestingly, a greater number of mtDNA haplotypes (four) were detected at a single sample site (Sullivan Arm) in Kinbasket Reservoir than were detected in all other lacustrine and adfluvial samples from CRDWK combined (see Appendix A). Compared to CRDWK, Sullivan Arm is relatively close to Canal Flats.

Colonization above barriers in upper Kootenay tributaries would have occurred relatively early in postglacial history, as would colonization across Canal Flats into the upper Columbia. The southern Rocky Mountain Trench and southern Rocky Mountains of Alberta reveal older postglacial sediments than does south-central British Columbia (Clague 1975). Carbon dates (reviewed for British Columbia by Clague 1980) from tributary valleys in the East Kootenays (approximately 12 000 years before present) are older than those from sites in northern CRDWK (range: 9 990 – 9 490 years before present) and also older than reliable carbon dates from southern CRDWK (range: 11 000 – 10 100 years before present). Therefore, although deglaciation of CRDWK likely proceeded in a manner similar to that in the Rocky Mountain

Trench (Fulton, R. J., Geological Survey of Canada, unpulblished data), eastern access to the upper Columbia was possible from the upper Kootenay drainage prior to southern access from Columbia River's mainstem.

Support for a west-south relationship
Founding populations isolated from migratory upper Kootenay populations in habitats above waterfalls and north of Canal Flats were likely more similar to one another than to the nearby migratory populations of today, which have remained accessible to incoming alleles for the last 10 000 - 12 000 years. On the basis of diploid microsatellite data, migratory populations in the upper Kootenay drainage were grouped together not with populations above waterfalls in the same drainage, but with populations in the south of CRDWK (see Figure 2.8). Further, the dominant restriction fragment pattern of mtDNA in southern CRDWK populations was Haplotype 1, which was the dominant haplotype in samples from Wigwam River (Taylor *et al.* 1999), an upper Kootenay site accessible to migratory bull trout. Also, the dominant mtDNA haplotype in migratory populations in the Duncan River system had restriction patterns consistent with Haplotype 1 (D.S. O'Brien, UBC Dept. of Zoology, personal communication).

The Duncan River is upstream of Bonnington Falls from CRDWK, but it was open to bi-directional gene flow with migratory upper Kootenay populations prior to recent hydroelectric developments. As in CRDWK (see below), populations above barriers accounted for a large component of the total mtDNA diversity detected in the Duncan drainage (O'Brien 1999), including haplotypes consistent with Taylor *et al.*'s (1999) haplotypes 13 and 19. These haplotypes commonly occurred above waterfalls in CRDWK and above barriers elsewhere in southeastern British Columbia, whereas Haplotype 1 was more common below waterfalls. For example, only Haplotype 13 was found above a barrier on Salmo River (Taylor *et al.* 1999), and only Haplotypes 13 and 19 were found above a barrier on Hoder Creek (SJL, unpublished data), in the Slocan drainage. This is more circumstantial evidence that a second wave of colonization occurred at a time when access to above-barrier habitats was no longer available. This second wave carried mostly Haplotype 1, made contributions to migratory populations in CRDWK, upper Kootenay, and Duncan drainages, and came from a source not deeply diverged from that which fuelled the first colonization. In support of this second source being further west than the first, Haplotype 1 dominates British Columbia's interior (Taylor *et al.* 1999), to which migratory corridors through the Okanagan valley (Fulton 1969) were used by numerous fish species (McPhail and Lindsey 1986).

Comparative phylogeography of populations above waterfalls in CRDWK
The distinct postglacial histories of northern and southern CRDWK, described above, are supported by strong differentiation between migratory populations to the north and south of Slewiskin Creek. Concordance of geological characteristics (e.g., paleozoic versus granitic rock in the north and south, respectively; Reesor and Moore 1971) and commonness of bull trout populations above falls in the north of CRDWK (relative to their paucity in the south) are also supportive of my hypothesis. Although populations above waterfalls in CRDWK clustered as a group with migratory northern CRDWK populations and with populations above falls in the East Kootenays, they displayed little cohesiveness (see Table 2.4). Woden Creek is south of Slewiskin Creek and is the only such habitat above waterfalls in CRDWK known to harbour a bull trout population. (Whatshan River drains into Lower Arrow Reservoir, but the tributary sampled, Fife Creek, is north of Slewiskin Creek.) Woden A had the lowest RDIA score of any A sample (see Figure 2.9), in accordance with its southern geography. This indicated that the source for colonization of habitat above the Woden Creek waterfall was different from that

which colonized the northern populations. Among the more northern samples, no relationship was obvious between genotypic 'northerness' (RDIA) and geographic position in CRDWK.

Interestingly, the different history of Woden A was not reflected by allelic diversity, allelic identity, or genetic differentiation from the lacustrine sample (see Figures 2.4 and 2.5). Instead, Payne A and St. Leon A were remarkable in each of these traits: the few alleles they contained were rare, and they were strongly differentiated from the lacustrine samples. Payne A and St. Leon A also had the most northerly RDIA scores. That more extreme founder effects (and thus less allelic diversity) should correlate with greater divergence from the lacustrine sample is not surprising. Genetic drift at founding, however, should promote random loss of alleles in independent populations. The absence of common alleles at a given locus and apparent fixation of the same rare one in Payne A and St. Leon A (i.e., anti-nestedness, see Figure 2.5) therefore requires explanation. The similarity of Payne A and St. Leon A to one another cannot be explained by dependence resulting from stream capture – that is, one population could not have directly founded the other – because they are spatially separated from one another by Halfway A. Also, populations that I studied above waterfalls are unlikely to have founded one another because they are so strongly differentiated from one another. (Despite their similarities, apparently fixed differences exist between Payne A and St. Leon A at *Sco*1 and *Sco*19.) Thus the anti-nestedness of Payne A and St. Leon A suggests founding from a source strongly differentiated from that which provided founders for other populations above falls. Populations above waterfalls on St. Leon and Payne creeks were founded from a source that had a higher frequency of allele 156 at *Sfo*18, and probably a lower frequency of allele 201 at *Sco*19, than is generally found in CRDWK (see Appendix A).

Thus, my data suggest that founders of populations above waterfalls in CRDWK were drawn from a minimum of three source populations. Whereas Woden Creek's population reflects a spatial distinction in source population, the cohesion of St. Leon A and Payne A indicates a non-spatial mechanism for differentiation from Halfway A and other populations above waterfalls in northern CRDWK. Confined drainages and differentiation among populations above falls argue against evulsion and stream capture, respectively, suggesting that populations above waterfalls resulted from proglacial flooding. Though flooding implies access from a single source, accessibility of various habitats above waterfalls in northern CRDWK was probably unequal. Such access may have occurred at different times or for different durations. Because the shape of a trunk glacier (if present), degree of melt in tributary valleys, ancient habitat quality, and warping of the continent's surface would all contribute to accessibility, easily measurable physical characteristics such as elevation would not reliably indicate temporal accessibility of habitats above waterfalls. If access to habitat above barriers on some creeks ended earlier than did access to other habitats, however, we may expect to find less allelic diversity, and the alleles found may better reflect the constitution of an earlier source population. My analyses support this temporal mechanism for differentiation among populations, as St. Leon A and Payne A had both the lowest diversity and the highest RDIA scores. Costello *et al.* (unpublished data) recently analysed seven microsatellite loci in bull trout from the Beaver River in Glacier National Park, a tributary to Kinbasket Reservoir (see Figure 1.1). This population is in the proposed east-north corridor upstream of CRDWK and may better represent early-founding populations for northern CRDWK. At some genetic loci discussed in this thesis and at other genetic loci, alleles relatively common in Glacier National Park were rare in CRDWK (in limited sampling) except in St. Leon A and Payne A, where they were the only alleles detected (Costello *et al.*, unpublished data), further supporting early isolation of those populations.

Bull trout in Whatshan A also displayed genetic variation that was not strongly nested within the genetic variation of populations that contain more allelic diversity (see Figure 2.5 and Appendix A). Like St. Leon A, Whatshan A bull trout displayed a high frequency of allele 174 at *Sco*19, an allele that is otherwise very rare in CRDWK. But Whatshan A was quite unlike St. Leon A at other loci (e.g., *Sfo*18 and mtDNA). Depending on the presence of a trunk glacier, the source population for Whatshan Lake may have been spatially distinct and may have been shared by other early-founded populations above waterfalls in northwest CRDWK. Above falls, only the two populations in east-flowing streams in CRDWK contained mtDNA haplotypes detected nowhere else in CRDWK, and more populations should be surveyed. Regardless of the mechanism, Whatshan A was strongly differentiated from both highly diverse and genetically depauperate populations, which shows that the above suggestion of three colonizing sources probably underestimates differentiation among source populations. Thus, independent evolution of the resident life history might have occurred several times within the study area.

Genetic diversity versus species diversity

Other species also evolved resident life histories above waterfalls in CRDWK. Two salmonids, westslope cutthroat trout, *Oncorhynchus clarki lewisi*, and rainbow trout, *O. mykiss*, and one cottid, *Cottus cognatus*, were found in sympatry above waterfalls with the resident bull trout populations that I studied. Species diversity above waterfalls was nested and indicated that habitats accessible by – and able to sustain – populations of *Oncorhynchus* species were also accessible and colonizable by *C. cognatus* and bull trout. As opportunities for colonization by *Oncorhynchus* species increased, so did opportunities for larger numbers of bull trout, perhaps from a greater diversity of source populations. This is supported by the high allelic diversity of resident bull trout populations that are sympatric with *Oncorhynchus* species. (A similar relationship exists between stream order, number of fish species, and genetic diversity in Trinidadian guppies; Shaw *et al.* 1991). When sculpins and *Oncorhynchus* species were present above waterfalls, they tended to be caught most frequently in reaches just upstream of the falls and less frequently further upstream. In contrast, apparent catchability (and presumably population density) of bull trout was lower in habitat shared with other species. As allopatric A populations tend to be genetically depauperate despite their population densities, allelic diversity probably reflects mechanisms of founding, as described above, rather than population sizes since isolation.

Dolly Varden, *Salvelinus malma*, a close relative of bull trout, is a relatively early colonizer of newly postglacial habitats compared to several *Oncorhynchus* species (Milner and Bailey 1989, Milner *et al.* 2000). They can feed more effectively at low light intensities than can *O. clarki* (Schutz and Northcote 1972), and this ability may confer an advantage on them in colonizing streams that run thick with suspended glacial sediment. Their numbers in new streams do not seem to be affected by habitat complexity (Milner *et al.* 2000). Arctic char (*Salvelinus alpinus*) is a cold-adapted fish that is also a capable postglacial disperser (Balon 1984). Bull trout may share these advantages with their congeners, in addition to their documented physiological and competitive advantages over other species in cold water (McPhail and Murray 1979, Haas 2001). Therefore, I expect that habitats colonized by *Oncorhynchus* species were open to colonization later in postglacial history (until conditions became less glacial) than were populations where bull trout were found alone. If allopatric bull trout populations were founded and isolated a longer time ago, it is not surprising that I found lower levels of differentiation between resident bull trout populations sympatric with *Oncorhynchus* species and the present-day adfluvial population.

Westslope cutthroat trout were found above a barrier in the Jordan River and exist above several waterfalls in northern CRDWK but not, in my experience, in sympatry with rainbow trout. Behnke (1992) suggested that westslope cutthroat trout colonized postglacially from a refuge they shared with bull trout that was centered in the Clark Fork drainage. "Redband" rainbow trout, he wrote, expanded from a different refuge to the south and west, and were unable to colonize above barriers in the Kootenay, Clark Fork, or Spokane drainages. His account of postglacial colonization by westslope cutthroat and rainbow trout parallels my suggestion that CRDWK was colonized by two groups of bull trout. If postglacial dispersal of these species occurred along the same routes used by bull trout, habitats accessible to cutthroat trout as they colonized northern CRDWK from the upper Kootenay River (as I proposed for bull trout) and accessible to "redband" trout as they colonized CRDWK from their southwestern refuge via Columbia River's mainstem should have also been accessible to bull trout that colonized along those routes, respectively. That is, the difference in colonization routes of *O. c. lewisi* and "redband" trout should be indicated by more northern RDIA scores of bull trout in Jordan A versus those of Halfway A and Kuskanax A. "Redband" trout tend to eliminate *O. c. lewisi* where the two come into contact (Behnke 1992), however, and the distribution of the two species in CRDWK may also be explained by late colonization of *O. c. lewisi* up Columbia River's mainstem, followed by even later colonization by "redband" trout. Thus, Jordan A may have been accessible to southern bull trout and therefore have lower RDIA scores than would be expected under the former scenario. Persistence of *O. c. lewisi* above more northerly migration barriers may result, then, from failure of *O. mykiss* to reach those barriers before they became impassable. The similarity of alleles present within Jordan A, Halfway A, and Kuskanax A support the latter explanation.

Because of biological barriers to gene flow or undetected geomorphic barriers to migration, populations above waterfalls were genetically subdivided among sample sites. This subdivision within populations above waterfalls left open the possibility that sampling at a finer scale was required to detect congruence between bull trout genetic variation and distributions of other species above waterfalls. It could also help explain why populations above waterfalls in northern CRDWK did not have convincingly more northern RDIA scores than their downstream counterparts (see Figure 2.9). In Halfway River, rainbow trout seemed to dominate the first two sample sites above the waterfall, whereas only bull trout were caught at the uppermost site. Above the waterfall on Kuskanax Creek, rainbow trout dominated the lowermost site, only bull trout were captured at the most upstream site, and the middle site was intermediate. Were habitats above waterfalls colonized first by bull trout from an early source, and then downstream parts of these habitats colonized again, from a more southern source, when rainbow trout gained access? Although RDIA scores generally appeared low in downstream sites that were dominated by rainbow trout (see Figure 2.20), the downstream site also seemed more southerly in a stream with bull trout only (Woden Creek). Further, increases in RDIA with distance above falls were not statistically significant ($P = 0.29$). Finally, any resolution of a relationship between species distributions and genetic distributions with these data is confounded by recent human activities. Management initiatives on Halfway and Jordan rivers may have influenced genetic constitutions of sites above the waterfalls. As logging proceeds to steeper terrain, roads are providing increased access to resident populations in east-flowing streams in northern CRDWK, and those populations should be studied before opportunities increase for anthropogenic gene flow. Anthropogenic alterations confound interpretations and also have implications for conservation.

Conservation of bull trout populations above waterfalls

Impoundment by Hugh Keenleyside and Revelstoke dams reduced spawning and rearing habitat that supported Arrow Lakes' sport fishery for bull trout, and habitat above waterfalls was appropriated in compensation. The waterfall on Halfway River was dynamited to make upstream habitat available to adfluvial bull trout, and hatchery-reared bull trout were released above the Jordan River waterfall to make use of habitat there. Jordan A and Halfway A are the only two bull trout populations above falls known to be associated with such potentially influential anthropogenic activities on their genetic diversity. Jordan A and Halfway A had low RDIA scores, they were relatively undifferentiated from the B samples immediately downstream of them and from lacustrine samples, and they had high allelic diversity. These attributes were shared with Kuskanax A and were predicted by the presence of *Oncorhynchus* species. Jordan A and Halfway A had substantially lower RDIA scores than did Kuskanax A, however, indicating that management initiatives may have resulted in upstream gene flow from populations in southern CRDWK. This possibility is particularly compelling for Jordan A because access may have ended prior to colonization by rainbow trout (so RDIA should actually be higher for Jordan A) and because males from southern CRDWK streams were often used to fertilize eggs at Hill Creek Hatchery. Cross-assignment of individuals from both Jordan A (five fish) and especially Halfway A (12 fish) to the B populations below and assignment ratios relative to those in other A/B pairs (see Figure 2.1) also support recent influxes of genotypes from migratory populations.

If the symptoms described above faithfully indicate anthropogenic influences on genetic variation, adfluvial bull trout may readily invade and recruit into populations above barriers, if given the opportunity. The anti-nestedness of allelic diversity among populations above waterfalls also hints at this possibility. If habitat was accessible above other waterfalls when Payne A and St. Leon A were founded, then why were the alleles that were 'fixed' in Payne A and St. Leon A not detected in more diverse populations? Whether the original founding populations were competitively inferior or were incapable of withstanding other biotic (e.g., disease organisms, changed food source, interspecific competition) or abiotic (e.g., flooding) stresses associated with secondary access, populations resident above falls and their genetic diversity appear vulnerable to displacement or replacement by migratory individuals. Thus, costs in the form of lost genetic attributes of resident populations must be evaluated when considering the use of habitats above falls to support or enhance migratory populations.

These costs may be prohibitive. For instance, although individual populations above falls generally have reduced genetic variation compared to adfluvial populations, they comprise a considerable number of unique or rare genetic variants as a whole. These populations have been isolated and independently evolving for approximately 10 000 years, and they are genetically distinct from adfluvial populations and from each other. Some were founded from distinct source populations, different lineages in space-time which may have been evolving independently for significantly longer than the habitats above falls have existed, some of them perhaps descended from distinct subpopulations of the Pleistocene refuge. Clearly, populations above waterfalls are an important component of bull trout biodiversity in CRDWK. These populations (and populations above waterfalls outside of CRDWK) not only represent the evolutionary legacy of bull trout in British Columbia and elsewhere, but they are also valuable because they present diverse raw material for the species' future evolution and persistence. These populations would meet the viable salmonid population guidelines of the U.S. N.M.F.S. (McElhany *et al.* 2000) if not the criteria for designation as evolutionarily significant units.

It is obligatory in conservation biology to describe which conservation goals should be compromised most readily in the face of pressures for increased use and extraction of natural resources. My results suggest that such prioritization will be difficult in CRDWK. The island theory of biogeography predicts that diversity increases with size of islands (MacArthur and Wilson 1967), and the same mechanisms predict similar trends in biodiversity preserves (Brown 1986). When islands or reserves represent or were founded from a single source, the diversity of smaller islands or reserves can be only a subset of that occurring on larger reserves. Conservation efforts may then be focussed mainly on large reserves with minimal sacrifice of conservation goals. For example, Ranta *et al.* (1999) found that plant species diversity was nested among islands near Copenhagen, and therefore rare species only occurred on the largest, most speciose islands. Because of low *beta* diversity (i.e., nested species complements) among the islands, only a few of the largest islands needed to be protected to ensure representation of all species. Even species distributions that are significantly nested among fragmented habitats (using analyses that detect nestedness liberally; see Methods), however, can support a system of several small reserves (see Boecklen 1997 and references therein). Because bull trout populations above waterfalls in CRDWK represent more than a single colonizing source, high *beta* diversity and anti-nestedness of allelic variation was observed in this study (see Figure 2.5), and representation of that diversity requires that a relatively large set of populations are set aside. A related issue further complicates matters: allelic diversity within populations is not positively associated with the presence of rare genetic variation, and thus both diverse and depauperate populations must be admitted to the network of reserves. This conflict between conservation of variation within populations versus among them is reminiscent of the SLOSS (see Diamond *et al.* 1975, Lahti and Ranta 1985, Caughley 1994) debate.

SLOSS is the acronym for "Single Large or Several Small?", which is a question of how best to allocate resources in designing reserves. Most researchers now agree that it is better to put more eggs in fewer baskets as, on a per reserve basis, larger reserves more completely represent conservation values (more species, more ecosystem processes, fewer edge effects, and increased chances of long-term persistence) that outweigh the benefits of redundancy offered by multiple small reserves (Soule and Wilcox 1980, Brown 1986, Patterson and Atmar 1986). In terms of genetic variation, allelically diverse populations are analogous to large reserves, each probably more representative of its source population than populations founded by few individuals, and each perhaps with greater ability to adapt and persist in a changing environment. In the case of bull trout populations above falls in CRDWK, however, depauperate populations offer more than spatial redundancy. As suggested above, they represent different source populations and have been evolving independently for longer periods of time than have diverse populations. Also, allelic variation at microsatellite loci reflects historical effective population size rather than contemporary population size. Whereas variation at molecular markers should provide information on evolutionary potential at small population sizes (Vrijenhoek 1994), populations above waterfalls in CRDWK seem large. In such cases, variation at neutral marker loci is likely disconnected from potentially adaptive allelic variation (Lynch 1996) and may not reflect resilience of populations. For example, despite loss of genetic variation at allozyme loci via postglacial northward colonization, northern populations of pitcher plant mosquito displayed more variation in developmental rates and photoperiodic response than did southern populations (Armbruster *et al.* 1998). Because of these evolutionary considerations, this version of the problem of reserve design is not soluble without expanding both scientific and philosophical investigation beyond the scope of the general SLOSS debate.

Another practical problem in prioritizing populations for conservation is determination of which populations represent distinct sources. Strong differentiation (and low allelic redundancy) among populations above falls permits the argument that each represents a distinct source. I detected some cohesion, however, that argued for a southern source, an early northern source, and a late northern source. Further genetic study may reveal the origin of Whatshan A's unique collection of alleles or identify distinct sources for unstudied populations. Unfortunately, my data offer little hope that surrogates will permit adequate evaluation of biogeographic history without incorporating studies of molecular markers. Although the presence of other salmonid species may predict the amount of allelic diversity in an isolated population, it does not appear to predict the allelic identity contained therein. The presence of sculpins appears even less informative.

Other conservation implications

In addition to making prioritization of resident bull trout populations more difficult, incongruent inter- and intraspecific genetic distributions have another negative implication. As colonization mechanisms and abilities differ even among species that dispersed from shared refugia, different species above a given barrier likely represent contrasting colonization histories of source populations that differed in space-time. This influence of colonization may be a general phenomenon, as suggested by relatively congruent species and genetic diversities in vicariance-dominated biogeographic settings versus those dominated by vagility/dispersal (Bernatchez and Wilson 1998). This implies that, in CRDWK and other postglacially-colonized habitats, goals with respect to conservation of biodiversity within a species will often conflict with goals within other species and with goals for conservation at the species level.

Although populations of bull trout above falls may not yield information on biogeographical histories of the species with which they are sympatric, this study has demonstrated that they can provide biogeographic and biological information relevant to the conservation of *conspecific* populations that are *not* resident above barriers. For example, the temporal context provided by populations above waterfalls suggests that divergence between adfluvial bull trout spawning in northern and southern streams of CRDWK is ancient and is maintained by contemporary processes rather than being an artefact of them. And populations above waterfalls yield hints at why migratory populations tend to contain little genetic diversity within them (relative to other species).

The commonness of bull trout populations above waterfalls in northern CRDWK portends a propensity of bull trout to invade 'marginal' habitats. Despite severe inbreeding and loss of genetic variation during the founding of most of these populations (e.g., St. Leon A was invariant at all sampled loci, including a tetrasomic locus), these invasions resulted in successful colonization, and the populations have persisted. Thus, bull trout demonstrate resilience and some insensitivity to negative inbreeding effects (e.g., mutation meltdown; Lynch *et al.* 1995). Given the relatively high population densities of bull trout when they are alone above waterfalls (observed in this study, and quantified by J.J. Ladell, UBC Dept. of Zoology, unpublished data), it is possible that a greater proportion of all bull trout exist alone above barriers than exist in sympatry with other fish species in any other context. This may have been true throughout the Pleistocene glaciations. As habitats were created and erased, drainage connections made and broken, and bull trout populations founded and extirpated, all extant bull trout presumably come from strongly bottlenecked ancestries. Such a genetic history provides an evolutionary context promoting compensation via purging of deleterious recessive alleles, increased phenotypic plasticity, or reduced mutation rates. Other fish species, with metapopulation dynamics not so

immediately tied to opportunities afforded in glacial meltwaters, would maintain more allelic diversity, reflecting a legacy less influenced by large fluctuations in population size.

In their explanation of colonization order among salmonid fishes, Milner *et al.* (2000) suggest that Dolly Varden are early colonizers because they may have high straying rates from their natal streams and because they are little restricted by habitat conditions. Likewise, Arctic char are sometimes viewed as a dispersive, opportunistic, weedy, generalist species (*sensu* Balon 1984). Such notions are supported by the apparent ease of recruitment from migratory populations into resident ones above falls, and have important implications for management and conservation of char. It is judicious to examine these characteristics in greater detail, however, as they seem to be refuted by maintenance of genetic differentiation between stream populations in northern and southern CRDWK (see Figure 2.7) and by lack of downstream gene flow in A/B population pairs. Clearly, regulation of gene flow presents further challenges and opportunities for study. In the next chapter I examine gene flow among populations that are not separated by geomorphic barriers, and I investigate factors that limit and promote that gene flow.

Chapter 3: Contemporary and anthropogenic influences on adfluvial bull trout

Introduction

A brief history of salmon biology and management: an analogy

Within the range of most (if not all) sexually reproducing species, individuals do not mate randomly with one another in space and time, even when discreet geographic barriers do not divide the organism's distribution. Progeny from these non-random matings are distributed closer to their parents in space and time, in general, than would be expected if they were distributed randomly. Because environmental conditions are also positively autocorrelated in space and time, conditions necessary for adaptation to local environmental conditions are often met in nature. That is, organisms that live and reproduce relatively close to where (in space and time) their own parents lived and reproduced are likely to have traits that confer upon them a selective advantage over most relatively dispersive conspecific individuals with whom they compete. Thus, genetic variation in a species is structured not only by physical limitations to dispersal but also by selection against dispersive individuals that would otherwise promote high levels of gene flow (Ehrlich and Raven 1969). Local selection has been observed in bacteria, plants, and animals that have little control over the dispersal distance of their gametes or progeny (i.e., they rely on wind or currents for dispersal; e.g., Sork *et al.* 1993). Such selection has presumably led to reduced vagility in many species, such as stream-dwelling organisms with reduced planktonic life history stages (Hughes *et al.* 1996 and references therein). In some species individuals can utilize resources great distances from their natal sites but return to their natal sites to reproduce. Migratory birds, turtles, and whales are general examples (Papi and Wallraff 1992, Fitzsimmons *et al.* 1997, Baker *et al.* 1998), but salmon are most famous for this 'natal homing' migratory behaviour (Thorpe 1994).

Homing in salmon promotes local adaptation and local adaptation promotes homing, and this positive feedback has not gone unnoticed by evolutionary or conservation biologists (Northcote 1997). Salmonids display a high degree of local adaptation (reviewed by Taylor 1991), and populations that spawn in different river systems may be legally recognized as units for conservation because of their unique evolutionary traits and histories (Waples 1995). Molecular genetic techniques have detected population subdivision at very fine spatial (e.g., within streams, Spruell *et al.* 1999) and temporal scales (e.g., within spawning runs, Gharrett and Smoker 1993), and local selection can promote local adaptation at scales finer still than those described by neutral molecular techniques (Gharrett 1994). For example, whereas molecular genetic techniques may fail to detect strays among nearby populations (*Oncorhynchus nerka*), otolith analyses can independently demonstrate heritable spatial subdivision among salmon at adjacent spawning sites with different thermal regimes (e.g., Quinn *et al.* 1999). Heritable physiological and behavioural differences also exist between overlapping but temporally-differentiated salmon populations spawning at single spatial locations (Tallman 1986, Hendry *et al.* 1999).

That the productivity of locally adapted 'stocks' is difficult to replace has long been recognized by fisheries managers (Lichatowich *et al.* 1999) who therefore valued diverse, locally adapted populations (e.g., Ricker 1973). Nevertheless, evolutionary relationships between many of those populations and their environments were compromised in favor of hydroelectric development. Some compensatory measures were taken, as hatcheries were built to reduce requirements for natural habitat (Lichatowich *et al.* 1999). Also, for utilization of remaining habitat in affected rivers, salmon were transported between ocean and spawning grounds (via barges and fish ladders, for example; Blumm *et al.* 1998). Thus, remnants of locally adapted genetic variation still exist in the depleted populations of many stream systems. Small populations may be vulnerable to extinction because of stochastic, ecological, and genetic processes (Caughley 1994,

Frankham 1995a, Craighead *et al.* 1999). The perceived value of these populations to conservation has lead to discussion of dam removal on several rivers (e.g., see Blumm *et al.* 1998).

Measures taken to overcome degradation of spawning habitats and conserve small, vulnerable populations of salmon are hindered by exploitation during their feeding migrations. As a result of their homing behaviour, salmon utilizing a feeding area may comprise several reproductively isolated populations with relatively independent demographics. A given level of harvest at the feeding area (i.e., in a mixed-population fishery) will therefore have different effects on the various populations. Small populations may be more susceptible to stochastic effects related to estimates of sustainable yield, for example, and their declines would be relatively imperceptible in catch statistics (Ricker 1973). Perhaps more importantly, productivity and resilience of populations in some habitats will differ from the productivities of others at any given time, and more productive populations will better withstand a given fishing pressure than less productive ones. Populations successfully augmented by hatcheries, for example, may support an intense fishery that could drive less productive populations to extinction. For this reason, attempts to increase productivity of any but the least productive populations could have a net negative effect (Ricker 1973). Although mixed-population fisheries can alone promote extinction of some populations, the confluence of habitat degradation and inappropriate compensatory efforts increases the likelihood of degraded population structure and decayed genetic diversity.

Hatchery populations themselves have also maintained some remnants of endemic genetic characteristics. Populations are often supplemented or replaced by using adults taken from other populations for artificial spawning and hatchery production. The few hatchery populations that operate solely on endemic broodstock may retain distinctions of the original populations at supposedly neutral genetic marker loci, but deviations result from high variance in fitness in hatchery populations (Ryman and Laikre 1991). Hatcheries generally aim to produce as many salmon as possible from relatively few parents (usually, only a subset of returning spawners are used as broodstock), leading to increased inbreeding and rapid genetic drift. Hatcheries can reduce these random genetic effects by increasing and equalizing the numbers of males and females chosen as broodstock, by spawning individuals with multiple partners, or by equalizing family sizes (Hedrick *et al.* 1995). Implementation of these protocols, particularly the latter one, has been recent and rare because their benefits are uncertain and the risks they alleviate are negatively correlated with other risks (Hedrick *et al.* 1995, Waples 1999).

Non-random genetic effects are another source of concern. Populations have evolved at quantitative genetic loci in response to selection by hatchery regimes (see reviews by Reisenbichler and Rubin 1999, Einum and Fleming 2001). First, hatcheries select broodstock non-randomly with respect to morphological traits or spawning time (Waples 1999, Ford and Hard 2000). Progeny are then exposed to selection in high density, low cover, low complexity, low predation conditions. Thus, because not all adults are spawned artificially, and all family sizes are not equalized, the hatchery process changes locally adapted genetic variation at some loci. Discouraging selective forces by equalizing family sizes may also have negative effects. In attempting to reduce domestication, selection that purges deleterious variation in the wild is forgone (Waples 1991, 1999), and reduced fitness or even sterility can result (Grahn *et al.* 1998). By appropriating choice of mates from spawning fish, hatcheries eliminate natural behaviours that may promote the formation of fitter genotypes, and may therefore further reduce fitness of populations (Grahn *et al.* 1998, see also Whitlock 2000).

Finally, non-heritable influences (e.g., learning and development) in hatchery environments may hamper the ability of hatcheries to support the original locally adapted genetic variation of salmon populations. Hatchery-reared fish may compete poorly with wild fish while rearing in freshwater, have relatively poor marine survival, and often demonstrate reduced breeding success versus wild fish (Hesthagen and Johnsen 1989, Kelly-Quinn and Bracken 1989, Finstad and Heggberget 1993, Petersson and Jarvi 1997). Cumulatively, these heritable and non-heritable limitations to the success of hatchery fish may outweigh benefits in egg survival and reduced predation that are afforded by hatchery production. Further, ecological interactions with unfit hatchery releases can harm wild populations (reviewed by Einum and Fleming 2001).

Despite the demonstrated inferiority of hatchery fish in wild environments, with persistent attempts at supplementation, outplanted hatchery fish (those derived from non-local broodstock) often leave a significant genetic footprint on recipient populations (e.g., Utter *et al.* 1989, Garcia-Marin *et al.* 1991). These footprints represent successful contribution of hatchery fish to wild populations, but this introgression also signals the introduction to wild populations of potentially deleterious heritable traits (see reviews by Waples 1999 and Fleming and Petersson 2001). Nickelson *et al.* (1986) found that hatchery-released coho salmon parr had a negative effect on wild parr, and that hatchery releases had no positive influence on the abundance of returning adults. Releases of hatchery fish reduced wild production, however, and due to genetic influences of hatchery fish on wild populations, wild production remained depressed after hatchery releases ceased. Because the perceived risk of negative genetic influences sometimes exceeds the perceived benefits of hatchery production, conservation concerns have prompted the cessation of some hatchery operations (Ford and Hard 2000) and the frequency of releases of non-native hatchery fish has declined (Einum and Fleming 2001).

In contrast, there are also many cases in which extrinsic hatchery fish do not seem to contribute to wild, locally adapted populations (presumably owing to inferiority of hatchery fish in several traits; e.g., Moran *et al.* 1991, Hansen and Loeschcke 1994, Poteaux *et al.* 1998). If supplementation of fishing opportunities is the goal of hatchery production, poor fitness of hatchery fish may not represent failure (provided that hatchery fish grow and survive to contribute to the fishery; Fleming and Petersson 2001). Even very limited introgression justifies concerns regarding conservation of local adaptation and productivity, however, and the relationship between introgression and natural production must be understood to more fully evaluate the risks and potential benefits of hatchery programs (Hansen and Loeschcke 1994, Fleming and Petersson 2001). Such understanding demands knowledge of how local conditions can promote success of hatchery fish over wild fish. It also demands knowledge of the mechanisms promoting homing and straying that determine whether those local conditions are exploited over time (Quinn 1997). I examined these questions, so important in management and conservation of salmon and trout, in a less studied but related organism.

Study system

Columbia River has been subject to many anthropogenic perturbances, and dams are dominant structures along most of its length. In the Columbia River drainage of British Columbia's West Kootenay region (CRDWK), Hugh Keenleyside (1967), Mica (1973), and Revelstoke (1980 - 1984) dams were constructed on the mainstem for flow regulation as part of the Columbia River Treaty (Sebastian *et al.* 2000). These dams probably influenced the geographic structure of fish populations in the area because they blocked migration among the reservoirs they created. For example, bull trout (*Salvelinus confluentus*) were observed in large numbers at the base of Revelstoke Dam following its completion and were thought to be diverted to ascend and spawn

in the nearby Jordan and (perhaps) Illecillewaet rivers (Sebastian *et al.* 2000). Also, inundation of spawning habitat throughout CRDWK may have forced fish to abandon traditional spawning grounds and seek out other suitable habitat. In partial compensation for fisheries losses resulting from these dams, propagation of bull trout in hatcheries began twenty years ago and has continued at Hill Creek Hatchery since 1983. Initial goals for production included annual supplementation of 4000 catchable bull trout. Adults were collected from Arrow tributaries and from below Revelstoke Dam and taken to the hatchery where they were held until artificially spawned. Some adults died prior to spawning, some were sacrificed, some were spawned and released, and others were released without spawning. Progeny were reared in fiberglass raceways and circular tanks before release to Arrow tributaries.

The bull trout is a salmonid fish that displays several life histories. Fluvial and adfluvial forms are the best studied (Baxter and McPhail 1996): they are long-lived (more than 15 years; Donald and Alger 1993, McPhail and Baxter 1996), iteroparous, and may rear in spawning streams for up to four years (McPhail and Murray 1979, Fraley and Shepard 1989). Adfluvial fish migrate to a lake to feed and grow for some period before returning to spawning streams to reproduce. In CRDWK, dam construction and operation most certainly affected adfluvial bull trout, the form to which most angling and hatchery efforts have been directed. Relative to most other salmonines (Washington and Koziol 1993), experience in hatchery-production of bull trout is minimal. Because of its notoriety as a piscivorous pest, until recently management efforts have focussed on eradication rather than production. The bull trout is considered either endangered or of conservation concern in Nevada, Montana, Oregon, Washington, Idaho, Alberta, and British Columbia – throughout almost all of its declining range (Anon 1999). Conservation plans for bull trout in several of these jurisdictions may include artificial propagation via hatcheries (Leary *et al.* 1993, Kanda *et al.* 1997, Spruell *et al.* 1999).

In CRDWK, success of hatchery production has been largely unmonitored and otherwise questionable (Sebastian *et al.* 2000). Sebastian *et al.* (2000) estimated that more than 98% of approximately 1 300 bull trout caught annually in Arrow Reservoir are wild, giving an upper-bound estimate of 26 bull trout contributed to the fishery by the hatchery, annually. This estimate may be biased downward because it reflects early years of production, when culture techniques were still being refined (G. Thorp, Hatchery Manager, MELP, personal communication). Initial attempts at hatchery supplementation involved the capture of large numbers of bull trout for broodstock. These were mostly collected from immediately below Revelstoke Dam and from Jordan River. The broodstock collection program subsequently expanded southward to include other tributaries to Arrow Reservoir. Concomitant with this southward expansion, declines in bull trout spawners appeared to occur in tributaries used for broodstock collection (Winsby and Stone 1996).

In addition to the possible effect of reduced bull trout densities in broodstock streams, the hatchery program may have had genetic influences. For example, many males caught from northern tributaries were not running milt, so sperm from males caught in southern tributaries was often used to fertilize 'northern' eggs. The progeny were released mostly into downstream reaches of Upper Arrow tributaries. Although collections of broodstock and releases of progeny were somewhat correlated, Hill Creek Hatchery operated an outbreeding, outplanting program. Monitoring of hatchery success was not possible until after 1990, before which progeny from crosses were not systematically marked for post-release identification. Subsequently, several other changes have been made: the collection program has included more tributaries, though fewer broodstock are collected; crosses between adults from distant tributaries have been

avoided, in general; progeny have been reared for two years (rather than one); and progeny have been released closer to the collection sites of their parents. Such changes in protocol are logistically difficult, and their necessity requires evaluation.

Some subdivision of populations must occur to justify the hatchery's avoidance of outbreeding and outplanting. McPhail and Murray (1979) suggested that population structure in Arrow bull trout should be studied prior to supplementation efforts. Since that time, significant genetic structuring of other bull trout populations has been detected at both small (Spruell *et al.* 1999, Taylor *et al.* 2001) and broad spatial scales (Leary *et al.* 1993, Williams *et al.* 1997a, Taylor *et al.* 1999). Genetic differentiation among spawning populations in CRDWK has remained controversial, however, because of historical environmental impacts, described above, that promoted interbreeding among populations (Sebastian *et al.* 2000), and because of suggestions (see McPhail and Murray 1979) that Arrow bull trout switch spawning streams as they age. Also, radiotelemetry and genetic analyses suggested large amounts of interbreeding among tributaries of the nearby Duncan River (O'Brien 2001). In Chapter 2, I used molecular genetic techniques to demonstrate significant genetic subdivision of adfluvial bull trout among historical northern and southern groups of stream populations in CRDWK and among streams within those groups (see Table 2.4). In this chapter, I examine the degree of genetic variation among adfluvial samples in greater detail. I evaluate that differentiation with respect to its maintenance, its response to estimable anthropogenic impacts, and its consequences for management and conservation.

Molecular genetic data are often used to evaluate models of dispersal (e.g., isolation by distance; see Paetkau *et al.* 1997) and to distinguish between migration and gene flow (e.g., Baker *et al.* 1998, Neraas and Spruell 2001). For conservation of bull trout (and other species), this information about the genetic structure among populations should be used to relate the spatial scale of management to relevant scales of evolutionary and ecological processes (Rieman and McIntyre 1995; Dunham and Rieman 1999). I use genetic data to examine the roles of homing and straying in generating population structure observed in bull trout in CRDWK. I also use available data on captures of hatchery fish for this purpose. In addition, I compare potential influences of hatchery operations on stream populations to allele frequencies and measures of differentiation among streams (Figure 3.1), and I describe mechanisms of their effects. Finally, I evaluate the consequences of genetic structure remaining among streams for mixed-population fisheries and no-harvest zones of bull trout in CRDWK. Molecular genetic data are useful in analyses of mixed-populations in fish harvests (Shaklee *et al.* 1999). I use similar molecular genetic analyses to relate lacustrine catches to their streams of origin, and I use the genetic data collected from stream populations to examine potential differentiation in the demographies of those streams.

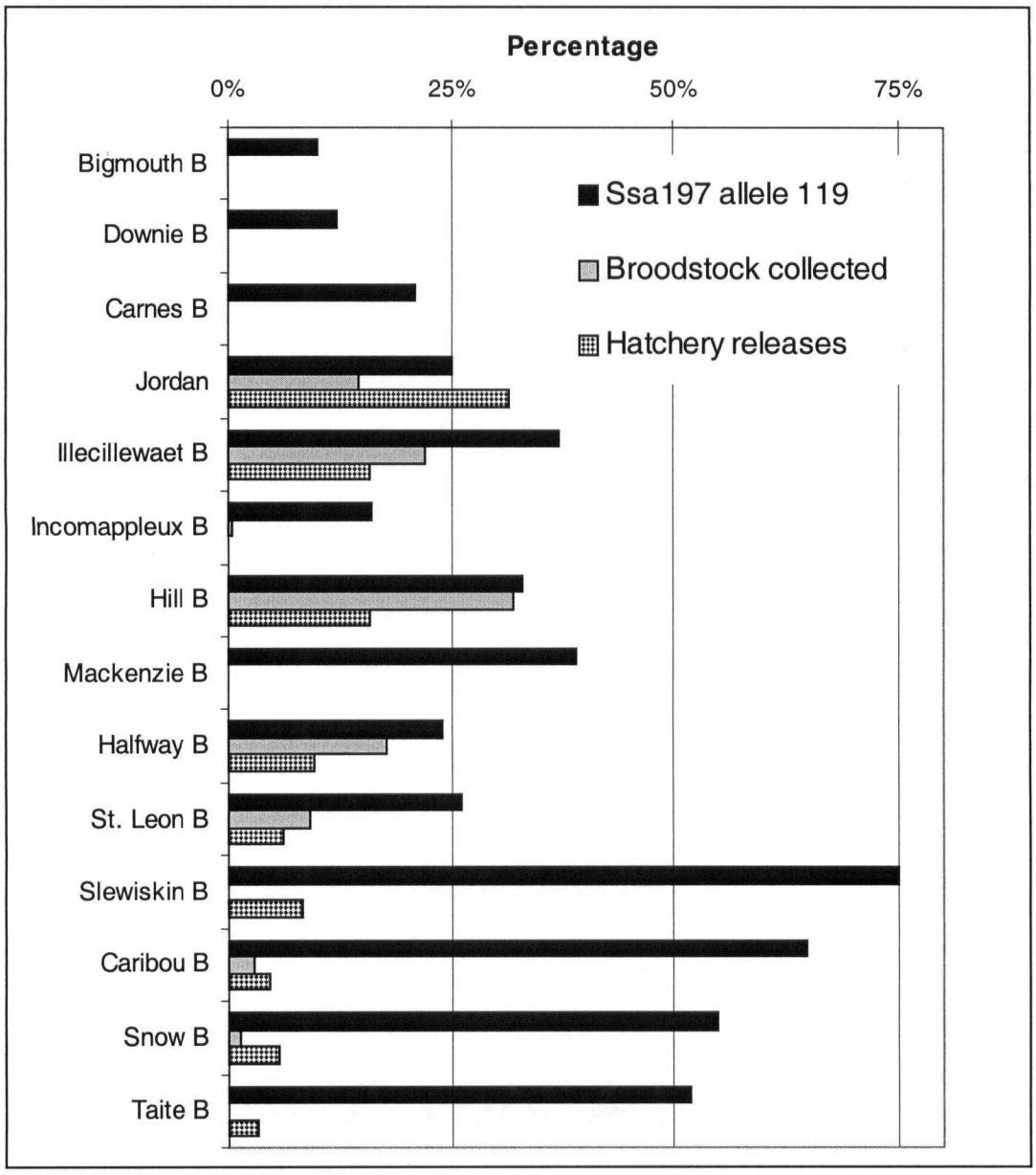

Figure 3.1. Frequencies of one *Ssa*197 allele, broodstock collections and releases of hatchery fish (in percentages of the total from 1989 – 1996) in adfluvial populations. Hatchery releases to Jordan River include those that were performed above the barrier there.

Methods

I used genetic analyses of haploid (mtDNA), diploid (*Sco*19, *Sco*23, *Sfo*18, *Ssa*197), and tetraploid loci (*Sco*1) to examine potential influences of homing, straying, exploitation, and hatchery supplementation on population structure. Data sets 1 and 2, described in Chapter 2, again served as the raw material for investigation. As in Chapter 2, "4 usat" refers to the four diploid microsatellite loci, and sample names are followed by "B" to indicate whether they are samples from below waterfalls in streams.

Fluvial differentiation

Drift of a population's allelic frequencies over time may be reflected in genetic differentiation between adults and juveniles. In addition, adfluvial adults within a spawning tributary may

represent a pool of migrants from differentiated populations and might or might not, as a group, display differences in allelic frequency compared to juveniles sampled within that tributary. To test for temporal differentiation, I used F-statistics (Weir and Cockerham 1984) to compare adults to juveniles within populations. Only Illecillewaet River, Halfway River, and a combination of Caribou and Snow creeks provided enough juveniles and adults to allow a reasonably powerful test. To determine whether samples from juveniles rearing in streams were representative of the spawning populations there, I also qualitatively compared numbers of alleles among adults and juveniles using the cumulative heterozygosity option of DOH (http://www.biology.ualberta.ca/jbrzusto/Doh.html) as in Chapter 2. In addition, self-assignment (the relative likelihood of an individual's genotype arising in its nominal population versus other populations) of adults and juveniles was compared using Wilcoxen signed-rank tests. Finally, differentiation among adfluvial populations and groups of adfluvial populations was measured (as in Chapter 2) using data only from adfluvial adults, and I compared the results to those obtained with combined data for adults and juveniles.

Comparable to estimates of temporal variation within tributaries (above), I described spatial variation within tributaries using traditional F-statistics. I also performed an AMOVA (Excoffier *et al.* 1992) using ARLEQUIN (Schneider *et al.* 1997), thereby partitioning genetic variation hierarchically among tributaries and among sites within those tributaries. To examine if observed differentiation was directional along an *a priori* axis, I used a distinct analytical tool described in Chapter 2. Within streams that I sampled at multiple sites below waterfalls, an estimate of a genotype's northern versus southern affinities, RDIA, allowed me to test whether upstream samples were more 'northern' and less 'southern' than downstream samples in the same tributary. For this comparison, probabilities associated with individuals' nominal populations (i.e., capture locations) were not considered; that is, geometric means included only probabilities of genotypes arising in adfluvial populations that were sampled at only one location. Direct comparison of RDIA calculated here to RDIA values calculated in Chapter 2 is impossible, as different samples were used to define the north-south axis. As in Chapter 2, these data were analysed with linear regression and ANCOVA.

Lacustrine differentiation

I used standard F-statistics and exact tests to examine differentiation among reservoirs. All F-statistics and exact tests in this chapter were calculated with GENEPOP (Raymond and Rousset 1995) or ARLEQUIN as described in Chapter 2. Samples from within the Arrow reservoir could be grouped into three semi-distinct sample areas. Weigh-in stations at the Nakusp and Shelter Bay fishing derbies (near Kuskanax Creek and opposite Incomappleux River, respectively; see Figure 2.2) provided two samples of bull trout caught from anywhere in the reservoir by anglers. I assumed that the distribution of capture locations for each derby was centered at the census station; that is, I assumed that samples from Shelter Bay Derby were caught approximately 50 km to the north of Nakusp Derby samples. The third sample area was another 50 km north where bull trout were collected for the hatchery's broodstock program from the Columbia River, below Revelstoke Dam. These fish were considered lacustrine because annually, and at the time of capture, Arrow Reservoir becomes quite full and this section of the Columbia River is probably unsuitable for spawning. I was able to bolster sample sizes from the Columbia River and derbies with lacustrine samples provided by a radiotelemetry project (K. Bray, unpublished data) because the capture locations of radiotagged fish are known.

Using the assignment calculator of DOH, I compared lacustrine bull trout to tributary samples and thus assigned them to their most likely their natal streams (of those streams I sampled) to

determine relative importance of tributary populations for the sport fishery. DOH also provided probabilities used to calculate RDIA, which tested whether the north-south pattern of genetic variation among streams was reflected also in the lacustrine environment. Calculations of RDIA here differed from those above as below-falls samples from all tributaries were included in defining "northern" and "southern" genotypes. Individuals' RDIA scores from each of the three lacustrine sample areas within Arrow Reservoir were compared using linear regression.

Demographic effects in adfluvial populations

The distribution of allelic frequencies at a given locus in a sample contains demographic information about the population. At equilibrium, a constant effective population size and mutation rate produces a predictable relationship between expected heterozygosity (H_e) and number of alleles (Ewens 1972). While over-representation of intermediate allelic frequencies together with under-representation of rare allelic frequencies may identify recently bottlenecked populations (Luikart and Cornuet 1998), a preponderance of rare alleles may indicate that populations with different rare alleles have been amalgamated, either by sampling methods or by recent historical events (Chakraborty *et al.* 1988). Because available statistical analyses for such demographic effects test against a null, equilibrium distribution, they were not appropriate for my data, which have likely been influenced by both opposing forces as a result of historical colonization and anthropogenic habitat manipulation. To investigate relative demographic effects among populations, I instead divided an estimate of a sample's unbiased, expected heterozygosity, H_e, by the number of alleles detected. Rather than making comparisons to a theoretical relationship within a given population, I made comparisons across populations.

Two loci, *Sco*1 and *Sco*19 were polymorphic enough to support this analysis. For *Sco*19, DOH's cumulative heterozygosity option provided estimates of both H_e and number of alleles, averaging over 1000 resamples of 20 individuals. This option of DOH does not accept non-diploid data, so analysis of *Sco*1 was not as straightforward. Like in Chapter 2, numbers of alleles were estimated as for *Sco*19, with *Sco*1 entered as two diploid loci instead of a single tetraploid locus. For each sample, I calculated heterozygosity by subtracting from 1 the homozygosity, p_i^4, of each allele, i. The estimate was not corrected for sample size but, as heterozygosities were close to 1 (range: 0.982 – 0.998), this was unlikely to affect results. To reduce variation caused by small samples, few loci, and these analytical problems, samples were ranked from low to high with respect to the H_e/#alleles ratio. Under this scheme, both low ratios and low ranks reflect some combination of relatively high population amalgamation and relatively little bottlenecking.

Little variation was generally detected in samples from above barriers (testifying to historical bottlenecks; see Chapter 2), and sample sizes were too low for this analysis. Samples from the population above the Halfway River waterfall were an exception. These samples, lacustrine samples, and an artificial sample generated by combining B samples (below barriers) from all streams were analysed to determine the utility of this analysis – they served as references for comparisons among adfluvial populations.

Isolation by distance

To examine restriction of gene flow by geographic distance, matrix correlations (i.e., Mantel tests; Mantel 1967) were performed between geographic and genetic distances among pairs of samples. Genetic distances chosen were D_S (Nei 1972, 1978) and D_{LR} (Paetkau *et al.* 1997). These two distances, though calculated in very different ways, performed well with empirical microsatellite data in a recent comparison of distance metrics (Paetkau *et al.* 1997). I also chose

them because available software allowed an examination of sensitivity to influences of sample size. DOH offers two ways of avoiding probabilities of zero, which arise from sampling that fails to detect some alleles in a population. One method "corrects" for sample size in calculation of D_{LR}: it adds one copy of missing alleles to a sample (smaller samples are given higher frequencies of missing alleles than are large samples). The other method assumes the same frequencies of missing alleles (0.01) in samples regardless of their size. Likewise, TFPGA allows calculation of Nei's D_S based purely on sampled allelic frequencies and on allelic frequencies "unbiased" by sample size. All six loci were used to calculate D_{LR}, whereas D_S was only analysed for diploid microsatellite loci.

Hatchery influences

Bull trout produced by the hatchery (identified by missing adipose fins) may have facilitated gene flow among populations. To understand their potential influences on genetic structure, I investigated geographic pattern in the recovery of hatchery bull trout. I used exact tests to compare the proportions recovered in Arrow Reservoir's sport fishery and in the hatchery's broodstock collection program. To determine whether the difference I found could be attributed to targeting of different sized (aged) fish by anglers and broodstock collectors, I used t-tests to compare sizes (fork lengths) of hatchery and wild bull trout in the two samples.

Of hatchery fish that were genetically examined, I compared their observed heterozygosity (H_o; there were too few in any particular stream to examine expected heterozygosity, H_e), and self-assignment to that of wild fish from the same locations.

In a manner similar to the evaluation of isolation-by-distance (see above), I investigated genetic differentiation using Mantel tests, except I compared matrices of pairwise genetic distance and hatchery use. Each cell of the latter matrix contained the sum of broodstock collected from – and, in a similar test, hatchery fish released to – the streams in the pairwise comparison. For example, the following matrix would result if 10, 100, 140, and 300 broodstock were collected from streams a, b , c, and d, respectively:

	b	c	d
a	110	150	310
b		240	400
c			440

with cell ab = 10 + 100, et cetera (analyses ignored diagonals). To account for other correlates (e.g., geographic distance), I also did partial Mantel tests (Smouse *et al.* 1986). Each test was performed with and without samples from above Revelstoke Dam, for both data sets 1 and 2. In every case, Mantel tests employed 5 000 permutations and were supported by the MANTEL program of the R-PACKAGE (Casgrain and Legendre 2000). Because of *a priori* expectations, these were 1-tailed tests.

Results

Differentiation of tributary samples

No significant differences were found between adfluvial adults and juveniles within a tributary; they provided similar sample information about stream populations. Estimates of F_{ST} ranged from –0.045 to +0.053 (combined across loci, $P > 0.5$ for all populations). Resampling of adults and juveniles from the same streams provided no evidence of differing allelic diversity between adults and juveniles (not shown). Also, although adults had lower self-assignment ratios than

juveniles within Illecillewaet River (uncorrected $P = 0.044$) using DOH's "add-one-in" option, differences were not detected in general (Fisher's combined $P > 0.1$, both options). Using adults only, population structure estimated among the northern and southern tributaries was similar to that calculated using juveniles (see Table 3.1).

Table 3.1. Differentiation across ungrouped tributaries, F_{ST}, and across the northern and southern groups of tributaries, F_{CT}, for different loci comparing samples of adults (Adults only) to samples including juveniles (Both). Significant difference from zero is indicated by * ($P < 0.05$) and ** ($P < 0.005$).

Locus	Sample type	F_{ST}	F_{CT}
MtDNA	Both	0.401**	0.503**
	Adults only	0.353**	0.575*
*Sco*1	Both	0.023**	0.003
	Adults only	0.001*	0.006
4 usat	Both	0.105**	0.084**
	Adults only	0.033*	0.063*

In spatial comparisons within adfluvial populations, AMOVA demonstrated that while differences among sites within tributaries accounted for a small but statistically significant amount of variation at diploid microsatellite loci (1.3%, $P < 0.05$), differences among tributaries accounted for much more (10.5%, $P < 0.001$). Using mtDNA, this discrepancy was more evident (1.9%, $P > 0.2$; 41%, $P < 0.001$). Estimates of F_{ST} among subsamples within tributary populations ranged from less than – and not significantly different from – zero for each locus, to 0.096 ($P = 0.14$, Incomappleux River), 0.043 ($P = 0.006$, Illecillewaet River), and 0.026 ($P = 0.002$, Slewiskin Creek) for mtDNA, combined diploid microsatellite loci (4 usat), and *Sco*1, respectively. Combining probabilities across loci and correcting for multiple comparisons, only Slewiskin Creek samples were significantly different from one another. Combining across tributaries, however, yielded a significant result ($P < 0.01$) suggesting that genetic differentiation among accessible locations within tributaries is a more general phenomenon.

Within tributaries, RDIA increased significantly with distance (genotypes became more northerly upstream) within Illecillewaet River and Slewiskin Creek ($r^2 = 0.06$ and 0.10, respectively; one tail, uncorrected for multiple comparisons $P = 0.05$ and 0.02; Figure 3.2). ANCOVA detected no significant difference among slopes but did demonstrate that RDIA differed among tributaries ($P < 0.0001$) and decreased within these tributaries at sites closer to their mouths ($P = 0.004$, one-tail).

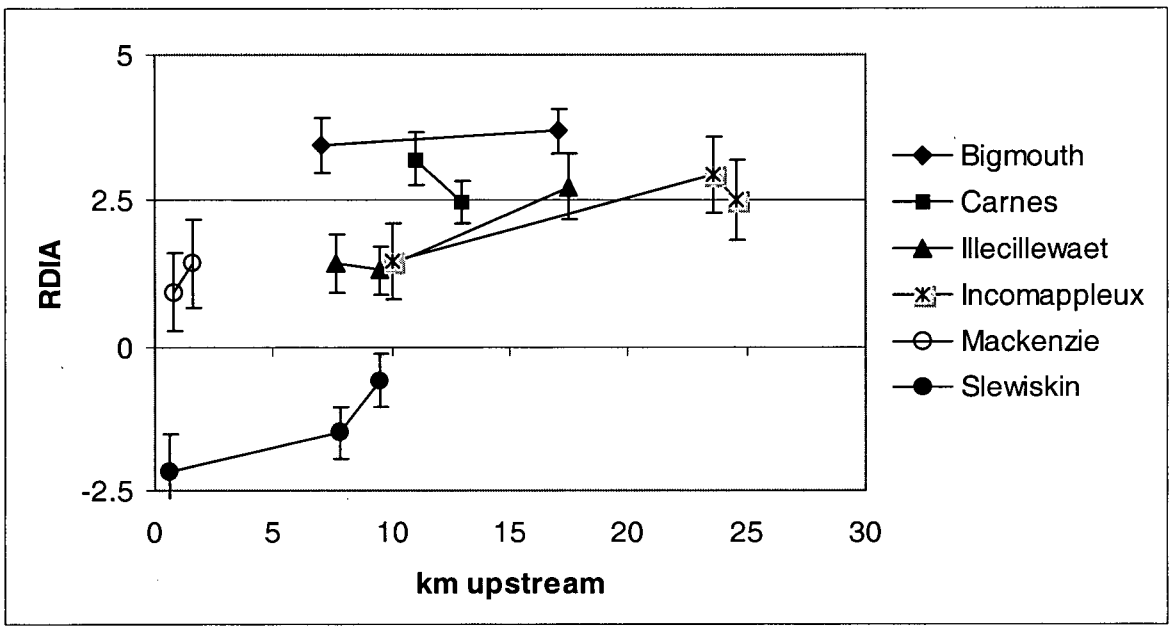

Figure 3.2. The relationship between RDIA, a measure of genotypic affiliation to northern versus southern tributaries, and distance of a sample site within a tributary upstream of its entry into Arrow or Revelstoke reservoir. Bars represent standard errors.

Lacustrine differentiation

Lacustrine samples were well differentiated among reservoirs, but differences within Arrow reservoir were not so obvious (Table 3.2). Of five nuclear loci, a significant difference among Arrow samples was only found at *Sco*19 ($P = 0.035$, uncorrected), and samples from Shelter Bay and Nakusp derbies were not distinguished by any locus, including mtDNA (not shown). Examination of RDIA, however, showed that lacustrine adults sampled from more northerly locations within Arrow Reservoir tended to have more northerly genotypes ($r^2 = 0.15$, $P < 0.0001$; Figure 3.3).

Table 3.2. Differentiation among lacustrine adults sampled from Kinbasket, Revelstoke, and Arrow reservoirs, and differentiation among samples from within Arrow Reservoir.

Locus	Sample comparisons	F_{ST}	*P*-level
mtDNA	Among reservoirs	0.109	< 0.001
	Within Arrow	0.083	0.014
*Sco*1	Among reservoirs	0.002	0.27
	Within Arrow	0.001	0.26
4 usat	Among reservoirs	0.033	< 0.001
	Within Arrow	0.006	0.18

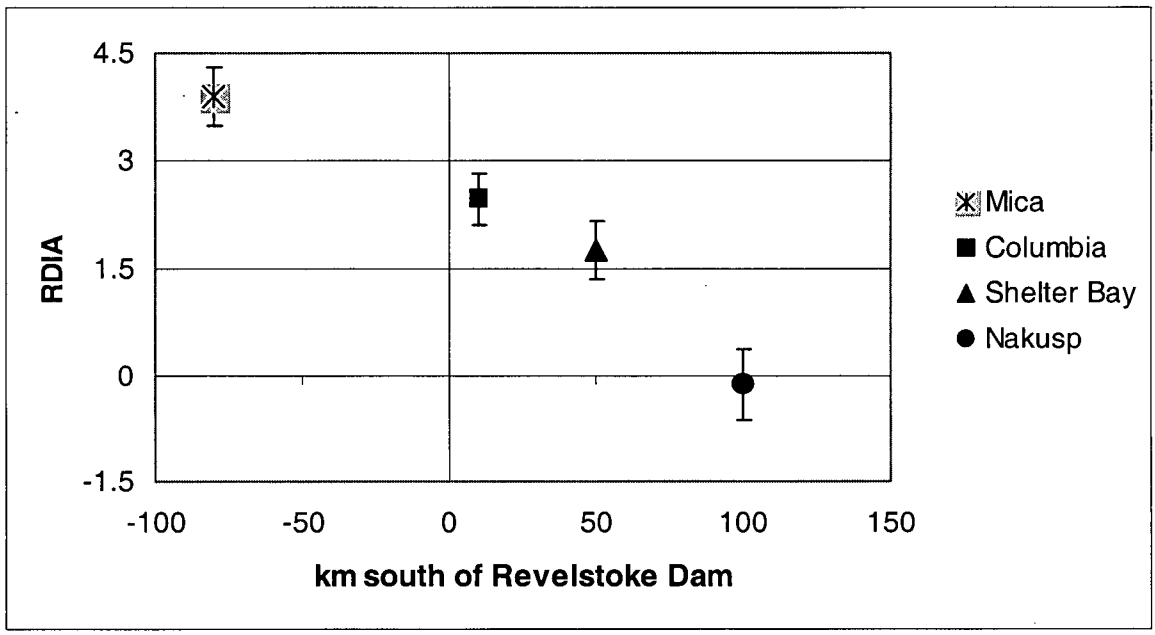

Figure 3.3. RDIA of lacustrine samples versus their geographic location. Samples called "telemetry" (see Table 2.1) were added to derby samples for totals of eight, 26, 36, and 38 individuals from Mica Derby, Columbia River, Shelter Bay Derby, and Nakusp Derby, respectively. RDIA decreases (becomes more 'southern') in samples south of Revelstoke Dam (located at 0 km). Data set 2 (excluding fallers) was used in calculations. Results are shown for the "add-one-in" option of DOH. Bars reflect standard errors. Standard errors for capture location (x-axis) were not estimable, but expected to be large.

Assignment of lacustrine bull trout to potential streams of origin on the basis of their genotypes provides a different view of the variation within and among lacustrine samples (Figure 3.4). Halfway River was the only tributary to which no assignments were made, and Snow Creek was only implicated in the assignments of one lacustrine sample (Nakusp Derby). All other tributaries were implicated in assignments at multiple sample locations (see Figure 3.4). Individuals from the Nakusp Derby sample were assigned to 12 tributaries, and Columbia River and Shelter Bay samples included assignment to 10 tributaries each. Despite this variance within samples, assignment of lacustrine samples to tributaries followed a geographically predictable pattern (as expected from RDIA scores, see Figure 3.3); assignments tended to be made to geographically proximal streams. For instance, a relatively high proportion of bull trout from Columbia River was assigned to Jordan and Illecillewaet rivers and to tributaries above Revelstoke Dam, whereas 33% of fish caught near Shelter Bay were assigned to Incomappleux River (see Figure 3.4). More commonly than in the other samples, lacustrine bull trout caught near Nakusp were assigned to southern tributaries. Although accounting for only 20% of total assignments, southern tributaries (Slewiskin, Caribou, Snow, and Taite creeks) accounted for 40% of the Nakusp Derby sample (versus 11% and 4% of Shelter Bay and Columbia River samples, respectively).

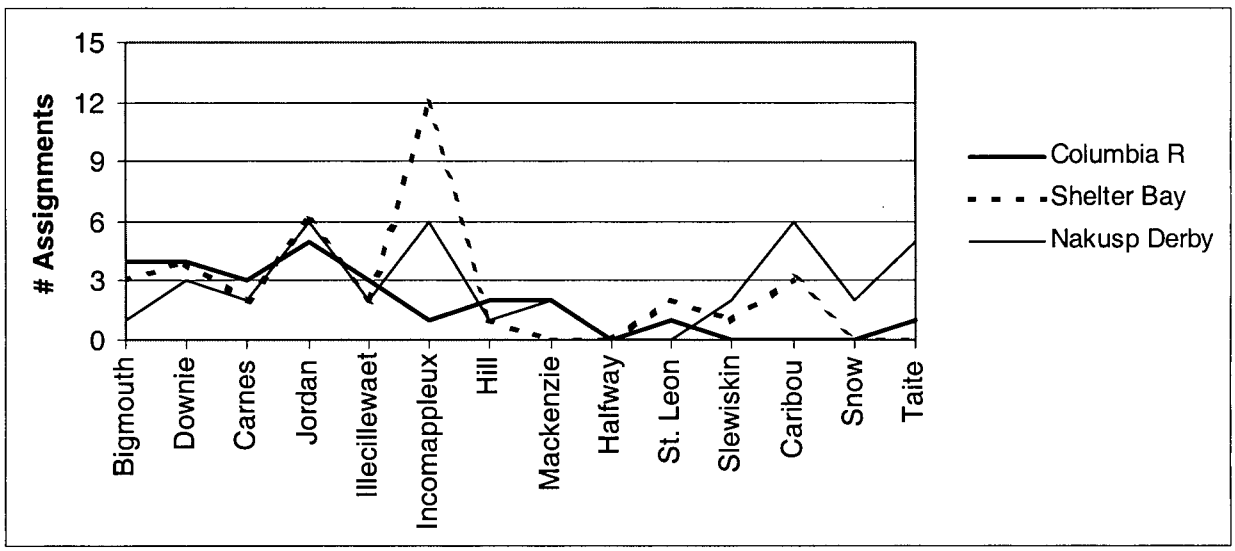

Figure 3.4. Genotypic assignment of lacustrine bull trout from different capture locations in Arrow Reservoir to tributaries in CRDWK. Data set 2 was used for assignment. Results for the "add-one-in" option are shown. Telemetry samples were added to derby samples for a total sample size of 100 (26, 36, and 38 individuals from Columbia River, Shelter Bay Derby, and Nakusp Derby, respectively). Tributaries are arranged with the most northerly to the left and the most southerly to the right.

Demographic analysis

The relationship between numbers of alleles detected and heterozygosity was ranked for samples that consisted of 20 or more individuals (Figure 3.5). High ranks reflect an excess of heterozygosity relative to allelic diversity, as would be expected in a bottlenecked population. The rankings predicted with each locus were somewhat congruent, as rankings at the two polymorphic loci were positively correlated (Spearman Rank Correlation, $r = 0.45$, $P = 0.03$). The strongest exception was an amalgamated sample (Shelter Bay Derby) which ranked lowest (i.e., amalgamated) at *Sco*1 but ranked highly (fourteenth out of 18 samples) at *Sco*19. At *Sco*1, amalgamated samples (Shelter Bay Derby, Nakusp Derby, Telemetry, and "all streams" samples) had significantly lower ranks than did stream samples of adfluvial populations (Wilcoxen signed-rank test, $P = 0.01$), but not at *Sco*19 ($P = 0.23$). Nevertheless, using the sum of a sample's ranks, amalgamated samples had lower ranks than tributary samples. A sample from a presumably bottlenecked population above a physical barrier, Halfway A (see Chapter 2), was ranked second highest. Demographic signals varied strongly and tended not to demonstrate a geographic pattern across tributary populations, as samples with high and low rankings were collected from neighboring streams. For example, Hill Creek had the lowest rank, whereas Incomappleux River and Mackenzie Creek, the most proximal streams sampled, ranked thirteenth and sixteenth, respectively. Samples collected downstream of known resident populations reflected a variety of rankings, and these were insensitive to whether or not fallers were removed (not shown).

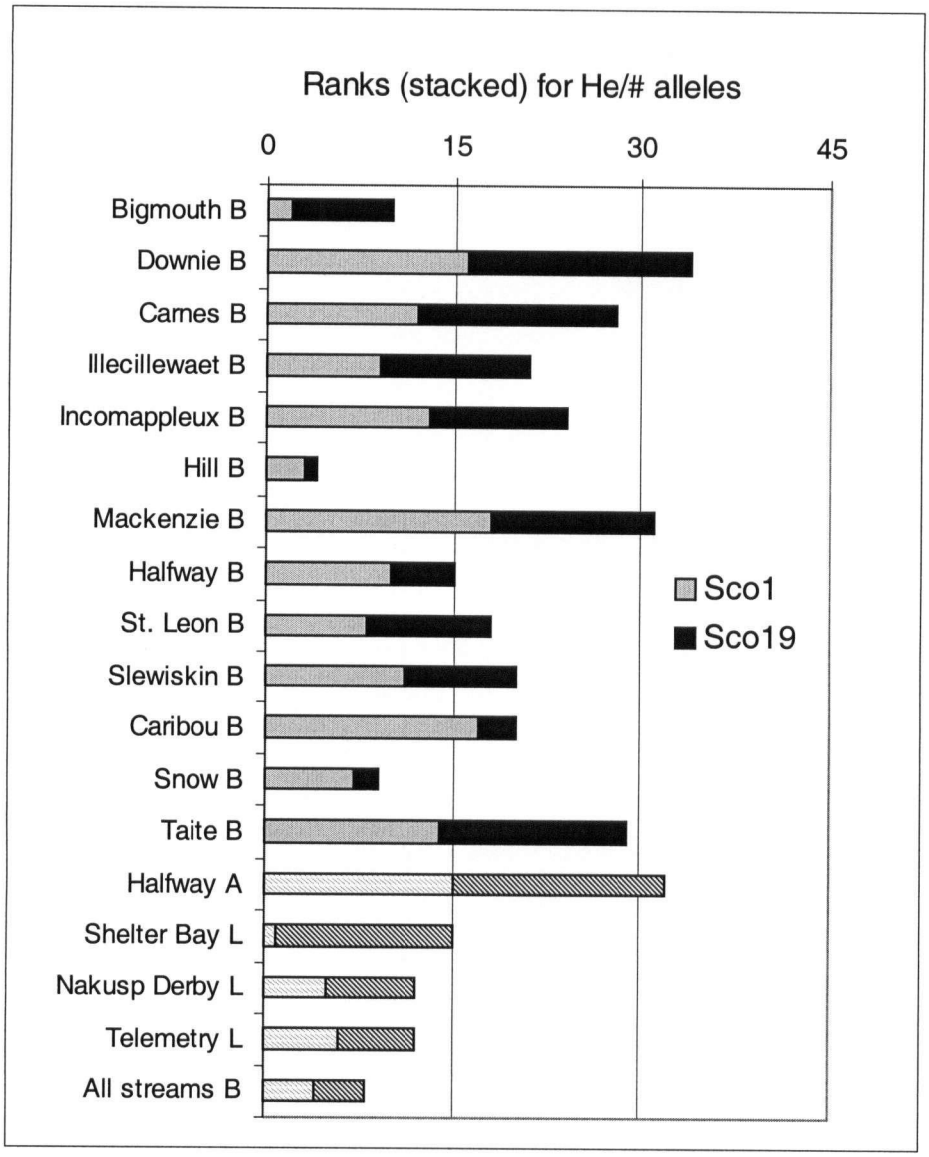

Figure 3.5. Relative comparison of demographic signals for two loci among stream populations (solid bars) with sample sizes of at least 20. High ranks represent a high ratio of H_e to number of alleles, indicating relatively severe population bottlenecks or relatively little mixing of populations. Bottlenecked and amalgamated samples for reference are included (hatched bars). Ratios were calculated using resamples of 20 individuals from populations (see text).

Isolation by distance

Using D_{LR}, streams were demonstrably isolated by geographic distance ($r = 0.50$, $P < 0.001$; Figure 3.6). Variation in statistical results caused by different ways of dealing with sample size differences and inclusion/exclusion of fallers was negligible. A marginal relationship was detected using D_S ($r = 0.25$, $P < 0.1$; see Figure 3.6), but a non-significant *negative* relationship was indicated for D_S within the northern and southern groups ($r = -0.03$ and -0.09, respectively). Even using D_{LR}, isolation by distance was not obvious visually or statistically ($r < 0.2 < P$) within either the northern or southern groups of tributaries.

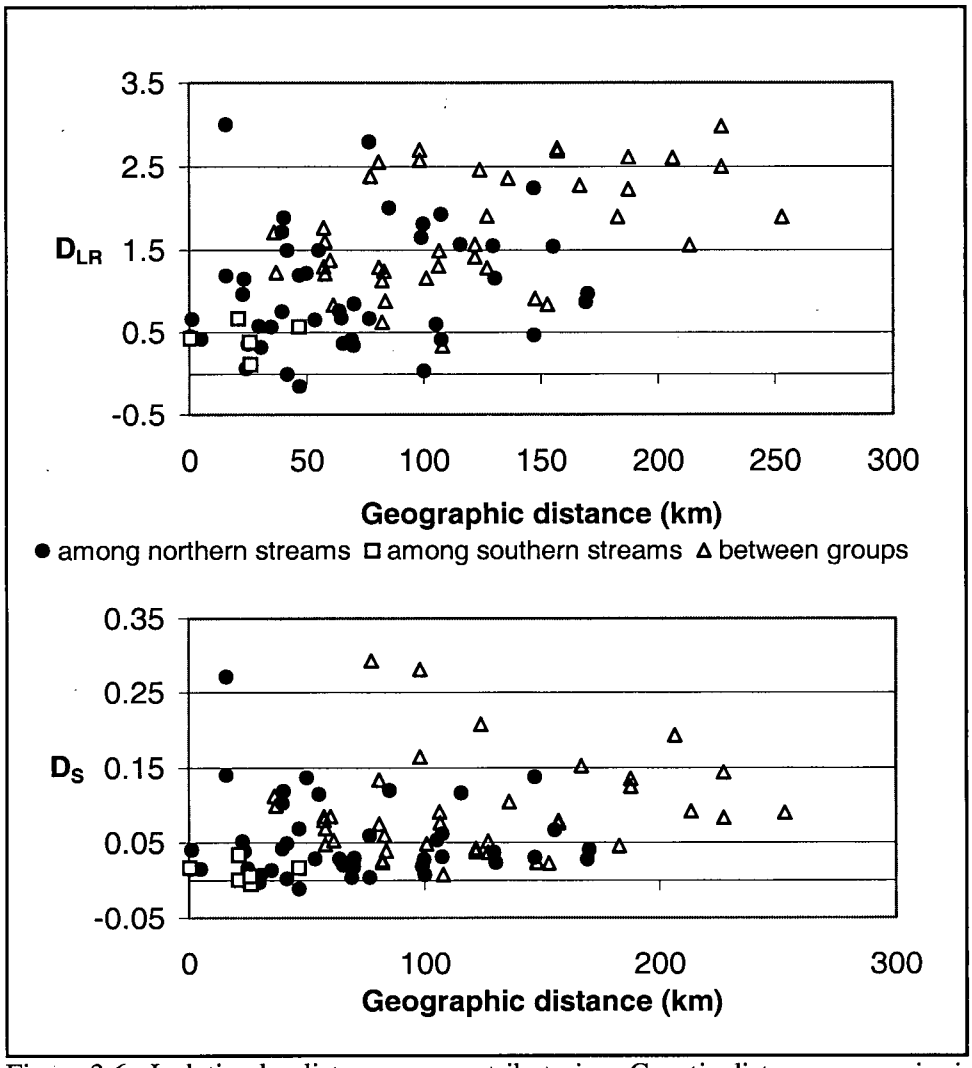

Figure 3.6. Isolation by distance among tributaries. Genetic distances are pairwise estimates of D_{LR} and D_S (among northern tributaries, among southern tributaries, and between northern and southern tributaries).

Returns of hatchery bull trout

A disproportionate number of hatchery bull trout were recaptured at Hill Creek (the hatchery site) and in broodstock collections, in general, relative to recaptures in the fishery. Thirty-five adults (3.4% of the total) collected for broodstock between 1990 and 1998 were fish produced by the hatchery. Less than 1% of the reservoir's fishery over the same time period, however, was composed of marked (i.e., hatchery) fish (British Columbia Ministry of Environment, Lands and Parks, unpublished data). The difference between these proportions is highly significant (exact test, $P < 0.001$). Of streams used for broodstock collection, Hill Creek had the highest recapture rate (16 recaptures, or 9.3%). Combined, 2.2% of broodstock collected from other streams were of hatchery origin (significantly different from the recapture rate at Hill Creek, $P < 0.001$). Broodstock tagged in previous years were also more frequently recovered at Hill Creek than at other broodstock collection sites ($P < 0.001$). Thirteen adults (7.5%) collected at Hill Creek had tags, compared to only six (0.7%) of the adults collected in all other streams.

Bull trout captured for broodstock averaged 53.0 cm in length and bull trout captured in the fishery averaged 55.3 cm, but this difference was not significant (one-tailed, $P > 0.2$). Similarly,

within broodstock samples, recaptured hatchery fish were not smaller than wild fish ($P = 0.24$, one-tailed). Neither observed heterozygosity nor self-assignment of recaptured hatchery bull trout differed significantly from that of wild adfluvial adults ($P = 0.4$ and 0.55, respectively).

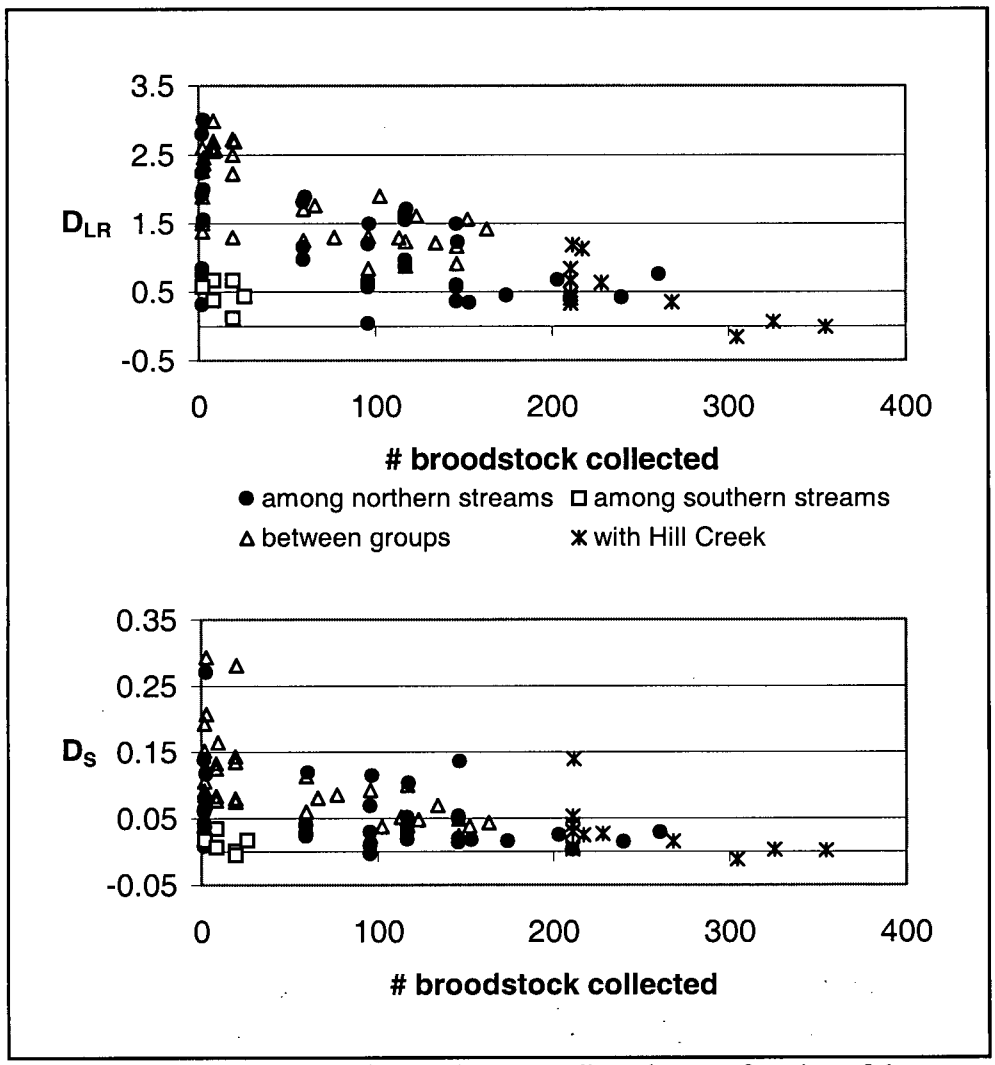

Figure 3.7. Pairwise genetic distance between tributaries as a function of the summed total number of broodstock collected from those streams (Mantel tests for D_{LR} (top) and D_S (bottom); r = -0.62, $P < 0.001$ and r = -0.47, $P < 0.001$, respectively). Pairwise comparisons are identified as among northern tributaries, among southern tributaries, between northern and southern tributaries, and any comparison involving Hill Creek (the site of the hatchery).

Genetic influences of hatchery bull trout

Neither number of hatchery fish released into a stream nor the number of broodstock collected from it was significantly correlated with H_e ($r^2 = 0.08$ and 0.0002, respectively; $P = 0.3$ and 0.9). Genetic differentiation among tributaries was negatively related to broodstock collection and releases of hatchery fish. Regardless of whether I performed the Mantel tests differently (D_S versus D_{LR}, including sample size corrections and fallers or restricting analyses to only Arrow tributaries, et cetera; all combinations were examined), the number of fish collected from a stream population for broodstock was strongly negatively related to its genetic distance from

other stream populations (Figure 3.7). The statistical relationship between genetic distance and releases of hatchery fish was more sensitive to analytical procedures.

Partial Mantel tests also strongly supported the homogenizing effect of broodstock removal, though statistical results were more variable than above. For example, residuals of the relationship between geographic and genetic distances were still negatively associated with broodstock collection ($-0.38 > r > -0.59$; and $0.02 > P > 0.001$). Interestingly, Mantel tests between broodstock collection and genetic distance, partialling-out effects of hatchery releases, revealed a significant or marginally significant negative relationship ($-0.27 > r > -0.45$; and $0.062 > P > 0.003$). This is in contrast to the reciprocal partial Mantel test, in which the influence of hatchery releases on genetic differentiation was never statistically significant and sometimes even estimated to be *positive*.

Discussion

Mechanisms maintaining differentiation: homing

Comparisons of adfluvial adults and juveniles sampled from the same streams failed to detect temporal changes in allelic frequencies. No statistical differences among juveniles and adults were encountered at all with respect to diversity, assignment, or amount of differentiation (among tributaries; see Table 3.1). Homing is therefore implicated as an important mechanism maintaining the genetic differentiation among tributaries found in Chapter 2. It is surprising that recovered hatchery fish, with their history of potential mixing, did not differ significantly from wild fish. Low sample size or relatively low survival of genetically mixed hatchery fish may explain this discrepancy.

Migration to natal sites by spawning adfluvial adults is also implicated by frequent recovery of marked hatchery fish in broodstock collections (from spawning tributaries) relative to the sport fishery. If adfluvial bull trout were panmictic and chose spawning streams randomly, the proportion of hatchery fish in every stream should be the same as that in the lacustrine fishery. I could not attribute this difference to the targeting of different-sized (and presumably different-aged) bull trout in the fishery versus broodstock collection. Broodstock collections and hatchery releases are geographically correlated, and higher frequency of hatchery fish in these streams probably results from homing to either ancestral streams or release sites.

Some adult bull trout captured as broodstock from other locations in previous years were subsequently recaptured during broodstock collection at Hill Creek. In addition, proportions of hatchery fish were highest in Hill Creek among broodstock streams. Both results implied that factors other than only cues at release and 'smolting' sites are important in homing migrations of bull trout. Age is negatively related to homing precision in other salmonids (Quinn 1993 and references therein), and learning or other effects of transporting adults among staging and spawning sites should not be discounted. Other explanations include Nordeng's (1971, 1977) pheromone hypothesis of homing which proposes that migrating adults are attracted to population-specific odors produced by rearing juveniles – bull trout may mistakenly 'home' to Hill Creek and other release sites by following chemical trails from related individuals that have been transported there. High proportions of hatchery fish at Hill Creek are also consistent with imprinting at the egg stage, which was previously thought unimportant in salmonids relative to later imprinting (Dittman *et al.* 1996, but see Quinn *et al.* 1999).

Lacustrine and demographic analyses

Traditional genetic analyses detected differences among distant lacustrine samples separated by dams (Table 3.2), but measurement of differentiation along a pre-defined axis (RDIA) was more useful for examining lacustrine genetic structure within a single reservoir. Genetic analysis of lake-caught samples showed that spawning populations differ in their lacustrine distributions (see Figure 3.3), and this lacustrine philopatry might have influenced the results discussed above. Information from telemetry, however, suggests that any physical limitation of lacustrine movement is minor. For example, within a period of one month, one bull trout tagged and released below Revelstoke Dam traveled south to Lower Arrow Reservoir and then north to Incomappleux River (K. Bray, CBFWCP, Revelstoke, B.C., personal communication), a minimum distance of approximately 180 km. Historical tag data also show that bull trout in CRDWK can migrate long distances – fish tagged in Arrow Reservoir had their migrations curtailed by Mica Dam (see Figure 1.1 in Chapter 1) prior to construction of Revelstoke Dam (Sebastian *et al.* 2000). It is possible that spawning populations differ in the distances and directions of movement during their feeding migrations (e.g., Jonsson 1982, Healey 1983, Pascual and Quinn 1994), but genotypic assignments indicated that long-distance lacustrine migrations are possible and commonly undertaken by at least some members of most stream populations (see Figure 3.4). If these genotypic assignments are accurate, the statistically significant result shown in Figure 3.3 represents a probability distribution of occurrence effected by a limited proportion of individuals migrating far from natal/spawning streams (or by a limited time or season that they do so) rather than a physical or behavioural limitation to dispersal at the population level. This possibility could be further evaluated by analysis of lacustrine samples collected at different times.

Note that not all streams within CRDWK were sampled, thus limiting the inferential power of genotypic assignment. Assignment was based on the relative likelihood of a genotype arising in one stream population versus others, but the genotypes of some lake-caught bull trout were expected to arise only very rarely in any of the sampled stream populations. Assignments of bull trout caught in Arrow Reservoir to tributaries above Revelstoke Dam (see Figure 3.4) were more frequent than expected as the dam and its turbines should constitute a significant barrier (there are no fish passage facilities). From similar results for bull trout collected below Cabinet Gorge Dam (Idaho), Neraas and Spruell (2001) concluded that downstream movement is frequent. My results may also be explained, however, by a lack of samples from tributaries between Shelter Bay and Revelstoke. These tributaries could contain genotypically more 'northern' populations than Jordan and Illecillewaet rivers (which have been influenced by hatchery operations; see below), and it is possible that bull trout from these unsampled populations were mistakenly assigned to tributaries above Revelstoke Dam. Thus, evaluation of which streams are most productive from a fisheries perspective, and other interpretations from assignment of lacustrine bull trout in CRDWK, should employ caution. As measures of RDIA (Figure 3.3) were perhaps more robust, the strongest conclusion from genotypic analysis of lacustrine bull trout is that bull trout captured from northern locations within Upper Arrow Reservoir have a more northern origin than bull trout captured from more southern locations within the reservoir.

Limited lacustrine mixing may be responsible for population amalgamation scores of derby and telemetry samples. These lacustrine samples signaled amalgamation less strongly than an artificial mixture of stream populations ("All streams" in Figure 3.5). In agreement with Figure 3.3, the artificially amalgamated stream sample probably represents a greater degree of panmixia than occurs during the lacustrine life history phase of bull trout in CRDWK. Even some individual stream samples (from Hill, Snow, and Bigmouth creeks) appeared more amalgamated

(ranked lower in H_e/#alleles) than lacustrine samples (see Figure 3.5). Despite this, and despite variance in ranking (only weak correlation among loci), significantly low ranks of samples known to be amalgamations instills confidence in this analysis. Also, the sample from Hill Creek, which ranked lowest, actually supports the analysis – hatchery operations there promoted a high degree of amalgamation (see below). Further, low genetic variation above the waterfall on Halfway River indicated bottlenecking (though ranking only second highest, perhaps owing to recent upstream gene flow and amalgamation among sites above the falls; see Chapter 2), suggesting that interpretations are valid throughout the range of rankings. Heterozygote deficiencies (indicative of population admixtures called Wahlund effects) in lacustrine samples were insignificant after correcting for multiple tests (see Chapter 2), underscoring the potential utility of the above analysis.

Stream populations varied widely in their H_e/# alleles rankings, and little of this variation was spatially predictable. In particular, ranks of streams near to the break point between northern and southern tributaries did not indicate a high degree of amalgamation, as might be expected if they exchanged more migrants across the break point than did more distal populations. As demographic effects influence ranks, this indicates a measure of demographic independence among even neighbouring streams, which is consistent with the homing behaviour described above. Bigmouth and Downie creeks are good examples of adjacent samples yielding different demographic signals, with the former seeming relatively amalgamated and the latter appearing relatively bottlenecked. Some theoretical analyses suggest that dynamics of populations can be independent if immigrants number fewer than 10% of the recipient population (Hastings 1993). For conservation purposes, managers of salmonid populations attempt to recognise independent population dynamics (McElhany *et al.* 2000), but although migration rates can be estimated from allelic frequencies (see Appendix D), determining the independence of population dynamics is difficult. Previous evidence that demographies varied among stream populations in CRDWK was limited to observations of differences in age of first lakeward migration and size at reproduction among bull trout populations in different streams, and alternate hypotheses could not be refuted (McPhail and Murray 1979). Despite the limited genetic data on demographic independence (only two loci) and thereby a limited utility for comparing spawning populations, my results support the possibility that life history differences among stream populations are related to demographic independence.

Results of demographic analyses have further implications: they suggest that environmental disturbances at broad scales in reservoirs (e.g., flooding and nutrient settlement by dams) do not overwhelm effects on population genetic data of local disturbances within streams (e.g., appropriation of water on Mackenzie Creek). Disturbance and other ecological and evolutionary processes relevant to management may occur at the scale of tributaries or smaller. My analysis endorses further examination and consideration of demographic differences among tributary populations. Differences in demography may owe to a variety of causes that are influenced by management initiatives. For example, the most important food species for lacustrine bull trout in CRDWK, kokanee (*O. nerka*), is patchily distributed in Arrow reservoir (Sebastian *et al.* 2000) and may therefore be differentially accessible by bull trout populations. As it will influence productivity of kokanee, fertilisation of Arrow Reservoir to compensate for nutrient settlement has implications for management of the bull trout fishery.

The bull trout fishery in Arrow Reservoir is currently managed as a single unit (e.g., Sebastian *et al.* 2000). Genetic differentiation among tributary populations (see Table 3.1) and assignment of catch to various tributary populations (see Figure 3.4) demonstrate that it is a mixed-population

fishery. Demographic analyses (see Figure 3.5) suggest that populations will respond differentially to a given fishing pressure. If bull trout were panmictic in lacustrine environments, no-harvest zones (such as the one from Mica Dam to Bigmouth Creek) would protect all adfluvial populations equally, and only differing productivities among them would have to be considered by management. Spatial variation in fishing effort (Lindsay 1987, Sebastian *et al.* 2000) and differentiation within the lacustrine fishery (see Figure 3.3) ensure that stream populations will be exposed to unequal harvest rates, however, further complicating their differential responses. For example, a poorly located no-harvest zone may only protect productive tributaries nearby and may deflect excess fishing pressure to other populations that can not sustain it. Alternation of low and high demographic indices in streams near protected and fished areas, respectively, would be expected in such a case. In contrast, judicious use of no-harvest zones in reservoirs could facilitate conservation of the most vulnerable populations, after such populations have been identified.

Patterns of straying

Genetic influences of strays did not appear to be strongly limited by distance. If strays are more likely to spawn successfully at locations near to their natal streams, a monotonic increase in differentiation may be expected with increasing distance among samples. Limited lacustrine dispersal, suggested above, should promote this trend. Not enough migrants are exchanged by nearby populations, however, to result in a spatial correlation of tributary ranks with respect to demographic inferences (see above), in agreement with the weak relationship found here between genetic and geographic distances. This relationship was significant for D_{LR} (a genetic measure based on all six loci) but not with D_S. Nearby streams were sometimes more divergent than the most geographically distant samples. A quantum effect among groups of northern and southern tributaries was largely responsible for the positive relationship between distance and genetic differentiation among tributaries – within the northern and southern groups, no significant isolation by distance was detected (see Figure 3.6).

Intuitively, straying rates should generally be higher among nearby populations, at some scale, than among relatively distant ones. Also, reproductive success of strays may be higher among neighboring populations than among distant populations (Reisenbichler 1988). In other studies of bull trout genetic variation, grouping populations on a regional scale revealed significant cohesion within regions (e.g., Taylor *et al.* 1999, 2001), but at more local scales (e.g., 25 km; Spruell *et al.* 1999) strong associations between genetic and geographic distances were not found. One possible explanation is that no effective migration occurs among populations and the pattern of differentiation among all populations is only an artefact of founding conditions and subsequent effective population sizes. But observed differentiation among populations in CRDWK suggests some mixing, as total isolation since deglaciation would likely produce greater differentiation than was observed (see Appendix D). Assuming a constant rate of decay since founding, differentiation between northern and southern groups of populations in CRDWK has been eroded at a rate of less than four effective migrants (strays) per generation (and even fewer effective female migrants; see Appendix D). Freshwater brown trout also rarely demonstrate isolation by distance, perhaps because geomorphological barriers are rarely considered (Carlsson and Nilsson 2001 and references therein). Given that straying occurs, and given my attempts to account for geomorphic structures (i.e., waterfalls), alternative explanations are relevant. If few migrants are exchanged among populations that differ greatly in demographic parameters (e.g., size), then differential drift could overwhelm straying patterns (e.g., isolation by distance). Influences of founding and demographics are not mutually

exclusive, and both are probably important in CRDWK (together with other influences on straying and the success of strays; see below).

Differentiation within tributary populations

Though less dramatic than differentiation among tributaries, allelic frequencies differed significantly within adfluvial tributary populations in general, with the most obvious differences within Slewiskin Creek. Analysis of RDIA revealed a trend whereby, relative to one another, upstream and downstream samples were genotypically more like samples from northern and southern tributaries, respectively. Again, this was most obvious within Slewiskin Creek (see Figure 3.2). Isolation by distance and other non-random straying patterns have been observed in other studies of salmonids (e.g., Bams 1976, Mills 1994), but propensity to migrate a particular distance in a non-natal stream is without precedent in literature known to me. Perhaps relevant to my results, Pascual and Quinn (1994) found that, after accounting for proximity, salmon are more likely to stray to streams that share physical characteristics of their natal streams (e.g., stream order or size, direction of flow) than to other streams that do not. Heritability, either from imprinting or additive genetic variance, with respect to temperatures or flow regimes of spawning sites, or migration distances to them, may explain increasing RDIA with distance upstream. Migrators that miscue among tributaries may still "home" such that strays from northern tributaries seek out spawning sites at colder locations or further upstream in southern tributaries. Conversely, strays from shorter, relatively warm southern tributaries may seek warmer downstream spawning sites in northern tributaries. During a single spawning period, individuals of some salmonid species commonly select multiple sites, kilometers apart, at which to spawn (e.g., Taggart *et al.* 2001). The significant result found here (see Figure 3.2) implies that spawning site fidelity for bull trout in CRDWK occurs on a more localized scale. This hypothesis (above) is one of two alternative explanations for the positive relationship between a sample's distance upstream within a tributary and its RDIA. It has a phylogeographic basis: colonists from the north have experienced relatively good access to upstream habitats, and bull trout from the south have had better access to downstream habitats than to upstream habitats. This is akin to expectations for RDIA among sites above barriers (see Chapter 2); but, in migratory populations, this hypothesis requires that southern bull trout gain less access to upstream habitat because of intrinsic biological differences rather than chance geological differences.

A second hypothesis is that access is equal but local selection regimes promote northern genotypes in upstream habitats and southern genotypes in downstream habitats. Upstream habitats are generally characterised by faster flows and lower temperatures than downstream habitats, and selection among these environments could act upon quantitative traits, characteristic of northern and southern bull trout owing to their respective histories. Adaptive divergence in morphometric and meristic traits exists in several salmonid populations differing in migration distance and flow regime, and temperature is a key agent of environmental selection that acts on various quantitative traits (see Taylor 1991 and references therein). Selection could also act upon the sampled loci themselves (or physically linked loci). Under neutral expectations, molecular DNA markers reflect the demography of populations. Selection on these markers can divorce their demography from that of the organism. Temperature is a potential selective agent that varies similarly with both latitude and altitude and could influence both north-south and upstream-downstream genetic patterns. It has influenced variation at molecular marker loci in other studies (see Taylor 1991 and references therein, Glemet *et al.* 1998).

I suspect that temperature is not acting on the genetic markers examined here, however, for several reasons. First, even without correcting for multiple comparisons, genetic variation in migratory populations did not deviate from Hardy-Weinberg equilibrium. Second, frequencies of alleles within the northern and southern groups of samples do not reflect well the temperatures observed in tributaries. For example, Incomappleux B is very glacial (and therefore cold), yet it has the highest frequency of the southern haplotype (Haplotype 1) of any northern population (see Figure 2.2). Third, except for shared differences among northern and southern groups of samples, frequencies of 'northern' alleles were not correlated among loci. That is, if temperatures of tributaries were responsible for observed genotypic frequencies, one should expect that bull trout from the coldest streams would have the highest frequencies of 'northern' alleles at each locus. Likewise, the warmest streams should have the most southerly alleles at each locus. This was not the case. For example, although Incomappleux B was relatively 'southerly' in terms of mtDNA, it was one of the most 'northerly' at *Ssa*197; Illecillewaet B displays the opposite pattern (see Appendix A).

Hatchery influences on genetic structure

Loss of heterozygosity is a common worry associated with hatchery supplementation of wild populations (Verspoor 1988 and references therein). By using large numbers of broodstock, spawning each individual with more than one mate, and equalizing family size, however, hatcheries can actually increase N_e, thereby preventing reductions to H_e (e.g., Hedrick *et al.* 1995). In the case of Arrow bull trout, artificially spawned adults in a given year were few, and family sizes were not equalized. Still, for several reasons, I did not expect to detect strong impacts of the hatchery on either H_e or differentiation among populations. First, recaptured hatchery fish did not display genetic attributes significantly distinguishing them from wild fish (see above). Second, genetic effects in non-equilibrium conditions depend upon relative effective numbers of wild and stocked fish, respectively (Gharrett 1994, Hedrick *et al.* 1995, Waples 1999). Early data on releases of hatchery fish were poor, and neither N nor N_e is yet known for recipient streams. Thus, tests of perturbations on systems had uncorrected variance and reduced analytical power. Third, increased variance in reproductive success, promoted by hatchery operations, may have been nullified – statistically only – by promotion of outbreeding. That is, over-representation of a few individuals in a stream by planting their hatchery-reared offspring would tend to increase homozygosity and thus increase differentiation among streams; introduction of exogenous genetic variation via outcrossing and outplanting would have the opposite effect. Fourth, few hatchery-clipped fish had been recaptured in the sport fishery at the time of this study, possibly because of poor survival. Poor post-release survival of hatchery fish would limit their influence on the distribution of genetic variation.

Expected heterozygosity (unbiased) had no significant relationship with releases of hatchery fish and, in contrast to demographic analyses using allelic diversity, the rank of Hill Creek was not exceptional. Populations in this study did display somewhat low heterozygosity relative to that found within populations of other freshwater fish (see DeWoody and Avise 2000), but not compared to other studies of bull trout (e.g., see Figure 2.4, Chapter 2). In contrast, comparison of pairwise genetic differentiation among tributary populations supported the notion that their involvement with the hatchery program has promoted homogenization (see Figure 3.7). Comparisons of hatchery activity and genetic distance among streams could not account for differentiation within streams (broodstock capture and hatchery release locations were not complete), but differentiation within streams appeared to have no effect on the results. Estimates of differentiation were not less for stream populations sampled at multiple locations than were populations sampled at a single location (see Appendix C). Also, analyses suggested that this

effect was not merely correlative. That is, I obtained similar results when I tried to remove background influences of geography by doing partial Mantel tests, whether or not I included samples north of Revelstoke Dam. Therefore, prior to supplementation efforts, there was more allelic differentiation among populations than measured in this study.

Among populations with reciprocal migratory access below waterfalls, subdivision of genetic variation in CRDWK at diploid loci (F_{ST} = 0.11) was greater than that found in six of seven studies of anadromous species and in 15 of 49 freshwater species (see review by Ward *et al.* 1994). This degree of differentiation was also greater than that found in 7 of 14 studies of anadromous and freshwater salmonids sampled over broad geographical ranges (see Wenberg *et al.* 1998). As these sources did not distinguish differentiation among accessible populations from differentiation across barriers for freshwater species, differentiation observed within CRDWK should be interpreted as impressively high. Furthermore, despite a striking homogenizing effect of the hatchery program (Figure 3.7), tributaries to Upper Arrow Reservoir, where most hatchery activity occurred, were more distinct from each other than were Lower Arrow and Revelstoke Reservoir tributaries (Table 3.3). Genetic structure comparable to that found in more pristine watersheds remains in CRDWK (see Table 2.6 in Chapter 2) and may yet be conserved. My results testify to the strength of the north-south division among tributaries and to a limitation of hatchery effects.

Table 3.3. Distribution of genetic differentiation within CRDWK. Estimates are F_{ST}, measured among B samples (adfluvial bull trout in tributaries) with fallers removed (data set 1).

Tributaries compared	mtDNA	4 usat	*Sco*1
Revelstoke Reservoir	0.02	0.05	0.02
Upper Arrow Reservoir	0.27	0.11	0.02
Lower Arrow Reservoir	0.10	0.01	0.02
all CRDWK	0.41	0.11	0.03

Though obvious, the mixing effect of the hatchery appeared to be very localized. For example, two of the most genetically distinct tributary populations in the study were the nearest sample sites (Incomappleux River and Mackenzie Creek) to the most affected tributary (Hill Creek), which was the least distinct (see Figure 3.7 and Appendix C). If outplanted hatchery fish have high fitness, such limitation is unexpected because they have less opportunity to imprint and are expected to express relatively poor homing ability (Leary *et al.* 1993, Quinn 1993, Waples 1999). This is especially true when release sites are near the mouths of spawning streams (Quinn 1993 and references therein). Genetic components to homing in other salmonines have also been reported (McIsaac and Quinn 1988, Labelle 1992), so the observed localization is impressive. It is not unique, however. For example, hatchery-mediated genetic introgression occurred in stocked streams but not in accessible unstocked streams in studies of steelhead (*O. mykiss*; Williams *et al.* 1997b) and sockeye salmon (*O. nerka*; Hendry *et al.* 1996).

Limitation of hatchery-mediated gene flow to stocked streams could result from either strong homing to release sites or low relative fitness of strays from that location (Williams *et al.* 1997b). Numerous studies of salmonids have demonstrated low relative fitness of hatchery fish (e.g., *Oncorhynchus*, Altukhov and Salmenkova 1987; *Salmo*, Kelly-Quinn and Bracken 1989, *Salvelinus*, Finstad and Heggberget 1993), and limited representation of hatchery fish in the Arrow sport fishery is suggestive here. Given low relative fitness and poorly refined homing

expected for hatchery fish, how could hatchery operations so strongly influence genetic variation within recipient streams?

Mechanisms of hatchery influence

When controlling for effects of broodstock collection, releases of hatchery fish did not significantly reduce differentiation. In contrast, broodstock collection remained a significant negative influence on pairwise differentiation among stream populations in partial Mantel tests that controlled for hatchery releases. In general, hatchery fish seem to contribute to natural productivity best in the absence of conspecific and heterospecific competition (reviewed by Fleming and Petersson 2001). If, in tributaries involved in hatchery operations, population densities of wild bull trout are depressed by broodstock removal, then hatchery-produced fish may avoid competitive interactions that would otherwise impede their success. When straying to tributaries not as heavily utilized for broodstock collection, where rearing habitats are closer to carrying capacity, exogenous fish or their progeny may suffer low reproductive success or high mortality. Thus, impacts of hatchery fish would be limited to broodstock streams. In support of this competition-based explanation, stocked brown trout (*S. trutta*) could not make genetic contributions in streams with native populations (Moran *et al.* 1991). Also, hatchery fish released into stocked streams only survived when wild fish were first reduced via electroshocking and never increased stream populations beyond an apparent carrying capacity (Kelly-Quinn and Bracken 1989).

Note that successful exogenous fish may be artificial or wild in origin. Wild strays from other streams may also enjoy greater success in depressed populations (with available habitat) than they would in relatively dense populations. That is, success of hatchery progeny is not required to explain reduced differentiation of hatchery streams. Rather, reduced differentiation may be effected indirectly by the reduction of population densities. Of streams involved in the broodstock collection program, southern tributaries 'donated' few broodstock (see Figure 3.1), and only one hatchery fish has been captured from there. Nevertheless, southern tributaries were poorly differentiated from one another (see Table 3.3 and Figure 3.6). Lower Arrow populations had relatively high frequencies of 'northern' alleles compared to Slewiskin B (see Figures 2.1 and 3.1). Unlike Slewiskin B, Lower Arrow populations have borne the brunt of poaching efforts (J. Beck, Penticton Conservation Office, personal communication), and strays may do well there. Within Slewiskin B, high frequencies of 'northern' alleles were restricted to upstream habitats (Figure 3.2) which may have been empty until recent habitat manipulations. Also note that diversion of individuals to Jordan River, caused by blockage of their migrations to northern tributaries by Revelstoke Dam (Sebastian *et al.* 2000), should have made Jordan B a very genotypically 'northern' sample. Instead, coincident with the hatchery's later activities there, Jordan B had a remarkably 'southern' RDIA score (compare Figure 2.9 in Chapter 2 to Figure 3.2). Whereas some studies have found that fishing pressure reduces influences of hatchery fish (e.g., hatchery fish may be more vulnerable to angling; Garcia-Marin *et al.* 1998), my results suggest that introgression may be facilitated by harvest in this case.

The above explanation for hatchery impacts allows that wild fish stray at a *constant* rate, but proposes that their fitness is relatively high in habitats with depressed population densities. Above, in the section on homing, I discussed two ways in which hatchery operations could *increase* straying by wild fish. I alluded to Nordeng's (1971, 1977) pheromone hypothesis of homing and suggested that even unfit juveniles rearing at the hatchery and in recipient streams could produce familial odors and that these tributaries could mimic the natal streams of wild fish. As wild fish strayed to these streams at an increasing rate, genetic differentiation of those

streams would decrease. That is, outplanting of juveniles could increase straying in migratory bull trout. Second, rates of straying generally increase with age (Quinn 1993 and references therein), and I suggested that experiences interfere with homing ability. Unknown are the influences of capturing migratory adults from other streams and holding them at Hill Creek. This could make them more likely to use non-natal streams in future spawning events. A possible result is that genetic distances may decrease between these non-natal stream populations and those from which the broodstock were collected. Evidence for this possibility is limited to high recapture rates of previously used broodstock at Hill Creek.

As discussed above there are several explanations that are not mutually exclusive for how hatchery operations could have reduced genetic differentiation among streams. Because I found little evidence of high stray rates (e.g., adults and juveniles from the same streams were not differentiated), my results are perhaps most consistent with reduced population density permitting the existence of exogenous genes in streams that received hatchery-produced outplants. This warrants concern about hatchery-mediated detriments to 'bioheritage', local adaptation, fitness, production, and evolutionary potential of bull trout in CRDWK. Nevertheless, a large amount of genetic variation exists among tributaries in the area, reflecting historical and potentially adaptive evolutionary differences. Efforts by the hatchery program to reduce genetic mixing are justified and should probably be increased. For example, restriction of broodstock collection and release of hatchery progeny to Hill Creek could retard further anthropogenic homogenization.

Understanding mechanisms of homogenization – and the efficacy of measures to prevent it – requires information about homing, carrying capacities of streams, and other limits to natural bull trout production (Bams 1976, McPhail and Murray 1979). It may be found that even production of highly fit hatchery fish can only supplant rather than supplement wild production (Hilborn 1999) of bull trout. In this chapter, I have shown that harvest of bull trout can negatively affect bull trout population structure in two ways: via differentiated responses of populations in a mixed-stock fishery and via increased introgression from hatchery fish or wild strays in a less competitive environment. Results of this thesis also suggest that previous assumptions and interpretations made regarding bull trout management in CRDWK need to be further scrutinized and in some cases discarded. Chapter 4 reviews contrasts between those assumptions and my findings.

Chapter 4. Evaluation of methods, findings, implications, and future research

In this thesis, I used a single data set to make many inferences and conclusions. Support for each conclusion was not equal. In this chapter I review some of the findings of this thesis and evaluate my own work. I highlight the most strongly supported conclusions and identify related issues that I think most urgently require more research. These issues generally revolve around interactions of population densities, selection and migration in generating genetic patterns presented in earlier chapters. In this context and with respect to my findings, I compare and contrast conservation of resident and adfluvial bull trout populations.

Evaluation of findings and methods

Among the most strongly supported conclusions in Chapter 2 were that populations separated by waterfalls were genetically distinct and that (at least some) populations above waterfalls did not have strong, persistent genetic influences on the populations below them. Related to this, individual populations resident above waterfalls contained only a small proportion of the total genetic variation of bull trout in CRDWK but a considerable proportion as a group. Chapter 3 showed that adfluvial populations are also genetically diverged from one another and that homing is an important mechanism that maintains their distinctions. In addition, streams most involved in the operation of Hill Creek Hatchery's bull trout program are less genetically distinct than other streams. Finally, derby samples and other summer lacustrine samples showed that adfluvial bull trout are not generally panmictic during their feeding migrations in Arrow Reservoir. Studies of downstream fish populations rarely consider genetic influences from adjacent, potential source populations upstream of barriers (but see Neraas and Spruell 2001). Although my findings were not attributable to contemporary downstream gene flow (data sets 1 and 2 yielded the same qualitative results), downstream migration did occur, and testing for influences was necessary and improved the study.

Alone, the four diploid microsatellite loci supported this study's strongest findings. Relative to use of only these loci, however, combined use of all haploid, diploid, and tetraploid loci (via assignment; e.g., RDIA) generally provided stronger support and more information. Unfortunately, not all analyses could incorporate every locus. To answer some questions, I needed to combine my data with the work of others, none of which examined genetic variation at *Sco*1. Nevertheless, incorporation of mtDNA data (from Taylor *et al.* 1999) and data from *Sco*19, *Sco*23, *Sfo*18, and *Ssa*197 (from Costello and Taylor, in preparation) was fruitful and elucidated phylogeographic relationships among groups of populations. The scant geological literature available for the study area was beneficial in interpreting those relationships. With respect to examination of hatchery operations, I found creel surveys and the marking programs of broodstock and hatchery releases essential for relating my data to the ecology of bull trout in CRDWK. Without recapture rate estimates in reservoirs and streams, I would have no perspective on the magnitude or mechanism of the hatchery's potential influences. Radiotelemetry studies (K. Bray, CBFWCP, Revelstoke, personal communication) provided tissues useful for analysis and an appreciation for the migratory capacity exhibited by some adfluvial bull trout. In all, despite limitations on the compatibility of data, collaboration amongst studies was beneficial and should be increased, if possible. For example, both tissue samples and creel data from the fishery in Lower Arrow Reservoir would extend the relevance and scope of my findings. Also, radiotelemetry of broodstock would either support or refute my allegations of an effect of hatchery operations on their future migrations. More comprehensive mtDNA analyses in the East Kootenays could confirm phylogeographic inferences.

I do not recommend that future studies include examination of *Sco*1. Many allelic variants were detected at this locus, giving it utility in demographic comparisons and analyses that employed genotypic assignment. It was also informative in contrasting genetic variation among isolated populations. Other features, however, were undesirable. Undetermined characteristics of *Sco*1's tetrasomy (e.g., frequencies of crossovers between the centromere and locus; Ronfort *et al.* 1998), combined with the apparently null allele, made generally simple analyses prohibitively difficult (e.g., testing Hardy-Weinberg and linkage equilibria). Because of complications related to dosage at this locus (see Methods, Chapter 2), allele visualization and genotype identification were time consuming and even impossible in some cases. Finally, putative biological effects on *Sco*1 were often statistically insignificant at this locus (perhaps owing to its greater effective population size), whereas other loci yielded significant results in the same analyses (e.g., see Figure 2.7, Table 3.2). Several diploid microsatellite loci not used in this thesis are available and have been used in other studies of bull trout (e.g., Spruell *et al.* 1999). If more "hyper-variable" loci (like *Sco*1, but more easily interpretable) can be identified, combination with existing markers can replace and improve upon the contributions of *Sco*1.

The role of natural selection

Genetic loci, even the four diploid microsatellite loci, differed in the type and strength of signal that they indicated. Such variation is expected under a neutralist biogeographic interpretation. For example, some loci were strongly differentiated among northern and southern stream populations putatively owing to founder effects or drift among the source populations that colonized them. But drift toward different allelic frequencies is not assured in isolated populations, and the randomness of drift likely explains lack of congruent north-south patterns at some loci (see Appendix A). Differential selection on the studied loci (or at physically linked loci) was not considered a viable alternative because there was no significant deviation from Hardy-Weinberg or linkage equilibria within stream populations (after fallers were removed, see Chapter 2). Further, presumed stream temperatures (a potential agent of selection) and allelic frequencies were not coordinated across genetic loci (see Chapter 3). Because tests of Hardy-Weinberg equilibrium are weak (Jin and Chakraborty 1995) and temperature is only one of many potential agents of selection, other mechanisms and effects of selection require more scrutiny. I hypothesized that natural selection maintains the phylogeographic signals of genetic loci by acting on the historical adaptive background of individuals, rather than generating the north-south pattern in the genetic loci examined by this study. That is, historical population structure was responsible for the allelic differentiation observed in this study and for locally selected differences among populations; but maintenance of the allelic distribution is a byproduct of local selection against other traits of strays, rather than selection against variants at the loci I studied. If selection acted on the variants present at the loci I studied, one could expect upstream sample locations above waterfalls to yield more 'northern' genotypes (similar to the trend observed below falls). I found no evidence for such an association (see Figure 2.10).

This study was not designed to detect natural selection. Comparisons between adults and juveniles among stream populations can be used for such estimates (especially in a sampling design within and across cohorts; e.g., Bert and Arnold 1995). I could demonstrate that adfluvial adults home to some degree in this study (see Table 3.1), but too few adult samples were available to test for reduced genotypic influences of adult strays on recruiting populations. That is, future study including more adfluvial adults could answer the important question of whether selection (rather than homing alone) helps to maintain differentiation among populations –

whether migration exceeds effective migration. Similarly, genotypic study of parents and offspring permits estimation of fitness among families and, if applied to broodstock and to releases and recaptures of hatchery fish, such study could estimate effects of selection on the success of hatchery supplementation (e.g., Marsden *et al.* 1993, Hansen *et al.* 2001).

Implications of intraspecific invasion of A populations

With respect to genetic variation among samples within CRDWK, I ascribed importance to competition and natural selection because hatchery effects were associated with removal of broodstock from streams and because faller genotypes had limited introgressive effects on migratory populations below waterfalls. Utter (2001) found that freshwater salmonid populations were more invasible by exogenous genotypes than were anadromous populations. He attributed the empirical phenomenon to the importance of local selection for environmentally appropriate migratory behaviour. That is, freshwater populations differ from one another less in important ways (e.g., smoltification, migration) than do anadromous populations, with respect to their response to local selection regimes. My data are consistent with Utter's (2001) analysis: genotypic profiles of individuals in two resident populations (Jordan A and Halfway A) suggest that gene flow occurred in the upstream direction (see Chapter 2). Anthropogenic facilitation of this gene flow was mild (limited releases of hatchery fish above Jordan River's barrier and reduction of Halfway River's waterfall with dynamite) relative to attempted facilitation in migratory populations (via hatchery operations). Nevertheless, two thirds of bull trout from the two downsteam sites within Halfway A, and nearly half the Jordan A sample were assigned (on the basis of their genotypes) to below the Halfway and Jordan waterfalls, respectively.

Perhaps selection for appropriate migratory behaviour limits successful recruitment of exogenous alleles in adfluvial bull trout populations, whereas the simple life history of resident populations is not so discriminating. The steep slopes within CRDWK, however, shorten and simplify adfluvial migrations. Thus, local selection on migratory behaviour may be unimportant. If so, one could argue that resident populations should be more specialized and less invasible than migratory populations. This is because residency above a barrier should enhance the relationship between a population and the habitat there (e.g., seasonally harsh conditions could not be avoided via migration) and because a barrier prevents migration that dilutes both local selection and adaptation (e.g., Riechert 1993). My data appear to favor the importance of locally adapted migratory behaviour over this latter argument, but other possible reasons for relative invasibility of resident populations should be considered.

I imagine two alternative explanations for apparent invasibility of resident populations in CRDWK. One possibility is that, as individual movements among fluctuating habitats are restricted, temporal variance in selective regime is magnified within resident populations and overlaps and exceeds spatial variance in selective regime among populations. Under such conditions, endemic resident individuals may have little or no local advantage over most exogenous individuals. Another reason that endemic individuals may have less local advantage over exogenous individuals in habitat above barriers may relate to differences in effective population sizes. In CRDWK for example, populations resident above some waterfalls seem very large but have historically low effective population sizes, as indicated by their low genetic diversity. Inbreeding depression and lack of genetic diversity may actually promote exogenous genotypes because they reduce fitness and limit adaptation in local populations (Vrijenhoek 1994, Ingvarsson and Whitlock 2000, but see Armbruster *et al.* 1998), respectively, relative to at least some exogenous individuals that gain access to that environment. These mechanisms,

potentially responsible for the phenomenon of differential invasibility, have different managemnet and conservation implications than Utter's (2001) explanation.

The importance of a peripheral population (in this case, a stream resident population) for conservation depends largely upon its replaceability (see Chapter 2). Whereas peripheral habitats are important for a species' conservation (Channell and Lomolino 2000), extinction of a population that is not unique in evolutionarily important ways can be compensated by translocation from another population. That is, such a population has little value relative to its habitat. Simplistically, success of exogenous genotypes in populations resident above barriers suggests that those populations are replaceable and have little conservation value. But if populations are invasible despite or because of their evolutionary distinctions, they are less replaceable and of greater concern for conservation. For example, a population that is inbred may nevertheless harbour locally adapted genetic variation, and a population with limited but unique genetic variance will evolve unique solutions to environmental challenges (given time and opportunity).

It is unpopular to conserve unfit populations (e.g., McElhany *et al.* 2000), but such populations can persist in the absence of strongly negative biotic interactions or drastic abiotic change. For example, the aurora trout (*Salvelinus fontinalis timagamiensis*) was eliminated from all of its endemic habitats by acid rain in Ontario. Six males were spawned with three females (Patrick and Graf 1961), and a hatchery population was maintained for approximately thirty years in artificial habitat. Following this bottleneck and subsequent domesticating selection, aurora trout were successfully re-introduced to their endemic lakes, which were somewhat rehabilitated but devoid of other fish species (Snucins *et al.* 1995). Such success may not be expected in a highly competitive environment. This case study illustrates three principles: first, special populations should not be exposed to drastic environmental change; second, changes in selective regime may be withstood more easily in the absence of negative competitive influences; third, evolutionarily distinct populations can be valuable for conservation despite being inbred or otherwise unfit.

Thus, it is important to determine whether resident populations are invasible despite their evolutionary distinction, or because they lack evolutionary distinction. In the case of bull trout populations in CRDWK, more work needs to be done to better evaluate whether resident populations are indeed invasible. Enhancement initiatives exposed Jordan A and (perhaps) Halfway A to exogenous individuals. Those populations seemed to contain individuals recently descended from downstream B populations, but no control or baseline data were available. I could only compare them to other populations above falls of which only one was, like them, sympatric with other fish species (and accessible, therefore, to colonization relatively late in postglacial history; see Chapter 2). Bull trout populations above waterfalls in CRDWK are raw material for comparative study of the relationship between invasibility (or fitness) and neutral genetic variation, but subdivision and interspecific interactions within A populations in CRDWK complicate the issue of invasibility of resident populations. Gene flow from upstream sites within A populations, where other fish species were not found, may reduce appropriate adaptation at downstream sites where interspecific interactions are more common. Individuals with an ancestral legacy of evolution with other fish species below waterfalls may then have an advantage over native genotypes at sites immediately above waterfalls. (A summary of relevant future studies identified here and elsewhere in this thesis is provided in Appendix E.)

Impacts of exploitation and migration among B populations

Adfluvial populations and populations resident above waterfalls differ also with respect to implications of harvest. Resident populations may be more sensitive to a given fishing pressure (resident females have fewer eggs and a lower maximum recruitment per spawner than migratory populations) but generally attract less fishing pressure because of the small size of individual fish. Also, they appear less vulnerable to problems inherent in mixed-harvests. And, whereas catch-per-unit-effort (CPUE) may be replaced – and continued fishing pressure thereby encouraged – by migration among migratory populations, CPUE in an overexploited population will not be maintained if that population is resident above a barrier falls. Intraspecific replacement of CPUE should be considered potentially problematic within subdivided populations above waterfalls, however, as should interspecific replacement in resident populations sympatric with *Oncorhynchus* species.

Below waterfalls, legal fishing for bull trout in CRDWK is restricted to the lacustrine life history phase, and these fisheries are not mixed interspecifically (i.e., bull trout are not exposed to fishing pressure directed toward rainbow trout; Lindsay 1986). Intraspecific population mixture is substantial (see Figures 3.4, 3.5), although spatial subdivision in the lacustrine phase (see Figure 3.3) may offer some refuge to migratory populations via declining CPUE in overfished regions of the reservoir. This protection would be negated, however, if lacustrine bull trout follow an ideal-free distribution (Fretwell and Lucas 1970) to some degree (i.e., bull trout removed from an overfished region are replaced by bull trout dispersing from others), or if the amount or geography of angler effort was insensitive to CPUE. Immigration from populations upstream of dams could also have profound influences on downstream lacustrine fisheries. If bull trout do pass through Revelstoke Dam into Arrow Reservoir, a high CPUE may be maintained at the expense of local bull trout populations.

In streams, where broodstock collection and poaching occur, effort is probably very insensitive to declining CPUE in diminished populations. Poaching, like broodstock collection, is common in pools at the base of waterfalls (J. Beck, Penticton Conservation Office, personal communication), because even depleted populations will yield high CPUE there. In addition, poachers tend to restrict their activities to easily accessible but concealed locations. Thus, opportunity rather than population density plays a large role in where poachers direct their efforts, and negative feedback via CPUE will be a less effective agent for conservation.

Like movement in the lacustrine environment, natural migration or dispersal among spawning streams also has important consequences for the impacts of harvest on populations and their management. I could not tell if natural migration or hatchery-mediated migration was responsible for introducing exogenous genotypes into streams used for broodstock collection. If natural immigration is low, populations recover from overharvest mostly via endogenous production, and their recovery can be monitored by counting spawners in streams. If natural immigration is high and recovery of populations is exogenous and rapid, spawner counts will not detect the erosion of genetic variation as indigenous genetic variation is replaced by potentially maladapted exogenous genetic variation.

Migration of A individuals into B populations

My data suggest that immigration from populations above waterfalls into those below ("falling") is common, and that fallers comprise a considerable proportion of bull trout in some streams immediately below waterfalls. I concluded that effective immigration (successful recruitment into the migratory population), however, was rare. This interaction has the same potential

implications as natural immigration from adfluvial populations. If frequency or success of F_1 or later matings between life history types increases when migratory populations are reduced, then genetic variation of locally adapted B populations that are disturbed or exploited may be increasingly threatened by A populations directly upstream.

Even if fallers have no reproductive fitness under any circumstance, they may still present a danger to the productivity or persistence of endemic migratory populations. There are several likely examples of interactions that harm migratory populations. Fallers may compete for food or space with migratory juveniles or may eat them. Fallers may consume the eggs of migratory spawners or may fertilize them (and thus make them unavailable to fertilization by migratory males). Making habitats above waterfalls accessible to migratory individuals could increase the frequency of such interactions. Conditions that facilitate negative interactions between migratory and resident bull trout (e.g., releasing migratory fish above waterfalls, dynamiting waterfalls) should be identified and avoided.

Denouement

Compensation and management initiatives related to the Arrow Reservoir bull trout fishery were predicated on or at least implied a variety of assumptions. Some of these assumptions are contradictory, and some are contradicted by results presented in this thesis (Table 4.1). Early compensation for hydroelectric development was directed to mitigating lost spawning habitat for sport fish species, and efforts such as hatchery supplementation and destroying waterfalls were justified on the assumption that stream habitat was limiting. In contrast, more recent fertilization of Arrow Reservoir implies that lacustrine productivity is the factor limiting at least some sport fish species. Neither assumption has been tested for bull trout. With respect to population structure in bull trout, possible subdivision was ignored by early hatchery protocols but was implied by implementation of the no-harvest zone from Bigmouth Creek to Mica Dam in Revelstoke Reservoir. While my analyses demonstrate genetic distinctions both among tributaries used for spawning and among angling locations within Arrow Reservoir, no data are available regarding stock structure among lacustrine sites within Revelstoke Reservoir.

Regarding bull trout management strategies, only concerns regarding mutual limitations of angling and bull trout production are consistent with management practices (see Table 4.1). A recent review of Arrow Reservoir fish populations by Sebastian *et al.* (2000), however, did not detect an impact of hydroelectric developments on angling for bull trout (potential immigration from above Revelstoke Dam was not considered) and suggested that fishing effort is small and has only negligible effects on bull trout population size. Spatial variation in the size and recapture rates of bull trout within Arrow Reservoir, spatial variation in production of kokanee (the most important prey item for bull trout), and spatial variation in fishing effort were ignored in evaluation of exploitation rates (see Sebastian *et al.* 2000). Because the spatial scale of genetic distinction in bull trout is also small (see Chapter 3), disregard for these spatial distinctions in evaluation of exploitation rate will underestimate the impacts of angling on some populations. The sex ratio of bull trout caught in the fishery is unknown and could also be important. Smith and Slaney (1980) found spatial variation in sex ratio in the catch of Dolly Varden (*Salvelinus malma*) such that anglers targetted mature females most. Combined, erroneous assumptions and uncertainties with respect to spatial variation in bull trout and their harvest invalidate the conclusion of Sebastian *et al.* (2000).

Table 4.1. Assumptions of various management and compensation initiatives. Y indicates that an assumption is consistent with the management activity, N indicates that the opposite assumption is consistent, S indicates that stream habitat limits bull trout production, R indicates that lacustrine habitat is limiting and NA indicates that the assumption is irrelevant to the management activity. The results of this thesis invalidate assumptions that bull trout are panmictic among lacustrine and spawning sites. Results further suggest that angling may be a conservation concern, but they do not directly address limitations of bull trout production.

Management strategy	Implied Assumption			
	Limiting habitat (R/S)	Spawning-site panmixia (Y/N)	Lacustrine panmixia (Y/N)	Angling is a concern (Y/N)
Hatchery supplementation	S	Y	Y	Y
Outbreeding & outplanting	S	Y	NA	NA
Reducing waterfalls	S	NA	NA	Y
No-take zones in reservoir	NA	N	N	Y
Fertilization of reservoir	R	NA	Y	NA
Management (regulations & population estimates) at the reservoir level	NA	Y	Y	NA

In this chapter, I have described the implications of my findings and the consequences of various management strategies. I have also tried to identify questions (raised by, but not answerable with my data) whose answers have different conservation and management implications. In summary, population subdivision of bull trout in CRDWK occurs at a finer scale than historically assumed for purposes of management and compensation. A general result is that perturbations with deleterious effects assumed to be diffused or insignificant for putatively large, robust management units may actually have very acute effects on small, vulnerable populations. The nature and extent of those deleterious effects, and management's ability to recognize them, in some cases depend on the role of natural selection and whether recovery of populations is mostly endogenous or exogenous. Experiments and other study can resolve several of these issues. I cannot conclude that information gained will provide insights on how to eliminate biodiversity losses, but risk management and impact assessment could be improved and erosion of biodiversity could be reduced. Previously, many assumptions of management plans were not tested prior to their development, and impacts of perturbations and compensation activities were generally poorly monitored. This must change. The brief history of bull trout management and compensation in CRDWK seems to indicate that convenience of a working hypothesis plays a role in its adoption. This is a potentially harmful practice that may be common in fisheries management and elsewhere (c.f. Lichatowich 1999).

Chapter 5: Appendices

This chapter provides additional information on data and analyses that were not described in great detail in earlier chapters. All information is provided in summary form, either in figures or tables. Appendix A shows frequencies of allelic variants detected at each sampled location. Dendrograms created using 2 distances (Nei's D (Nei 1972, 1978) and Cavalli-Sforza's cord distance (Cavalli-Sforza et al. 1967)) and relating all stream populations in CRDWK are shown in Appendix B. Appendix C provides pairwise measures of genetic differentiation among B populations, and allows comparison of data sets 1 and 2. In Appendix D, I describe attempts to relate genetic variance among groups of northern and southern populations to gene flow (or effective migration rate, N_em) using both equilibrium and non-equilibrium methods. Tributary populations were not examined individually in this analysis because population number and size estimates are lacking. Finally, Appendix E summarizes research questions relevant to the implications of this thesis.

Appendix A

Table 5.1. Allele frequencies in geographic samples at six loci (data set 1). Samples from above waterfalls in streams, below waterfalls in streams, and in lacustrine environments are denoted by A, B, and L. Frequencies of null alleles that I could not confidently determine are marked N.

Sample	*Sco*1																			
location	173	175	177	179	181	206	214	222	225	227	229	231	233	235	237	239	241	243	245	null	N
Bigmouth B	0.18		0.03	0.08	0.04		0.02	0.01		0.06	0.13	0.04	0.05	0.06	0.06	0.03	0.06	0.02	0.04	0.10	
Downie B	0.11		0.09	0.24	0.01						0.06	0.04	0.09	0.14	0.08	0.03	0.01			0.11	
Carnes B	0.07	0.03	0.10	0.20	0.02					0.03		0.11	0.13	0.05	0.09	0.03	0.05	0.02	0.01	0.06	
Jordan B	0.23		0.05	0.13			0.03	0.08		0.03	0.13	0.08	0.05	0.10	0.05		0.05	0.03		0.00	
Illecillewaet B	0.18		0.02	0.14	0.08		0.01	0.02	0.01	0.01	0.05	0.08	0.07	0.08	0.07	0.02	0.02		0.01	0.13	
Incomappleux B	0.21		0.05	0.24	0.14		0.03		0.01		0.02	0.05	0.03	0.07	0.02		0.01		0.05	0.06	
Hill B	0.21		0.02	0.14	0.04		0.01	0.01	0.02	0.01	0.06	0.06	0.06	0.10	0.08	0.01	0.02		0.01	0.12	
Mackenzie B	0.36		0.04	0.04							0.08	0.08	0.08	0.16	0.01					0.16	
Halfway B	0.20			0.05	0.10		0.01	0.04	0.04		0.11	0.08	0.08	0.07	0.05	0.02	0.03	0.01	0.04	0.09	
St. Leon B	0.26			0.07	0.03			0.08		0.01	0.07	0.05	0.02	0.04	0.10	0.03	0.03	0.01	0.01	0.20	
Slewiskin B	0.19		0.09	0.04	0.07			0.07	0.02	0.03	0.08	0.09	0.08	0.09	0.06	0.01				0.09	
Caribou B	0.31		0.09	0.08	0.04						0.04	0.01		0.13	0.13	0.06	0.01		0.01	0.10	
Snow B	0.19		0.05	0.07	0.05	0.02	0.01	0.02	0.01			0.02	0.05	0.05	0.25	0.02	0.01			0.18	
Taite B	0.18		0.01	0.09	0.03	0.01					0.08	0.04	0.08	0.15	0.13	0.03		0.01		0.19	
Downie A	0.03			0.05										0.90						0.03	N
Jordan A	0.20			0.07				0.25	0.07		0.02	0.05		0.11	0.09		0.02			0.11	
Payne A															1.00					0.00	N
Halfway A	0.22			0.02	0.11	0.01		0.02		0.01	0.13	0.03	0.03	0.06	0.13	0.02	0.03	0.01		0.20	
St. Leon A	1.00																			0.00	N
Kuskanax A	0.42		0.07	0.03								0.11	0.01	0.03				0.25		0.08	
Whatshan A	1.00																			0.00	N
Woden A	0.32				0.18								0.02	0.14	0.05					0.30	
Kinbasket L	0.15		0.05	0.10	0.05					0.10	0.15			0.10		0.05	0.05	0.05		0.15	
Revelstoke L	0.25		0.06	0.16					0.03		0.03	0.13	0.06	0.06	0.03		0.06	0.03	0.09	0.00	
Arrow L	0.18		0.04	0.15	0.08		0.02	0.03	0.01	0.01	0.04	0.06	0.03	0.09	0.04	0.02	0.01	0.01	0.04	0.13	
Telemetry L	0.20		0.01	0.10	0.06		0.01	0.03	0.01	0.01	0.08	0.08	0.04	0.13	0.06	0.04	0.01	0.01	0.01	0.12	

Table 5.1. (Allele frequencies continued.)

Sample	*Sco*19											*Ssa*197		*Sfo*18		*Sco*23		mtDNA haplotype					
location	174	190	194	196	198	200	202	204	206	208	210	119	123	150	156	92	94	1	13	13a	19	4	8
Bigmouth B						0.75	0.13	0.08		0.02	0.02	0.10	0.90	0.85	0.15	0.50	0.50		1.00				
Downie B	0.10					0.53	0.08	0.30				0.13	0.88	0.93	0.08	0.23	0.78	0.05	0.95				
Carnes B	0.02					0.70	0.16	0.12				0.20	0.80	1.00		0.24	0.76		1.00				
Jordan B						0.80	0.10	0.10				0.25	0.75	0.95	0.05	0.35	0.65	0.10	0.90				
Illecillewaet B	0.02		0.02			0.62	0.07	0.26		0.01		0.37	0.63	0.92	0.08	0.30	0.70	0.12	0.88				
Incomappleux B	0.05		0.05			0.30	0.41	0.14	0.03	0.03		0.15	0.85	0.92	0.08	0.67	0.33	0.56	0.44				
Hill B		0.02		0.02		0.71	0.05	0.07	0.10	0.02		0.33	0.67	0.93	0.07	0.33	0.67	0.20	0.80				
Mackenzie B		0.05	0.02			0.48		0.09	0.30	0.05		0.39	0.61	0.80	0.20	0.09	0.91	0.46	0.54				
Halfway B	0.02	0.08	0.02			0.59	0.02	0.02	0.24	0.03		0.24	0.76	0.95	0.05	0.39	0.61	0.22	0.78				
St. Leon B	0.21	0.05				0.47	0.03	0.06	0.09	0.09		0.26	0.74	0.76	0.24	0.33	0.67	0.22	0.78				
Slewiskin B		0.08	0.03			0.61	0.06	0.15	0.08			0.75	0.25	1.00		0.31	0.69	0.80	0.15	0.05			
Caribou B		0.13	0.03			0.73		0.05	0.05	0.03		0.65	0.35	0.98	0.03	0.30	0.70	0.75	0.25				
Snow B		0.07				0.75		0.11	0.05	0.02		0.55	0.45	1.00		0.55	0.45	0.96	0.05				
Taite B		0.20				0.75		0.05				0.53	0.48	1.00		0.35	0.65	0.65	0.35				
Downie A						0.90	0.10						1.00	1.00			1.00		1.00				
Jordan A						0.86	0.14					0.73	0.27	1.00		0.23	0.77		0.82		0.18		
Payne A			0.95			0.05							1.00		1.00		1.00		1.00				
Halfway A		0.21				0.62	0.03		0.14			0.24	0.76	1.00		0.18	0.82	0.06	0.94				
St. Leon A	1.00												1.00		1.00		1.00		1.00				
Kuskanax A			0.53	0.08		0.28	0.06	0.03	0.03			0.07	0.93	1.00		0.61	0.39		1.00				
Whatshan A	0.50					0.50							1.00	1.00		0.86	0.14					1.00	
Woden A						1.00						0.38	0.62	1.00			1.00	1.00					
Kinbasket L	0.08					0.68	0.15	0.03			0.05	0.17	0.83	0.99	0.01	0.18	0.82	0.07	0.89		0.02		0.02
Revelstoke L						0.75		0.13	0.06	0.06			1.00	0.88	0.13	0.25	0.75		1.00				
Arrow L	0.04	0.03	0.03		0.01	0.59	0.17	0.11	0.01	0.01		0.30	0.70	0.93	0.07	0.42	0.58	0.27	0.73				
Telemetry L	0.01	0.03			0.01	0.58	0.19	0.14	0.03	0.03		0.26	0.74	0.93	0.07	0.47	0.53	0.24	0.76				

Appendix B

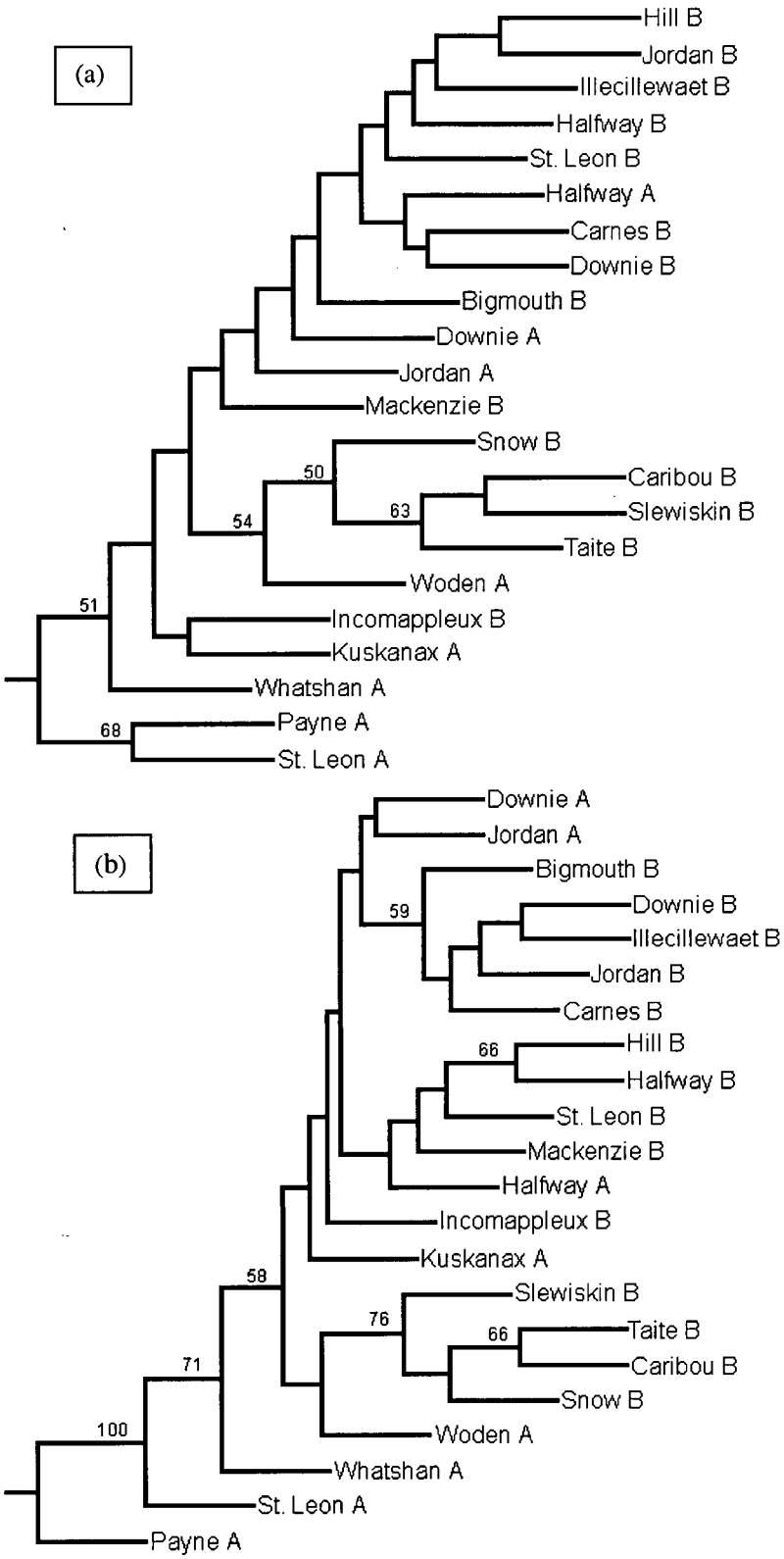

Figure 5.1. UPGMA dendrograms of stream samples (above and below waterfalls) from CRDWK. Nei's D_S (a) and Cavalli-Sforza chord distance (b) were calculated from allele frequencies for *Sco*19, *Sco*23, *Sfo*18, *Ssa*197, and mtDNA (data set 2). Numbers are the percentage of 5000 bootstraps (across loci) supporting each node. This analysis was performed with the PHYLIP program of Felsenstein (1995).

Appendix C

Table 5.2. Pairwise F_{ST} (lower triangle), measured with four diploid microsatellite loci, among migratory (B) populations for data set 1 (a) and data set 2 (b). Numbers 1 through 14 are stream populations, in order, from Bigmouth, Downie, Carnes, Jordan, Illecillewaet, Incomappleux, Hill, Mackenzie, Halfway, St. Leon, Slewiskin, Caribou, Snow, and Taite creeks. Mean pairwise differentiation between populations listed on the far left and all other populations is given. The upper triangle presents the statistical significance (* $P < 0.05$, ** $P < 0.005$, uncorrected for multiple tests) of each pairwise comparison.

(a)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	mean
1	-	**	**		**	**	*	**	**	**	**	**	**	**	0.11
2	0.08	-		*		**	*	**	**	**	**	**	**	**	0.10
3	0.06	0.02	-		*	**	*	**	**	**	**	**	**	**	0.08
4	0.02	0.06	-0.01	-		**		**			**	*	*	*	0.05
5	0.08	0.03	0.03	0.02	-	**		**	**	**	**	**	**	**	0.05
6	0.11	0.17	0.19	0.17	0.17	-	**	**	**	**	**	**	**	**	0.20
7	0.05	0.05	0.01	-0.02	0.00	0.18	-	**			**		*		0.04
8	0.18	0.09	0.10	0.10	0.06	0.28	0.06	-	**	**	**	**	**	**	0.11
9	0.05	0.06	0.04	0.01	0.04	0.14	0.01	0.07	-	**	**	**	**	**	0.06
10	0.06	0.03	0.05	0.02	0.03	0.14	0.02	0.04	0.02	-	**	**	**	**	0.06
11	0.25	0.21	0.17	0.14	0.07	0.32	0.08	0.12	0.14	0.13	-		*	*	0.13
12	0.20	0.20	0.14	0.09	0.06	0.31	0.04	0.11	0.10	0.10	0.00	-			0.11
13	0.13	0.18	0.13	0.07	0.06	0.21	0.04	0.17	0.07	0.10	0.06	0.03	-		0.10
14	0.14	0.14	0.09	0.04	0.04	0.26	0.01	0.11	0.06	0.07	0.03	-0.01	0.01	-	0.08

(b)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	mean
1	-	**	**		**	**	*	**	**	**	**	**	**	**	0.11
2	0.07	-	*			**	*	**	**	**	**	**	**	**	0.10
3	0.06	0.04	-		*	**	*	**	**	**	**	**	**	**	0.09
4	0.00	0.05	0.00	-		*					**	*	**	*	0.09
5	0.08	0.01	0.03	0.04	-	**		**	**	**	**	**	**	**	0.06
6	0.11	0.15	0.19	0.18	0.17	-	**	**	**	**	**	**	**	**	0.20
7	0.05	0.05	0.01	0.00	0.00	0.18	-	**			**		**		0.05
8	0.18	0.10	0.10	0.10	0.06	0.28	0.06	-	**	**	**	**	**	**	0.13
9	0.07	0.09	0.09	0.06	0.07	0.12	0.04	0.10	-		**	**	**	**	0.09
10	0.06	0.05	0.03	0.01	0.01	0.14	-0.01	0.07	0.01	-	**	*	*	*	0.05
11	0.25	0.19	0.17	0.21	0.07	0.32	0.08	0.12	0.15	0.09	-		**	*	0.14
12	0.20	0.18	0.14	0.16	0.06	0.31	0.04	0.11	0.13	0.06	0.00	-	*		0.12
13	0.16	0.20	0.22	0.21	0.11	0.21	0.11	0.25	0.10	0.09	0.14	0.12	-	*	0.15
14	0.14	0.13	0.09	0.09	0.04	0.26	0.01	0.11	0.09	0.03	0.03	-0.01	0.09	-	0.08

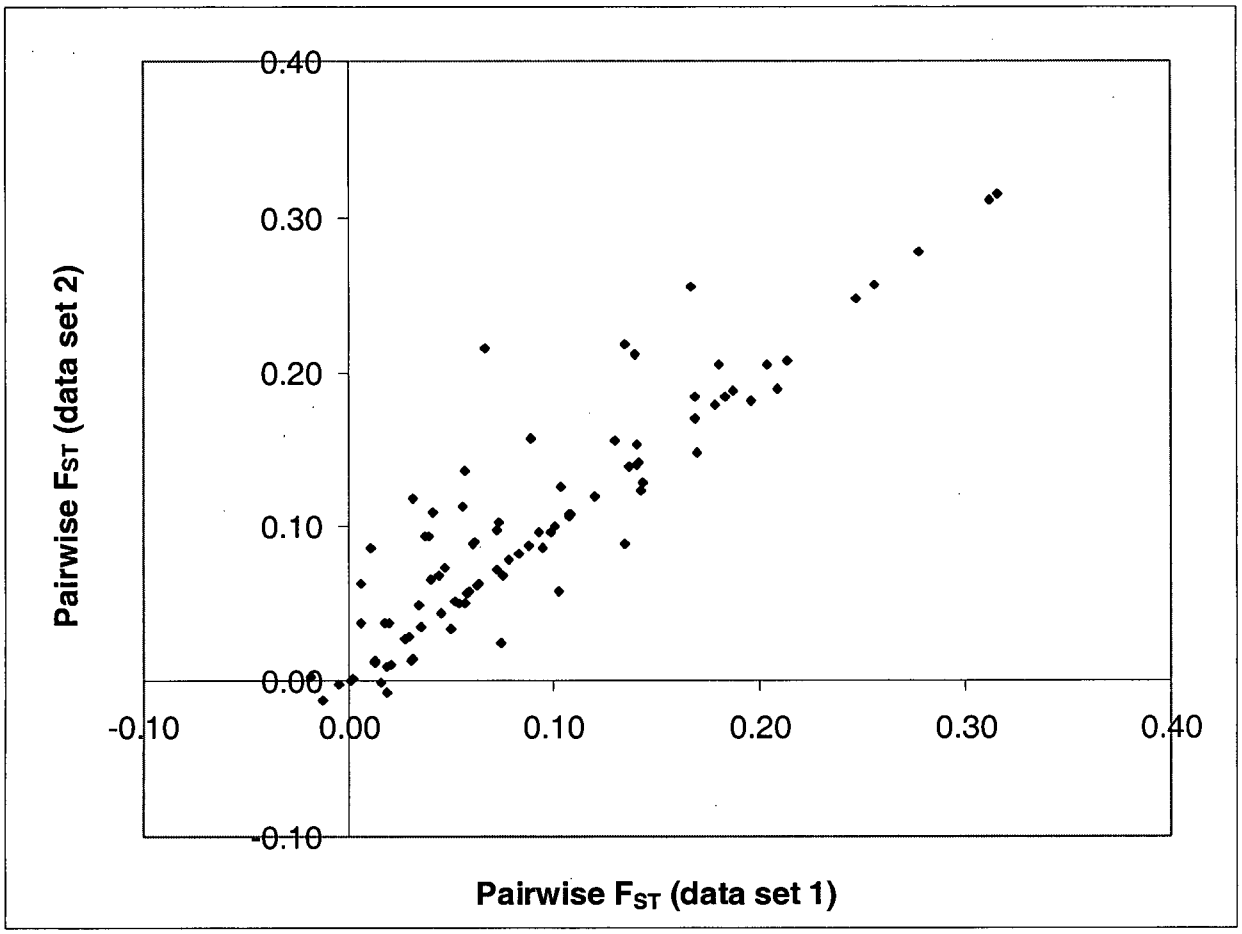

Figure 5.2. Comparison of pairwise differences (F_{ST}), measured with four diploid microsatellite loci, among B populations in CRDWK for data set 1 (including putative 'fallers') and data set 2. Data sets 1 and 2 are generally concordant and yield similar estimates of both average pairwise F_{ST} (0.092 and 0.103) and overall F_{ST} (0.105 and 0.114).

Appendix D

Estimates of gene flow from measures of genetic differentiation can differ depending on the methods and assumptions used. Here I use three methods to evaluate gene flow among northern and southern groups of populations. The first method estimates the number of years required to reach a given level of differentiation by populations diverging from a common source (Table 5.3). For diploid nuclear genetic differentiation (4 usat), fewer years would be required to reach the observed F_{CT} than have elapsed since the putative founding event. That is, given $N_e = 3180$ and other estimated parameters, gene flow would be required to prevent neutral genetic variation from diverging more than has apparently occurred. In contrast, estimated parameters suggest little or no flow of mtDNA among northern and southern groups of populations if they were founded from a similar source 10 000 years ago (see Table 5.3). This supports the hypothesis of founding from differentiated groups. (I have not quantified these inferences statistically because error in parameter estimation is unknown.) Because of residual phylogeographic influences, gene flow should be higher than expected when using a method that assumes the current F_{CT} is at equilibrium (the second method shown). Number of migrants is insensitive to N_e or N_{ef} using this second method, but F_{CT} must be at equilibrium or equilibrium F_{CT} must be predicted.

A method that does not assume F_{CT} to be at equilibrium is also described. The estimates resulting from this third method are up to nine times more than those estimated assuming that current differentiation is at equilibrium and up to three times those based on predicted equilibria. These estimates are still small, however, both for the rate of effective migration and for the number of effective migrants (see Table 5.3). Owing to large potential error in estimates of N_e, sensitivity of the third method to variation in this parameter was evaluated (Figure 5.4). Figure 5.4a shows that if the proportion of strays (migrants) is high, then either effective population size must be small (at $N_e = 300$, m is estimated at less than one in 100 effective migrants) or the fitness of strays must be very limited relative to the fitness of bull trout that spawn in their natal streams. Figure 5.4b shows that the proposed change in differentiation since founding requires only marginally larger numbers of effective migrants with increasing N_e.

The methods presented in Table 5.3 should tend to overestimate natural rates of gene flow because of recent anthropogenic outcrossing and outplanting, particularly for nuclear loci (see Chapter 3). This is not to say that straying rates are necessarily very low in Arrow bull trout, but more generally that strays in total contribute little to the productivity of populations. The migration rates derived from N_em estimates here are consistent with the evolution of local adaptations that could be constrained by artificial increases in gene flow (see Hendry *et al.* 2001). A common suggestion is that, for conservation purposes, anthropogenic gene flow should not exceed natural rates (e.g., Altukhov and Salmenkova 1987, Ryman 1991).

Table 5.3. Evaluation of gene flow from diploid nuclear loci (4 usat) and mtDNA, under a variety of assumptions.

Description (for nuclear data)			How	Notes
1. Method assuming no gene flow since founding				
Calculate the number of generations required to yield the observed differentiation	F_{CT} now (F_t)	0.084	From genetic data	
	N_e	3180	From census population size, adjusting for N_e/N	1
	Generations required	561	From $F_{CT} = 1-(1-1/(2N_e))^t$, solved for t	2
	Generation time	6.00	Estimated from otoliths	3
	Years isolated	**3365**	Product of (t)(generation time)	4
2. Method assuming differentiation at equilibrium				
Calculate the effective migration rate between the groups assuming F_{CT} is at equilibrium or by estimating an equilibrium F_{CT}	F_{CT} now (F_t)	0.084	From genetic data	
	Effective migrants per generation (N_em)	**0.68**	From $N_em = (1/F_{CT}-1)/(4(n/[n-1])^2)$	5
	Equilibrium F_{CT} ($F^\wedge$)	0.029	From alternate North/South groupings for F_{CT}	6
	Effective migrants per generation (N_em)	**2.12**	From $N_em = (1/F_{CT}-1)/(4(n/[n-1])^2)$	5
3. Method using change in differentiation over time				
Step 1. Estimate change in F_{CT} since founding	F_{CT} now (F_t)	0.084	From genetic data	
	Initial F_{CT} (F_o)	0.50	From inferred history (see text)	7
Step 2. Estimate number of generations since founding	Generation time	6.00	Estimated from otoliths	3
	Generations since founding (t)	1667	Carbon dating times divided by generation time	4
	N_e	3180	From census population size	1
Step 3. Solve for L numerically, then solve for m and N_em	L	0.9978	From $F_t = [1/(2N_e)][(L^t-1)/(L-1)] + F_oL^t$	8
	m	**0.0010**	From $m = 1-((1-(2N_e)^{-1})/L)^{1/2}$	
	N_em	**3.19**	Product of (N_e)(m)	

Table 5.3. (Evaluation of gene flow continued.)

Description (for mtDNA data)	N_{ef}/N_e (1)	N_{ef}/N_e (2)	How	Notes
1. Method assuming no gene flow since founding				
F_{CT} now (F_t)	0.50	0.50	From genetic data	
N_{ef}	1590	3180	From census population size, adjusting for N_{ef}/N	1
Generations required	2223	4446	From $F_{CT} = 1-(1-1/(N_{ef}))^t$, solved for t	2
Generation time	8.00	8.00	Estimated from otoliths	3
Years isolated	**17784**	**35571**	Product of (t)(generation time)	4
2. Method assuming differentiation at equilibrium				
F_{CT} now (F_t)	0.50	0.50	From genetic data	
Effective female migrants per generation ($N_{ef}m$)	**0.12**	**0.12**	From $F_{CT} = [1 + 2N_{ef}m(n/(n-1))^2]^{-1}$, solved for $N_{ef}m$	5
Equilibrium F_{CT} ($\hat{F}$)	0.27	0.27	From mean F_{CT} for other groupings	6
Effective female migrants per generation ($N_{ef}m$)	**0.34**	**0.34**	From $F_{CT} = [1 + 2N_{ef}m(n/(n-1))^2]^{-1}$, solved for $N_{ef}m$	5
3. Method using change in differentiation over time				
F_{CT} now (F_t)	0.50	0.50	From genetic data	
Initial F_{CT} (F_o)	0.95	0.95	From inferred history (see text)	7
Generation time	8.00	8.00	Estimated from otoliths	3
Generations since founding (t)	1250	1250	Carbon dating times divided by generation time	4
N_{ef}	1590	3180	From census population size	1
L	0.9985	0.9990	From $F_t = [1/(N_{ef})][(L^t-1)/(L-1)] + F_oL^t$	8
m	0.00044	0.00034	From $m = 1-((1-(N_{ef})^{-1})/L)^{1/2}$	
$N_{ef}m$	**0.69**	**1.08**	Product of $(N_{ef})(m)$	

Notes:

1, Sebastian *et al.* (2000) estimated 5 000 to 10 000 catchable bull trout in Arrow reservoir. I assumed northern and southern population sizes (N) of 10 000 each (as historical populations may have been larger than current ones), and applied corrections for effective size. Reported N_e/N ratios for fish average 0.32 (see Frankham 1995b and references therein, and Miller and Kapucinski 1997). For work with mtDNA, two estimates are provided for the effective number of females. If males and females are equal in abundance and also equal in other ecological parameters, then $N_{ef} = N_e/2$; however, females generally demonstrate lower variance in reproductive success than males (Fleming and Petersson 2001) thereby increasing the ratio. Still N_{ef} is probably less than N_e in general (see results for brown trout in Laikre et al. 1998).

2, This estimates the number of generations required to reach the current F_{CT} (F_t) among completely isolated groups (i.e., $m = 0$) that are diverging from a common source (Nei and Chakravarti 1977).

3, McPhail and Murray (1979) aged spawning bull trout; I used a larger number because there are older fish in the fishery and egg number is related to size (which is related to age). The estimate is lower for nuclear DNA because of potential influences of precocious males.

4, Clague (1981) and Fulton and Archard (1985) suggested the study area was deglaciated approximately 10 000 years ago.

5, Wright (1943) derived this formula for an infinite island model with low migration rates; for mtDNA, it becomes $F_{CT} = (N_{ef}m+1)^{-1}$ (Wright 1951, Hudson *et al.* 1992). Takahata (1983) developed the adjustment included here to account for the finite number of groups exchanging migrants. A given migration rate is more efficient at reducing differentiation among groups when there are fewer groups.

6, This comes from values of F_{CT} yielded by alternative groupings of northern and southern tributary samples (see Figure 2.7). At equilibrium the transition from northern to southern populations should be smooth and F_{CT} should reflect isolation by distance. By taking the means of F_{CT} for alternative North/South groupings, excluding those with divisions between Halfway and St. Leon creeks and between Slewiskin and Caribou creeks, I probably underestimate equilibrium F_{CT}.

7, Estimates of F_o are based upon expectations from variance in F_{CT} among loci and from diversity in founding populations (assessed via populations above falls). Given the estimates of other parameters, results of this method were very insensitive to error in estimates of F_o (not shown).

8, Whitlock (1992) developed this equation to deal with recently founded populations. The value L is defined as $(1-m)^2[1-(2N_e)^{-1}]$ for nuclear DNA. This calculation is not corrected for number of groups; thus estimates of m and N_em are inflated (see note 5 above). This calculation also fails to consider subdivision within groups.

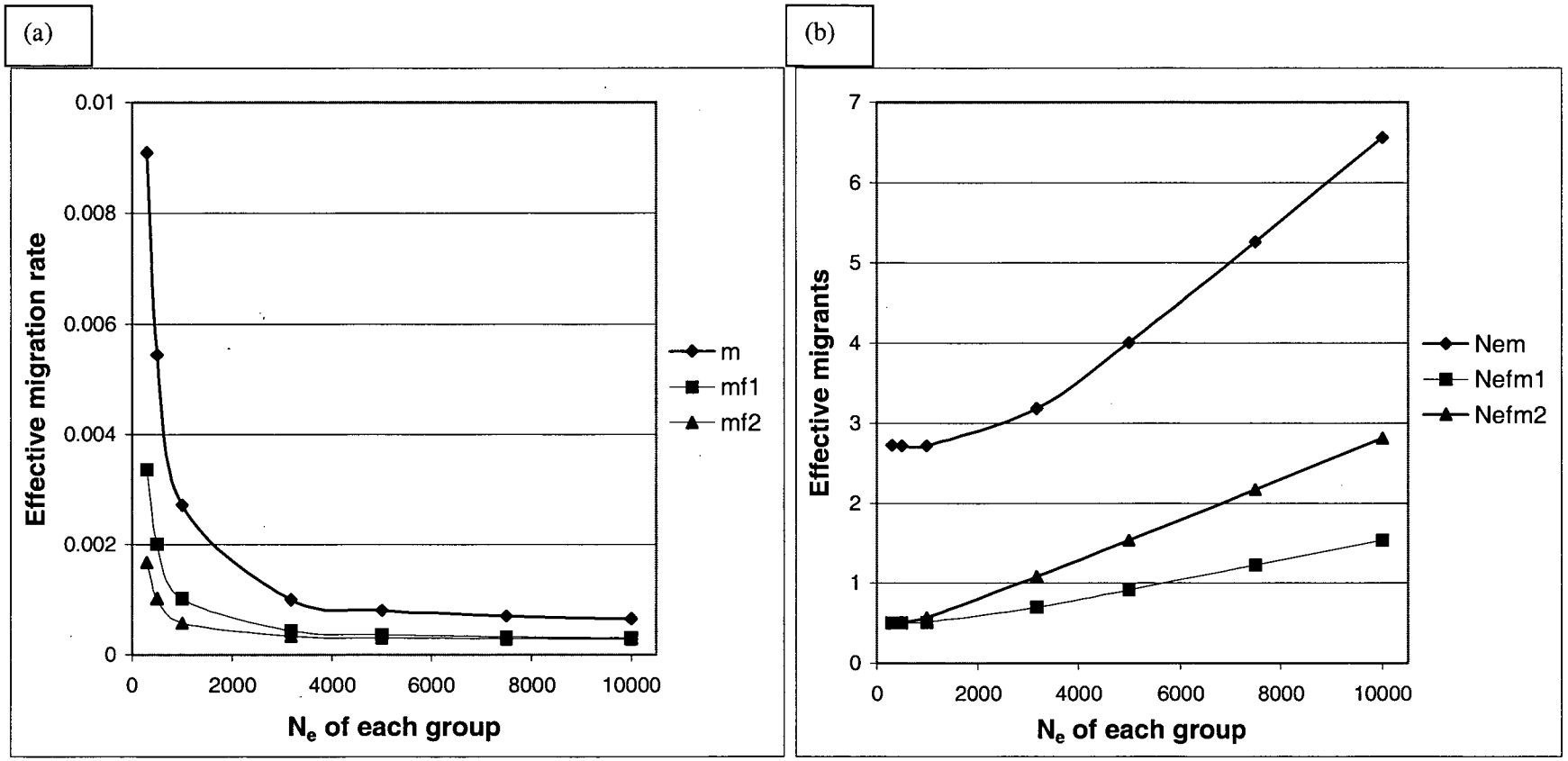

Figure 5.3. Estimated migration rates and numbers of migrants assuming differentiation among populations is not at equilibrium. Estimates of both effective migration rate (a) and total number of effective migrants (b) among northern and southern groups of populations are sensitive to variation in estimates of diploid effective population size (N_e). Because estimates of migration rate are more sensitive at smaller population sizes, estimates of number of migrants tends to increase with population size when N_e exceeds about 1000. Estimates are of m and N_em (effective migration rate and number of effective migrants, respectively, estimated for males and females combined from diploid microsatellite data) and of m_f and $N_{ef}m$ (effective migration rate of females and number of effectively migrating females, respectively, estimated from mtDNA). Estimates resulting if N_{ef} equals N_e or $N_e/2$ are displayed by mf1 and mf2, respectively, in (a) and by $N_{ef}m1$ and $N_{ef}m2$, respectively, in (b). The ratio N_{ef}/N_e was assumed not to have reciprocal influences on N_e.

Appendix E

Table 5.4. Summary of questions raised by this thesis as complimentary research opportunities.

Chapter 1
Chapter 2
How many distinct populations acted as sources for colonization of A habitats.
Do nuclear loci, like mtDNA, from A populations Kootenay Lake tributaries also suggest double invasion?
Do mtDNA data, like nuclear loci, from A populations in the East Kootenays, suggest double invasion?
What is the local phylogeography and colonization history of other CRDWK species?
What is the relationship between population subdivision of bull trout in A populations, and the distribution of other species?
How resilient are A populations? How vulnerable are they to extinction?
Do 'fallers' (precocious individuals from an isolated population) differ in reproductive success from non-migratory individuals (indigenous residents) in adfluvial populations.
Chapter 3
What is the seasonal nature of movement within lacustrine environments?
What is the post release survival of hatchery fish?
What effects do hatchery procedures have on broodstock adults?
How limiting are spawning sites versus feeding opportunites?
Is differentiation within streams an attribute of spawner behaviour or juvenile survival or behaviour?
Chapter 4
How many fish are caught, from where, and to what population do they belong?
Do genetic data predict migrations of radiotagged bull trout?
Are there other hypervariable loci in bull trout?
Is there selection on the studied loci or on loci physically linked to them?
Are different genotypic crosses more likely to contribute successfully to the fishery?
What is the nature of inbreeding depression in A populations?
Are A populations limited in their additive genetic variation?
What is the role of interspecific competition and gene flow in invasibility of A populations?
Do migratory bull trout follow an ideal-free distribution in either the lacustrine or stream environments?
Is there significant recruitment of bull trout into Arrow Reservoir from tributaries to Revelstoke Reservoir?
Does fishing pressure have stronger impacts on males than females?
How do anglers (including poachers) respond, in terms of geography and effort, to changes in CPUE?
What is the nature of interactions between fallers and B populations?
What changes the frequency of the negative interactions?

References Cited

Allendorf, F.W., and L.W. Seeb. 2000. Concordance of genetic divergence among sockeye salmon populations at allozyme, nuclear DNA, and mitochondrial DNA markers. Evolution, 54(2): 640-651.

Allendorf, F.W., and R.S. Waples. 1986. Conservation and genetics of salmonid fishes. In: (Avise, J.C., and J.L. Hamrick, editors) Conservation Genetics: Case Stories from Nature, pp. 238-280. Chapman and Hall, New York.

Altukhov, Y.P., and E.A. Salmenkova. 1987. Stock transfer relative to natural organization, management, and conservation of fish populations. In: (Ryman, N., and F. Utter, editors) Population Genetics and Fishery Management, pp. 333-343. University of Washington Press, Seattle.

Angers, B., and L. Bernatchez. 1997. Complex evolution of a salmonid microsatellite locus and its consequences in inferring allelic divergence from size information. Molecular Biology and Evolution, 14(3): 230-238.

Angers, B., and L. Bernatchez. 1998. Combined use of SMM and non-SMM methods to infer fine structure and evolutionary history of closely related brook charr (*Salvelinus fontinalis*, Salmonidae) populations from microsatellites. Molecular Biology and Evolution, 15: 143-159.

Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for brook char reveal strong population subdivision on a microgeographic scale. Journal of Fish Biology, 47(Supplement A): 177-185.

Angers, B., P. Magnan, M. Plante, and L. Bernatchez. 1999. Canonical correspondence analysis for estimating spatial and environmental effects on microsatellite gene diversity in brook charr (*Salvelinus fontinalis*). Molecular Ecology, 8(6): 1043-1053.

Anonymous. 1999. Endangered and threatened wildlife and plants; determination of threatened status for bull trout in the coterminus United States; final rule. Federal Register 64(210): 58909-58936.

Armbruster, P., W.E. Bradshaw, and C.M. Holzapfel. 1998. Effects of postglacial range expansion on allozyme and quantitative genetic variation of the pitcher-plant mosquito, *Wyeomyia smithii*. Evolution, 52(6): 1697-1704.

Baker, C.S., F. Cipriano, and S.R. Palumbi. 1998. Populaiton structure of nuclear and mitochondiral variation among humpback whales in the North Pacific. Molecular Ecology, 7(6): 695-707.

Balmford, A., M.J.B. Green, and M.G. Murray. 1996. Using higher-taxon richness as a surrogate for species richness: I. regional tests. Procedings of the Royal Society of London Series B, 263: 1267-1274.

Balon, E.K. 1984. Life histories of Arctic charrs: an epigenetic explanation of their invading ability and evolution. In: (Johnson, L., and B. Burns, editors) Biology of the Arctic Charr: Proceedings of the International Symposium on Arctic Charr, pp. 109-142. University of Manitoba Press.

Bams, R.A. 1976. Survival and propensity for homing as affected by presence or absence of locally adapted paternal genes in two transplanted populations of pink salmon (*Oncorhynchus gorbuscha*). Journal of the Fisheries Research Board of Canada, 33: 2716-2725.

Baxter, J.S. 1997. Aspects of the reproductive ecology of bull trout (*Salvelinus confluentus*) in the Chowade River, British Columbia. M.Sc. thesis. University of British Columbia, Vancouver, British Columbia.

Baxter, J.S., and J.D. McPhail. 1996. Bull trout spawning and rearing habitat data: summary of the literature. Fisheries Technical Circular No. 98. Dept. of Zoology, U.B.C.

Behnke, R.J. 1992. Native Trout of Western North America. American Fisheries Society, Bethesda, Maryland.

Bernatchez, L., and A. Osinov. 1995. Genetic diversity of trout (genus *Salmo*) from its most eastern native range based on mitochondrial DNA and nuclear gene variation. Molecular Ecology, 4(3): 285-297.

Bernatchez, L., and C.C. Wilson. 1998. Comparative phylogeography of Nearctic and Palearctic

fishes. Molecular Ecology, 7(4): 431-452.
Bert, T.M., and W.S. Arnold. 1995. An empirical test of predictions of two competing models for the maintenance and fate of hybrid zones: both models are supported in a hard-clam hybrid zone. Evolution, 49: 276-289.
Blumm, M.C., L.J. Lucas, D.B. Miller, D.J. Rohlf, and G.H. Spain. 1998. Saving Snake River water and salmon simultaneously: the biological, economic, and legal case for breaching the lower Snake River dams, lowering John Day Reservoir, and restoring natural river flows. Environmental Law, 28: 996-1054.
Boecklen, W.J. 1997. Nestedness, biogeographic theory, and the design of nature reserves. Oecologia, 112: 123-142.
Brown, J.H. 1986. Two decades of interactions between the MacArthur-Wilson model and the complexities of mammalian distributions. Biological Journal of the Linnean Society, 28: 231-251.
Brown, J.R., A.T. Beckenbach, and M.J. Smith. 1992. Influence of Pleistocene glaciations and human intervention upon mitochondrial DNA diversity in white sturgeon (*Acipenser transmontanus*) populations. Canadian Journal of Fisheries and Aquatic Sciences, 59: 358-367.
Buechner, M. 1987. Conservation in insular parks: simulation models of factors affecting the movement of animals across park boundaries. Biological Conservation, 41: 57-76.
Carlsson, J., and J. Nilsson. 2001. Effects of geomorphological structures on genetic differentiation among brown trout populaitons in a northern boreal river drainage. Transactions of the American Fisheries Society, 130: 36-45.
Casgrain, P., and P. Legendre. 2000. The R Package for Multivariate and Spatial Analysis, Version 4.0 (build 0018).
Caughley, G. 1994. Directions in conservation biology. Journal of Animal Ecology, 63: 215-244.
Cavalli-Sforza, L.L., and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution, 32: 550-570.
Chakraborty, R., P.E. Smouse, and J.V. Neel. 1988. Population amalgamation and genetic variation: observations on artificially agglomerated trbal populations of Central and South America. American Journal of Human Genetics, 43: 709-725.
Chan, T.Y., and A.J. Ribbink. 1990. Alternative reproductive behaviour in fishes with particular reference to *Lepomis macrochirus* and *Pseudocrenilabrus philander*. Environmental Biology of Fishes, 28: 249-256.
Channell, R, and M.V. Lomolino. 2000. Dynamic biogeography and conservation of endangered species. Nature, 403: 84-86.
Clague, J.J. 1975. Late Quaternary sediments and geomorphic history of the southern Rock Mountain Trench, British Columbia. Canadian Journal of Earth Sciences, 12: 595-605.
Clague, J.J. 1980. Late Quaternary geology and geochronology of British Columbia, part 1: radiocarbon dates. Geological Survey of Canada, paper 80-13.
Clague, J.J. 1981. Late Quaternary geology and geochronology of British Columbia, part 2: summary and discussion of radiocarbon-dated Quaternary history. Geological Survey of Canada, paper 80-35.
Cook, R.R., and J.F. Quinn. 1995. The influence of colonization in nested subsets. Oecologia, 102: 413-424.
Cook, R.R., and J.F. Quinn. 1998. An evaluation of randomization models for nested species subsets analysis. Oecologia, 113: 584-592.
Craighead, L.F., M.E. Gilpin, and E.R. Vyse. 1999. Genetic considerations for carnivore conservationin the Greater Yellowstone Ecosystem. In: (Clark, T.W., A.P. Curlee, S.C. Minta, and P.M Kareiva, editors) Carnivores in Ecosystems: the Yellowstone Experience, pp. 285-321. Yale University Press, New Haven, Connecticut.
Cross, T.F., C.P.R. Mills, and M. de Courcy Williams. 1992. An intensive study of allozyme variation in freshwater resident and anadromous trout, *Salmo trutta* L. in western Ireland. Journal of Fish Biology, 40: 25-32.
Currens, K.P., C.B. Schreck, and Li, H.W. 1990. Allozyme and morphological divergence of

rainbow trout (*Oncorynchus mykiss*) above and blow waterfalls in the Deschutes River, Oregon. Copeia, 1990(3): 730-746.
DeWoody, J.A., and J.C. Avise. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. Journal of Fish Biology, 56: 461-473.
Diamond, J.M., J. Terborgh, R.F. Whitcomb, J.F. Lynch, P.A. Opler, C.S. Robbins, D.S. Simberloff, and L.G. Abele. 1975. The island dilemma: lessons of modern biogeographic studies for the design of nature reserves. Biological Conservation, 7: 129-146.
Dittman, A.H., T.P. Quinn, and G.A. Nevitt. 1996. Timing of imprinting to natural and artificial odors by coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences, 53: 434-442.
Donald, D.B., and D.J. Alger. 1993. Geographic distribution, species displacement, and niche overlap for lake trout and bull trout in mountain lakes. Canadian Journal of Zoology, 71: 238-247.
Dunham, D.B., and B.E. Rieman. 1999. Metapopulation structure of bull trout: Influences of physical, biotic, and geometrical landscape characteristics. Ecological Applications, 9(2): 642-655.
Edmands, S. 2001. Phylogeography of the intertidal copepod *Tigriopus californicus* reveals substantially reduced population differentiation at northern latitudes. Molecular Ecology, 10(7): 1743-1750.
Ehrlich, P.R., and P.R. Raven. 1969. Differentiation of populations. Science, 165: 1228-1232.
Einum, S., and I.A. Fleming. 2001. Implications of stocking: ecological interactions between wild and released salmonids. Nordic Journal of Freshwater Research, 75, 56-70.
Ewens, W.J. 1972. The sampling theory of selectively neutral alleles. Theoretical population biology, 3: 87-112.
Excoffier, L., Smouse, P., and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131: 479-491.
Felsenstein, J. 1995. PHYLIP (Phylogeny Inference Package) version 3.57c. http://evolution.genetics.washington.edu/phylip.html.
Finstad, B., and Heggberget, T.G. 1993. Migration, growth and survival of wild and hatchery-reared anadromous Arctic charr (*Salvelinus alpinus*) in Finnmark, northern Norway. Journal of Fish Biology, 43: 303-312.
Fitzsimmons, N., C. Moritz, C.J. Limpus, L. Pope, and R. Prince. 1997. Geographic structure of mitochondrial and nuclear gene polymorphisms in Australian green turtle populations and male-biased gene flow. Genetics, 147(4): 1843-1854.
Fraley, J.J., and B.B. Shepard. 1989. Life history, ecology and population status of migratory bull trout (*Salvelinus confluentus*) in the Flathead lake and river system, Montana. Northwest Science, 63(4): 133-143.
Frankham, R. 1995a. Conservation genetics. Annual Review of Genetics, 29: 305-327.
Frankham, R. 1995b. Effective population size / adult population size ratios in wildlife: a review. Genetical Research, 66: 95-107.
Fretwell, S.D., and H.L. Lucas. 1970. On territorial behaviour and other factors influencing habitat distribution in birds. Acta Biotheoretica, 19: 16-36.
Fleming, I.A., and E. Petersson. 2001. The ability of released hatchery salmonids to breed and contribute to the natural productivity of wild populations. Nordic Journal of Freshwater Research, 75: 71-98.
Ford, M.J., and J.J. Hard. 2000. Does traditional hatchery production help conserve wild salmon – a comment on the Fall Creek coho hatchery controversy. NMFS-NWFSC- manuscript.
Fulton, R.J. 1968. Olympia interglaciation, Purcell Trench, British Columbia. Geological Society of America Bulletin, 79: 1075-1080.
Fulton, R.J. 1969. Glacial lake history, southern interior plateau. Geological Survey of Canada, paper 69-37.
Fulton, R.J., and R.A. Archard. 1985. Quaternary sediments, Columbia River valley, Revelstoke to the Rocky Mountain Trench, British Columbia. Geological Survey of Canada, paper 84-13.

Fulton, R.J., and G.W. Smith. 1978. Late Pleistocene stratigraphy of south-central British Columbia. Canadian Journal of Earth Sciences, 15: 971-980.

Garcia-Marin, J.L., P.E. Jorde, N. Ryman, F.M. Utter, and C. Pla. 1991. Management implications of genetic differentiation between native and hatchery populations of brown trout (*Salmo trutta*) in Spain. Aquaculture, 95:235-249.

Garcia-Marin, J.L., N. Sanz, and C. Pla. 1998. Proportions of native and introduced brown trout in adjacent fished and unfished Spanish rivers. Conservation Biology, 12: 313-319.

Gharrett, A.J. 1994. Genetic dynamics of a small population system: A model applicable to interactions between hatchery and wild fish. Aquaculture and Fish Management, 25(Supplement 2): 79-92.

Gharrett, A.J., and W.W. Smoker. 1993. A perspective on the adaptive importance of genetic infrastructure in salmon populations to ocean ranching in Alaska. Fisheries Research, 18: 45-58.

Glemet, H., P. Blier, and L. Bernatchez. 1998. Geographical extent of Arctic char (*Salvelinus alpinus*) mtDNA introgression in brook char populations (*S. fontinalis*) from eastern Quebec, Canada. Molecular Ecology, 7:1655-1662.

Grahn, M, A. Langefors, and T. von Schantz. 1998. The importance of mate choice in improving viability in captive populations. In: (Caro, T., editor) Behavioral Ecology and Conservation Biology, pp. 341-363. Oxford University Press, New York.

Guo, S.W., and E.A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics 48:361-372.

Haas, G.R. 2001. The mediated associations and preferences of native bull trout and rainbow trout with respect to maximum water termperature, its measurement standards, and habitat. In: (Brewin, M.K., A.J. Paul, and M. Monita, editors) Bull Trout II Conference Proceedings, pp. 53-55. Trout Unlimited Canada, Calgary.

Hamilton, K.E., A. Ferguson, J.B. Taggart, T. Tomasson, A. Walker, and E. Fahy. 1989. Post-glacial colonisation of brown trout, *Salmo trutta* L.: Ldh-5 as a phylogeographic marker locus. Journal of Fish Biology, 35: 651-664.

Hansen, M.M., and V. Loeschcke, 1994. Effects of releasing hatchery-reared brown trout to wild trout populations. In: (Loeschcke, V., J. Tomiuk, and S.K. Jain, editors) Conservation Genetics, pp. 273-289. Birkhauser Verlag Basel/Switzerland.

Hansen, M.N., Nielsen, E.E., and Mensberg, K.K.D. 1997. The problem of sampling families rather than populations: relatedness among individuals in samples of juvenile brown trout *Salmo trutta* L. Molecular Ecology, 6: 469-474.

Hansen, M.N., D.E. Ruzzante, E.E. Nielsen, and Mensberg, K.L.D. 2001. Microsatellite and mitochondrial DNA polymorphism reveals life-history dependent interbreeding between hatchery trout and wild brown trout (*Salmo trutta* L.). Molecular Ecology, 9: 583-594.

Hastings, A. 1993. Complex interactions between dispersal and dynamics: lessons from coupled logistic equations. Ecology, 74(5): 1362-1372.

Healey, M.C. 1983. Coastwide distribution and ocean migration patterns of stream- and ocean-type chinnok salmon (*Oncorhynchus tshawytscha*). Canadian Field Naturalist, 97: 427-433.

Hedrick, P.W., D. Hedgecock, and S. Hamelberg. 1995. Effective population size in winter-run chinook salmon. Conservation Biology, 9(3): 615-624.

Hendry, A.P., T.P. Quinn, and F.M. Utter. 1996. Genetic evidence for the persistence and divergence of native and introduced sockeye salmon (*Oncorhynchus nerka*) within Lake Washington, Washington. Canadian Journal of Fisheries and Aquatic Sciences, 53: 823-832.

Hendry, A.P., O.K. Berg, and T.P. Quinn. 1999. Condition dependence and adaptation-by-time: breeding date, life history, and energy allocation within a population of salmon. Oikos, 85: 499-514.

Hendry, A.P., T. Day, and E.B. Taylor. 2001. Population mixing and the adaptive divergence of quantitative characters in discrete populations: a theoretical framework for empirical tests. Evolution, 55: 459-466.

Hesthagen, T. and B.O. Johnsen. 1989. Lake survival of hatchery and pre-stocked pond brown trout, *Salmo trutta* L. Aquaculture and Fisheries Management, 20: 91-95.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature, 405: 907-913.

Hilborn, R. 1999. Confessions of a reformed hatchery basher. Fisheries, 24(5): 30-31.
Hudson, R.R., M. Slatkin, and W.P. Maddison. 1992. Estimation of levels of gene flow from DNA sequence data. Genetics, 132: 583-589.
Hughes, J.M., S.E. Bunn, D.A. Hurwood, S. Choy, and R.G. Pearson. 1996. Genetic differentiation among populations of *Caridina zebra* (Decapoda: Atyidae) in tropical rainforest streams, northern Australia. Freshwater Biology, 36: 289-296.
Hutchison, D.W., and A.R. Templeton. 1999. Correlation of pairwise genetic and geographic distance measures: Inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution, 53(6): 1898-1914.
Ingvarsson, P.K., and M.C. Whitlock. 2000. Heterosis increases the effective migration rate. Proceedings of the Royal Society of London, 267: 1321-1326.
Jin, L., and R. Chakrabory. 1995. Population structure, stepwise mutations, heterozygote deficiency and their implications in DNA forensics. Heredity, 74: 274-285.
Jones, A., D. Walker, K. Lindstrom, C. Kvarnemos, and J.C. Avise. 2001. Surprising similarity of sneaking rates and genetic mating patterns in two populations of sand goby experiencing disparate sexual selection regimes. Molecular Ecology, 10(2): 461-469.
Jonsson, B. 1982. Diadromous and resident trout *Salmo trutta*: Is their difference due to genetics? Oikos, 38: 297-300.
Kanda, N., R.F. Leary, and F.W. Allendorf. 1997. Population genetic structure of bull trout in the upper Flathead River drainage. In: (Mackay, E.C., M.K. Brewin, M.K., and M. Monita, editors) Friends of the Bull Trout Conference Proceedings, pp. 299-308. Bull Trout Task Force (Alberta, Canada). Trout Unlimited Canada, Calgary.
Kelly-Quinn, M. and J.J. Bracken. 1989. Survival of stocked hatchery-reared brown trout, *Salmo trutta* L., fry in relation to the carrying capacity of a trout nursery stream. Aquaculture and Fisheries Management, 20: 211-226.
Koseki, Y., and K. Maekawa. 2000. Sexual selection on mature male parr of masu salmon (*Oncorhynchus masou*): does sneaking behavior favor small body size and less-developed sexual characters? Behavioral Ecology & Sociobiology, 48(3): 211-217.
Labelle, M. 1992. Straying patterns of coho salmon (*Oncorhynchus kisutch*) stocks from southeast Vancouver Island, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences, 49: 1843-1855.
Laikre, L., P.E. Jorde, and N. Ryman. 1998. Temporal change of mitochondrial DNA haplotype frequencies and female effective size in a brown trout (*Salmo trutta*) population. Evolution, 52: 910-915.
Leary, R.F., F.W. Allendorf, and S.H. Forbes. 1993. Conservation genetics of bull trout in the Columbia and Klamath River drainages. Conservation Biology, 7: 856-865.
Leberg, P.L. 1993. Strategies for population reintroduction: effects of genetic variability on population growth and size. Conservation Biology, 7: 194-199.
Lahti, T., and E. Ranta. 1985. The SLOSS principle and conservation practice: an example. Oikos, 44: 369-370.
Lesica, P., and F.W. Allendorf. 1995. When are peripheral populations valuable for conservation? Conservation Biology, 9(4): 753-760.
Lichatowich, J. 1999. Salmon Without Rivers: A History of the Pacific Salmon Crisis. Island Press, Washington, D.C.
Lichatowich, J., Mobrand, L., and Lestelle, L. 1999. Depletion and extinction of Pacific salmon (*Oncorhynchus* spp.): a different perspective. ICES Journal of Marine Science, 56: 467-472.
Lindsay, R.A. 1986. Upper Arrow Reservoir sport fishery statistics. Unpublished manuscript, B.C. Fisheries Branch, Nelson, B.C.
Lindsay, R.A. 1987. Lower Arrow Reservoir sport fishery statistics. Unpublished manuscript, B.C. Fisheries Branch, Nelson, B.C.
Louis, E.J., and E.R. Dempster. 1987. An exact test for Hardy-Weinberg and multiple alleles. Biometrics 43:805-811.
Luikart, G., and J.M. Cornuet. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conservation Biology, 12: 228-237.

Lynch, M. 1996. A quantitative genetic perspective on conservation issues. In: (Avise, J.C., and J.L. Hamrick, editors) Case Histories from Nature, pp. 471-501. Chapman and Hall, New York.

Lynch, M., J. Conery, and R. Burger. 1995. Mutation meltdown in sexual populations. Evolution, 49: 1067-1080.

MacArthur, R.H., and Wilson, E.O. 1967. The Theory of Island Biogeography. Princeton University Press, Princeton.

Maekawa, K. T. Hino, S. Nakano, and W.W. Smoker. 1993. Mate preference in anadromous and landlocked Dolly Varden (*Salvelinus malma*) females in two Alaskan streams. Canadian Journal of Fisheries and Aquatic Sciences, 50: 2375-2379.

Maekawa, K., N. Shigeru, and S. Yamamoto. 1994. Spawning behaviour and size-assortative mating of Japanese charr in an artifical late-inlet stream system. Environmental Biology of Fishes, 39(2): 109-117.

Magurran, A.E. 1988. Ecological Diversity and its Measurement. Princeton University Press.

Mantel , N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research, 27: 209-220.

Margules, C.R., and R.L. Pressey. 2000. Systematic conservation planning. Nature, 405: 243-253.

Marsden, J.E., Krueger, C.C., Grewe, P.M., Kincaid, H.L., May, B. 1993. Genetic comparison of naturally spawned and artificially propagated Lake Ontario lake trout fry: Evaluation of a stocking strategy for species rehabilitation. North American Journal of Fisheries Management 13(2): 304-317.

Martin, A.D. 1976. Interim report of investigations on fish populations that will be affected by the Revelstoke 1880 Dam. Fish and Wildlife Branch, Nelson, B.C. Ministry of Environment.

McCusker, M.R., E. Parkinson, and E.B. Taylor. 2000. Mitochondrial DNA variation in rainbow trout (*Oncorhynchus mykiss*) across its native range: testing biogeographical hypotheses and their relevance to conservation. Molecular Ecology, 9: 2089-2108.

McElhany, P., M.H. Ruckelshaus, M.J. Ford, T.C. Wainwright, and E.P. Bjorkstedt. 2000. Viable salmonid populations and the recovery of evolutionarily significant units. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-42.

McGowan, C., and W.S. Davidson. 1992. Unidirectional hybridization between brown trout (*Salmo trutta*) and Atlantic salmon (*S. salar*) in Newfoundland. Canadian Journal of Fisheries and Aquatic Sciences, 49: 1953-1958.

McIsaac, D.O., and Quinn, T.P. 1988. Evidence for a hereditary component in homing behavior of chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences, 45: 2201-2205.

McPhail, J.D., and J.S. Baxter. 1996. A review of bull trout (*Salvelinus confluentus*) life-history and habitat use in relation to compensation and improvement opportunities. Fisheries Management Report No. 104, British Columbia Ministry of Environment, Lands and Parks.

McPhail, J.D., and C.C. Lindsey. 1986. Zoogeography of the freshwater fishes of Cascadia (the Columbia system and rivers north to the Stikine). In: (Hocutt, C.H., and E.O. Wiley, editors) The Zoogeography of North American Freshwater Fishes, pp. 616-637. John Wiley and Sons, Inc., New York.

McPhail, J.D., and C.B. Murray. 1979. The early life history and ecology of Dolly Varden (*Salvelinus malma*) in the upper Arrow Lakes. Report of the Institute of Animal Resource Ecology and Department of Zoology (University of British Columbia) to B.C. Hydro and Ministry of Environment, Fisheries Branch, Nelson, British Columbia.

Melnick, D.J., J.C. Morales, and R.L. Honeycutt. 2000. Conservation genetics: applying molecular methods to maximize the conservation of taxonomic and genetic diversity. In: (National Research Council, Board on Biology) Nature and Human Society: the Quest for a Sustainable World: Proceedings of the 1997 Forum on Biodiversity, pp. 264-275. National Academy Press, Washington D.C.

Merila, J., M. Bjorklund, and A.J. Baker. 1996. Genetic population structure and gradual northward decline of genetic variability in the greenfinch (*Carduelis choloris*). Evolution, 50(6): 2548-2557.

Merila, J., M. Bjorklund, and A.J. Baker. 1997. Historical demography and present day population structure of the greenfinch, *Carduelis choloris* – an analysis of mtDNA control-region sequences. Evolution, 51(3): 946-956.

Michael, J.H.Jr. 1983. Contribution of cutthroat trout in headwater streams to the sea-run population. California Fish and Game, 69: 68-76.

Miller, L.M., and A.R. Kapuscinski. 1997. Historical analysis of genetic variation reveals low effective population size in a northern pike (*Esox lucius*) population. Genetics, 147: 1249-1258.

Miller, M.P. 1997. Tools for population genetic analyses (TFPGA; version 1.3): A Windows program for the analysis of allozyme and molecular population genetic data.

Mills, D. 1994. Evidence of straying from wild Atlantic salmon, *Salmo salar* L., smolt transportation experiments in northern Scotland. Aquaculture and Fisheries Management, 25(Supplement 2): 3-8.

Milner, A.M., and R.G. Bailey. 1989. Salmonid colonization of new streams in Glacier Bay, Alaska. Aquaculture and Fisheries Management, 20: 179-192.

Milner, A.M, E.E. Knudsen, C. Soideth, A.L. Robertson, D. Schell, I.T. Phillips, and K. Magnusson. 2000. Colonization and development of stream communities across a 200-year gradient in Glacier Bay National Park, Alaska, U.S.A. Canadian Journal of Fisheries and Aquatic Sciences, 57: 2319-2335.

Moran, P., A.M. Pendas, E. Garcia-Vazquez, and J. Izguierdo. 1991. Failure of stocking policy of hatchery reared brown trout (*Salmo trutta*) in Asturias, Spain, detected using *LDH-5** as a genetic marker. Journal of Fish Biology, 39(Supplement A): 117-122.

Moritz, C. 1994. Defining 'evolutionarily significant units' for conservation. Trends in Ecology and Evolution, 9: 373-375.

Moritz, C., and D.P. Faith. 1998. Comparative phylogeography and the identification of genetically divergent areas for conservation. Molecular Ecology, 7: 419-429.

Munehara, H., and O. Takenaka. 2000. Microsatellite markers and multiple paternity in a paternal care fish, *Hexagrammos otakii*. Journal of Ethology, 18(2): 101-104.

Myers, N., R.A. Mittermeier, C.G. Mittermeier, G.A.B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature, 403: 853-858.

Nasmith, H. 1972. Engineering geology of the southern Cordillera of British Columbia. In: (D.J. Glass, editor) Excursion A08-C08, XXIV International Geological Congress, Montreal, Quebec.

Nei, M. 1972. Genetic distance between populations. The American Naturalist, 106(949): 283-292.

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

Nei, M., and A. Chakravarti. 1977. Drift variances of F_{ST} and G_{ST} statistics obtained from a finite number of isolated populations. Theoretical Population Biology, 11: 307-325.

Neraas, L.P., and P. Spruell. 2001. Fragmentation of riverine systems: the genetic effects of dams on bull trout (*Salvelinus confluentus*) in the Clark Fork River system. Molecular Ecology, 10(5): 1153-1164.

Nickelson, T.E., M.F. Solazzi, and S.L. Johnson. 1986. Use of hatchery coho salmon (*Oncorhynchus kisutch*) presmolts to rebuild wild populations in Oregon coastal streams. Canadian Journal of Fisheries and Aquatic Sciences, 43: 2443-2449.

Nordeng, H. 1971. Is the local orientation of anadromous fishes determined by pheromones? Nature, 233: 411-413.

Nordeng, H. 1977. A pheromone hypothesis for homeward migration in anadromous salmonids. Oikos, 28: 155-159.

Northcote, T.G. 1981. Juvenile current response, growth and maturity of above and below waterfall stocks of rainbow trout, *Salmo gairdneri*. Journal of Fish Biology, 18: 741-751.

Northcote, T.G. 1992. Migration and residency in stream salmonids – some ecological considerations and evolutionary consequences. Nordic Journal of Freshwater Research, 67: 5-17.

Northcote, T.G. 1997. Potadromy in Salmonidae – Living and moving in the fast lane. North American Journal of Fisheries Management, 17: 1029-1045.

Northcote, T.G., S.N. Williscroft, and H. Tsuyuki. 1970. Mersitic and lactate dehydrogenase

genotype differences in stream populations of rainbow trout below and above a waterfall. Journal of the Fisheries Research Board of Canada, 27: 1987-1995.

O'Brien, D.S. 1999. The Duncan bull trout telemetry project. A report submitted to the Columbia Basin Fish and Wildlife Compensation Program, Nelson, B.C. http://www.zoology.ubc.ca/~obrien.

O'Brien, D.S. 2001. Bull Trout (*Salvelinus confluentus*) Spawning migrations in the Duncan River: insights from telemetry and DNA. M.Sc. thesis. University of British Columbia, Vancouver, British Columbia.

O'Reilly, P.T., L. Hamilton, S. McConnell, and J.M. Wright. 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Canadian Journal of Fisheries and Aquatic Sciences, 53: 2292-2298.

Orti, G., D.E. Pearse, and J.C. Avise. 1997. Phylogenetic assessment of length variation at a microsatellite locus. Proceedings of the National Academy of Sciences, U.S.A., 94: 10745-10749.

Paetkau, D., L.P. Waits, P.L. Clarkson, L. Craighead, and C. Strobeck. 1997. An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. Genetics. 147(4): 1943-1957.

Papi, F., and H.G. Wallraff. 1992. Birds. In: (Papi, F., editor) Animal Homing, pp. 263-319. Chapman and Hall, London.

Park, L.K., M.A. Brainard, D.A. Dightman, G.A. Winans. 1993. Low levels of intraspecific variation in the mitochondrial DNA of chum salmon (*Oncorhynchus keta*). Molecular Marine Biology and Biotechnology, 2: 362-370.

Parkinson, E., R.J. Behnke, and W. Pollard. 1984. A morphological and electrophoretic comparison of rainbow trout (*Salmo gairdneri*) above and below barriers on five streams on Vancouver Island, B.C. Fisheries Management Report No. 83, B.C. Ministry of Environment.

Partridge, L., and P.H. Harvey. 1988. The ecological context of life history evolution. Science, 241: 1449-1455.

Pascual, M.A., and T.P. Quinn. 1994. Geographical patterns of straying of fall chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), from Columbia River (USA) hatcheries. Aquaculture and Fisheries Management, 25(Supplement 2): 17-30.

Patrick, N.D., and P. Graf. 1961. The effect of temperature on the artificial culture of aurora trout. The Canadian Fish Culturist, 30: 49-55.

Patterson, B.D., and W. Atmar. 1986. Nested subsets and the structure of insular mammalian faunas and archipelagos. Biological Journal of the Linnean Society, 28: 65-82.

Petersson, E., and T. Jarvi. 1997. Reproductive behaviour of sea trout (*Salmo trutta*) – consequences of sea ranching. Behaviour, 134: 1-22.

Pettersson, J.C.E., M.M. Hansen, and T. Bohlin. 2001. Does dispersal from landlocked trout explain the coexistence of resident and migratory trout females in a small stream? Journal of Fish Biology, 58:487-495.

Poteaux, C., D. Beaudou, and P. Berrebi. 1998. Temporal variations of genetic introgression in stocked brown trout populations. Journal of Fish Biology, 53: 701-713.

Pratt, K.L. 1992. A review of bull trout life history. In: (Howell, P.J. and D.V. Buchanan, editors) Proceeding of the Gearhart Mountain Bull Trout Workshop, pp. 5-9. Oregon Chapter of the American Fisheries Society, Corvallis, Oregon.

Quinn, T.P. 1993. A review of homing and straying of wild and hatchery-produced salmon. Fisheries Research, 18: 29-44.

Quinn, T.P. 1997. Homing, straying, and colonization. In: (Grant, S., editor) Genetic Effects of Straying of Non-native Hatchery Fish into Natural Populations, pp. 73-85. U.S. Department of Commerce, NOAA Tech. Memo. NMFS-NWFSC-30, Seattle, WA.

Quinn, T.P., E.C. Volk, and A.P. Hendry. 1999. Natural otolith microstructure patterns reveal precise homing to natal incubation sites by sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Zoology, 77: 766-775.

Ranta, P., A. Tanskanen, J. Niemela, and A. Kurtto. 1999. Selection of islands for conservation

in the urban archipelago of Helsinki, Finland. Conservation Biology, 13(6): 1293-1300.
Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, 83, 248-249.
Redenbach, Z. 2000. Molecular evidence of current and historical introgressive hybridization between bull trout (*Salvelinus confluentus*) and Dolly Varden (*S. malma*). M.Sc. thesis. University of British Columbia, Vancouver, British Columbia.
Reesor, J.E., and J.M. Moore Jr. 1971. Petrology and structure of the Thor-Odin gneiss dome, Shuswap Metamorphic Complex, British Columbia. Geological Survey of Canada Bulletin, 195.
Reisenbichler, R.R. 1988. Relation between distance transferred from natal stream and relative return rate for hatchery coho salmon. North American Journal of Fisheries Management, 8: 172-174.
Reisenbichler, R.R., and S.P. Rubin. 1999. Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. ICES Journal of Marine Science, 56: 459-466.
Remple, L.L., and D.G.Smith. 1998. Postglacial fish dispersal from the Mississippi refuge to the Mackenzie River basin. Canadian Journal of Fisheries and Aquatic Sciences, 55: 893-899.
Reynolds, J., B.S. Weir, and C.C. Cockerham. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. Genetics, 105: 767-779.
Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution, 43: 223-225.
Ricker, W.E. 1973. Two mechanisms that make it impossible to maintain peak-period yields from stocks of Pacific salmon and other fishes. Journal of the Fisheries Research Board of Canada, 30: 1275-1286.
Riechert, S.E. 1993. Investigation of potential gene flow limitation of behavioral adaptation in an aridlands spider. Behavioral Ecology and Sociobiology, 32: 355-363.
Rieman, B.E., and J.D. McIntyre. 1995. Occurence of bull trout in naturally fragmented habitat patches of varied size. Transactions of the American Fisheries Society 124: 285-296.
Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. In: Studies in Genetics VII. University of Texas Publication No. 7213. Austin, Texas.
Ronfort, J., E. Jenczewski, T. Bataillon, and F. Rousset. 1998. Analysis of population structure in autotetraploid species. Genetics, 150: 921-930.
Ryder, M.M. 1981. Biophysical resources of the East Kootenay area: terrain. B.C. Ministry of Environment, APD Bulletin, 7.
Ryman, N. 1991. Conservation genetics considerations in fishery management. Journal of Fish Biology, 39(Supplement A): 211-224.
Ryman, N., and L. Laikre. 1991. Effects of supportive breeding on the effective population size. Conservation Biology, 5: 325-329.
Schutz, D.C., and T.G. Northcote. 1972. An experimantal study of feeding behavior and interation of coastal cutthroat trout (*Salmo clarki clarki*) and Dolly Varden (*Salvelinus malma*). Journal of the Fisheries Research Board of Canada, 29: 555-565.
Scudder, G.G.E. 1989. The adaptive significance of marginal populations: A general perspective. Canadian Special Publications of Fisheries and Aquatic Sciences 105: 180-185.
Sebastian, D., H. Andrusak, G. Scholten, L. Brescia. 2000. Arrow reservoir fish summary: stock management report. British Columbia Ministry of Fisheries.
Shaklee, J.B., T.D. Beacham, L. Seeb, and B.A. White. 1999. Managing fisheries using genetic data: Case studies from four species of Pacific salmon. Fisheries Research, 43: 45-78.
Schneider, S., J.M. Kueffer, D. Roessli, and L. Excoffier. 1997. ARLEQUIN (version 1.1): an exploratory population genetics software environment. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
Shaw, P.W., G.R. Carvalho, A.E. Magurran, and B.H. Seghers. 1991. Population differentiation in Trinidadian guppies (*Poecilia reticulata*): patterns and problems. Journal of Fish Biology, 39(Supplement A): 203-209.
Shaw, P.W., G.R. Carvalho, A.E. Magurran, and B.H. Seghers. 1994. Factors affecting the distribution of genetic variability in the guppy, *Poecilia reticulata*. Journal of Fish Biology, 45: 875-888.

Sivasundar, A., E. Bermingham, and G. Orti. 2001. Population structure and biogeography of migratory freshwater fishes (*Prochilodus*: Characiformes) in major South American rivers. Molecular Ecology, 10: 407-417.

Skaala, O., and G. Naevdal. 1989. Genetic differenetiation between freshwater resident and anadromous brown trout, *Salmo trutta*, within watercourses. Journal of Fish Biology, 34: 597-605.

Slade, R.W., C. Moritz, A.R. Hoelzel, and H.R. Burton. 1998. Molecular population genetics of the southern elephant seal (*Mirounga leonina*). Genetics, 149: 1945-1957.

Smith, H.A., and P.A. Slaney. 1980. Age, growth, survival and habitat of anadromous Dolly Varden (*Salvelinus malma*) in the Keogh River, British Columbia. B.C. Fish and Wildlife Branch, Fisheries Management Report No. 76.

Smouse, P.E., J.C. Long and R.R. Sokal. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Systematic Zoology, 35: 627-632.

Sneath, P.H., and R.R. Sokal. 1973. Numerical taxonomy and practice of numerical classification. Freeman Press, San Francisco.

Snucins, E.J., J.M. Gunn, and W. Keller. 1995. Restoration of the aurora trout to its acid-damaged native habitat. Conservation Biology, 9: 1307-1311.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry, 3rd edition. W.H. Freeman and Co., New York.

Sork, V.L., K.A. Stowe, and C. Hochwender. 1993. Evidence for local adaptation in closely adjacent subpopulations of northern red oak (*Quercus rubra* L.) expressed as resistance to leaf herbivores. The American Naturalist, 142: 928-936.

Soule, M.E., and B.A. Wilcox. 1980. Conservation Biology: An Evolutionary Ecological Perspective. Sinauer Associates, Inc. Sunderland, Mass.

Spruell, P., and Allendorf, F.W. 1997. Nuclear DNA analysis of Oregon bull trout. Final Report to the Oregon Department of Fish and Wildlife, Report No. 97/5.

Spruell, P., Rieman, B.E., Knudsen, K.L., Utter, F.M., and Allendorf, F.W. 1999. Genetic population structure within streams: microsatellite analysis of bull trout populations. Ecology of Freshwater Fish, 8: 104-121.

Storfer, A. 1999. Gene flow and endangered species translocations: a topic revisited. Biological Conservation, 87: 173-180.

Taberlet, P., L. Fumagalli, A.G. Wust-Saucy, J.F. Cosson. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Molecular Ecology, 7: 453-464.

Taggart, J.B., Hynes, R.A., and Ferguson, A. 1992. A simplified protocol for routine total DNA isolation from salmonid fishes. Journal of Fish Biology, 40: 963-965.

Taggart, J.B., I.S. McLaren, D.W. Hay, J.H. Webb, and A.F. Youngson. 2001. Spawning success in Atlantic salmon (*Salmo salar*, L.): a long-term DNA profiling-based study conducted in a natural stream. Molecular Ecology, 10: 1047-1060.

Takahata, N. 1983. Gene identity and genetic differentiation of populations in the finite island model. Genetics, 104: 497-512.

Tallman, R.F. 1986. Genetic differentation among seasonally distinct spawning populations of chum salmon, *Oncorhynchus keta*. Aquaculture, 57: 211-217.

Taylor, E.B. 1991. A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. Aquaculture, 98: 195-207.

Taylor, E.B., S. Pollard, and D. Louie. 1999. Mitochondrial DNA variation in bull trout (*Salvelinus confluentus*) from northwestern North America: implications for zoogeography and conservation. Molecular Ecology, 8: 1155-1170.

Taylor, E.B., S. Pollard, Z. Redenbach, A. Costello, and C. Pacas. 2001. Nested analysis of genetic diversity in bull trout (*Salvelinus confluentus*): implications for speciation, biogeography, and conservation. Canadian Journal of Fisheries and Aquatic Sciences, 58: 406-420.

Templeton, A.R., E. Routman, and C.A. Phillips. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. Genetics, 140(2): 767-782.

Thorpe, J.E. 1994. Significance of straying in salmonids and implications for ranching.

Aquaculture and Fisheries Management, 25(Supplement 2): 183-190.
Uglem, I., G., Rosenqvist, and H.S. Wasslavik. 2000. Phenotypic variation between dimorphic males in corkwing wrasse. Journal of Fish Biology, 57(1): 1-14.
Utter, F.M. 2001. Patterns of subspecific anthropogenic introgression in two salmonid genera. Reviews in Fish Biology and Fisheries, 10(4): 431-446.
Utter, F.M., P. Aebersold, J. Helle, and G. Winans. 1984. Genetic characterization of populations in the southeastern range of sockeye salmon. In: (Walton, J.M., and D.B. Houston, editors) Proceedings of the Olympic Wild Fish Conference, pp. 17-32. Peninsula College, Port Angeles, Washington.
Utter, F.M., G. Milner, G. Stahl, and D. Teel. 1989. Genetic population structure of chinnok salmon, *Oncorhynchus tshawytscha*, in the Pacific Northwest, North America.
Verspoor, E. 1988. Reduced genetic variability in first-generation hatchery populations of Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences, 45: 1686-1690.
Vrijenhoek, R.C. 1994. Genetic diversity and fitness in small populations. In: (Loeschcke, V., J. Tomiuk, and S.K. Jain, editors) Conservation Genetics, pp. 37-53. Birkhauser Verlag Basel, Switzerland.
Waples, R.S. 1991. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. Canadian Journal of Fisheries and Aquatic Sciences, 48(Supplement 1): 124-133.
Waples, R.S. 1995. Evolutionarily significant units and the conservation of biodiversity under the Endangered Species Act. In: (Nielsen, J.L., editor) Evolution and the Aquatic Ecosystem: Defining Unique Units in Population Conservation, pp. 8-27. American Fisheries Society Symposium, 17, Bethesda, Maryland.
Waples, R.S. 1999. Dispelling some myths about hatcheries. Fisheries, 24(2): 12-21.
Ward, R.D., M. Woodwark, and D.O.F. Skibinski. 1994. A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. Journal of Fish Biology, 44: 213-232.
Waser, N.M., and M.V. Price. 1994. Crossing distance in *Delphinium nelsonii*: outbreeding and inbreeding in progeny fitness. Evolution, 48(3): 842-852.
Washington, P.M., and A.M. Koziol. 1993. Overview of the interactions and environmental impacts of hatchery practices on natural and artificial stocks of salmonids. Fisheries Research, 18: 105-122.
Wehrhahn, C.F., and R. Powell. 1987. Electrophoretic variation, regional differences and gene flow in the coho salmon (*Oncorhynchus kisutch*) of southern British Columbia. Canadian Journal of Fisheries and Aquatic Sciences, 44: 822-831.
Weir, B.S., and C.C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution, 38: 1358-1370.
Weiss, S., C. Scholtterer, H. Waidbacher, and M. Jungwirth. 2001. Haplotype (mtDNA) diversity of brown trout (*Salmo trutta*) in trubutaries of the Austrian Danube: massive introgression of Atlantic basin fish – by man or nature? Molecular Ecology 10(5), 1241-1246.
Wenburg, J.K., P. Bentzen, C.J. Foote. 1998. Microsatellite analysis of genetic population structure in an endangered salmonid: the coastal cutthroat trout (*Oncorhynchus clarki clarki*). Molecular Ecology, 7: 733-749.
Whitlock, M.C. 1992. Temporal fluctuations in demographic parameters and the genetic variance among populations. Evolution, 46(3): 608-615.
Whitlock, M.C. 2000. Rixation of new alleles and the extinction of small populations: drift load, beneficial alleles, and sexual selection. 2000. Evolution, 54: 1855-1861.
Whitlock, M.C., and D.E. McCauley. 1999. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. Heredity, 82: 117-125.
Williams, R.N., R.P. Evans, and D.K. Shiozawa. 1997a. Mitochondrial DNA diversity patterns of bull trout in the upper Columbia River basin. In: (Mackay, E.C., M.K. Brewin, and M. Monita, editors) Friends of the Bull Trout Conference Proceedings, pp. 283-297. Bull Trout Task Force (Alberta, Canada). Trout Unlimited Canada, Calgary.
Williams R.N., R.F. Leary, and K.P. Currens. 1997b. Localized genetic effects of a long-

term hatchery stocking program on resident rainbow trout in the Metolius River, Oregon. North American Journal of Fisheries Management, 17: 1079-1093.

Winsby, M., and E. Stone. 1996. Hill Creek Hatchery Assessment and Operating Plan Options Report. Report prepared for Columbia Basin Fish & Wildlife Compensation Program by Hatfield Consultants, West Vancouver, BC.

Wright, D.H., and J.H. Reeves. 1992. On the meaning and measurement of nestedness of species assemblages. Oecologia, 92: 416-428.

Wright, D.H., B.D. Patterson, G.M. Mikkelson, A. Cutler, W. Atmar. 1998. A comparative analysis of nested subset patterns of species compositions. Oecologia, 113: 1-20.

Wright, S. 1943. Isolation by distance. Genetics, 28: 114-138.

Wright, S. 1951. The genetical structure of populations. Annals of Eugenics, 323-354.