Population and Landscape Genetics of Arctic Grayling (*Thymallus arcticus*)

by

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Abstract

I investigated the population and landscape genetics of Arctic Grayling (*Thymallus arcticus*) distributed throughout several connected river systems in Alberta, Canada. Broad- and fine-scale population structure was examined by genotyping nine microsatellite loci in 1,116 Arctic Grayling captured from 40 sites in the Hay River, Peace River, and Athabasca River basins. Genetic diversity tended to decline from north to south (allele richness-latitude: Spearman's rank correlation rs = 0.793, $P < 0.05$), with the lowest level detected in a stocked population. Significant genetic divergence between and within major river basins was found (overall F_{ST} (θ) = 0.13) as well as strong isolation by distance patterns in the Peace River basin (Mantel r = 0.97, $P < 0.001$) and Athabasca River basin (Mantel r = 0.95, $P < 0.001$). Evidence for gene flow among sites in neighbouring rivers (i.e., 25–100 km apart) was common; significant genetic differentiation tended to occur at the sub-basin level. Allelic richness (Ar) was associated with variables describing post-glacial colonization route, spatial position in the stream network, and density of anthropogenic disturbance. These findings have important implications for species management and conservation, particularly in regards to management unit delineation, supplementation procedures, conservation priorities (i.e., protecting small and/or isolated stocks), and land-use planning.

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Preface

This thesis is an original work by Jessica Rae Reilly. Fish collection by University of Alberta staff was conducted in accordance to guidelines approved by the University of Alberta Animal Care and Use Committee (Protocol No. 758/09/13) and was permitted under provincial Fish Research Licences (Licence No. 12-2003 FRL, 12-0437 FRL, and 12-1201 FRL).

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Chapter 1

Population and Landscape Genetics of Arctic Grayling

Genetic analyses can provide insights into many characteristics of fish populations. For example, neutral molecular data have been used to elucidate seasonal migration patterns (Ruzzante et al. 2004), detect recent population declines (Peterson and Arden 2009), estimate effective population size (Serbezov et al. 2012), and characterize the spatial patterns of genetic differentiation and diversity (i.e., population structure; Rogers and Curry 2004). Genetic and spatial data have also been combined to identify barriers to fish movement (Small et al. 2007; Leclerc et al. 2008; Meeuwig et al. 2010) and explore how landscape characteristics and human activities in watersheds have influenced the genetic variability of populations (Angers et al. 1999; Costello et al. 2003; Tamkee et al. 2010). This knowledge has direct links to fisheries management through applications including Mixed-Stock Analysis (i.e., estimating the contribution of each source population to a mixed assemblage of indivudals), development of supplementation programs, delineation of Management Units, and land-use planning (Hallerman 2003). Consequently, genetic data are particularly valuable when managing exploited and vulnerable species, such as the Arctic Grayling (*Thymallus arcticus*). In this thesis I use genetic methods to study several aspects of Arctic Grayling in Alberta.

Species description

Arctic Grayling is a salmonid, sport fish closely related to salmon, trout and whitefish. The species' range extends from central Asia to eastern Canada, including St. Lawrence Island in the Bering Sea (Scott and Crossman 1973). In North America, Arctic Grayling are found from Alaska to Hudson Bay and south to the Athabasca River watershed in Alberta (Nelson and Paetz 1992). A disjunct population is located in the headwaters of the Missouri River, Montana. Arctic Grayling were once present in northern Michigan, but were extirpated before the 1940s (Scott and Crossman 1973). The species has also

been stocked to areas outside of its natural range, such as southern Alberta, Vermont, Utah, and Colorado (Scott and Crossman 1973; Berry 1998).

Arctic Grayling typically reside in riverine habitats within the southern portion of their range and complete three seasonal migrations to overwintering, summer feeding, and spawning areas. In late September to mid-October, lower water temperatures and flows trigger the downstream migration to watercourses that do not freeze to the bottom, contain sufficient water volumes to prevent hypoxic conditions, and have low occurrence of frazil ice (Armstrong 1986; Northcote 1995; Stanislawski 1997). Overwintering habitat is highly variable, ranging from deep pools to shallow, high-velocity riffles (Stanislawski 1997; Blackman 2002). Spawning migrations occur at the end of April to early May when water temperatures increase to approximately 1°C (Ward 1951; Tchir et al. 2003). Spawning occurs from early May to mid-June (Ward 1951; Nelson and Paetz 1992), followed by migration to summer habitat. Telemetry studies in Alberta and British Columbia have documented movement of adult and juvenile Arctic Grayling into different rivers to feed after adults breed, resulting in moderate to high levels of mixing between spawning assemblages (Stamford 2001; Blackman 2002). Young-of-the-year fish typically remain in the vicinity of the spawning habitat, although water currents may carry them downstream to backwater areas (Tack 1980; Armstrong 1986).

It is frequently reported that Arctic Grayling return to the same summer habitat and may also home to their natal streams to spawn (Tack 1980; Northcote 1995). However, the degree of fidelity is variable between watersheds (ASRD 2005). Telemetry studies in Alaska and British Columbia found that 20 to 99% of fish in unsilted, rapid runoff streams return to their summer habitat within a one-year cycle (Fish 1998; Tack 1980; Blackman 2002). Clark (1993) found that >50% of Grayling returned to the summer feeding habitat every year for 5 years. Unlike summer-habitat fidelity, natal philopatry has not been extensively investigated. Previous studies have reported tagged fish returning to the same stream during the spawning period, but also occasional straying between spawning streams (Craig and Poulin 1975; Jessop and Lilley 1975; Merkowsky 1989).

Conservation and management

Conservation status of Arctic Grayling varies throughout its range, with some stocks considered secure whereas others are critically imperiled. In Alberta, the abundance of Arctic Grayling has been declining since the 1950s and many populations have been extirpated or are severely depressed (ASRD 2005). These declines have resulted in catch rates of less than 1 fish/hour in many watercourses that historically had catch rates of 4 to 7 fish/hour (Berry 1998). Similar to other native fish species (e.g., Bull Trout [*Salvelinus confluentus*], ASRD and Alberta Conservation Association 2009), lower abundances have been attributed to overharvest, habitat fragmentation, and changes in water quality as a result of human disturbance (Sullivan 1988; ASRD 2005). Arctic Grayling is now designated provincially as a Species of Special Concern (ASRD 2001). This designation requires the provinicial government to develop and implement a species management plan (ASRD 2008).

Management of Arctic Grayling in Alberta was modified in 1998 in response to population declines. Specifically, winter and spring fishing seasons were closed and the minimum harvestable size limit was increased to allow fish to mature and spawn at least once (Berry 1998). Zero-limit fisheries were also established in several watercourses in the Athabasca and Peace River watersheds (Berry 1998). These regulations may not be sufficient to recover some Arctic Grayling populations within a short-time frame (i.e., several generations) if human-induced environmental change is a major force behind the declines. Currently, the relative role of anthropogenic disturbance in population declines is largely unknown (ASRD 2005, but see Scrimgeour et al. 2008) and should be addressed in future research to improve species management.

Population and landscape genetics

Management and status assessment of Arctic Grayling populations are also hampered by the paucity of genetic data (ASRD 2005). The potential effects of landscape characteristics and sportfishing on genetic diversity have not been investigated and

studies characterizing population structure are limited. Stamford and Taylor (2005) analyzed variation at five microsatellite loci in fish captured upstream, downstream, and within the Williston Reservoir, British Columbia. In this highly altered system, differentiation among nine sample sites was variable (F_{ST} average= 0.21, F_{ST} range 0.00 to 0.57). No significant differences in allele frequencies were detected at three locations situated approximately 200 km apart. Peterson and Arden (2009) investigated population structure in the upper Missouri River using 10 microsatellites loci. In this study, samples were collected from locations thought to represent spawning areas. Low differentiation (F_{ST} average <0.0055) was found between five fluvial spawning assemblages in the Big Hole River system. Low to moderate differentiation (F_{ST} range 0.071 to 0.174) was detected when these assemblages were pooled and then compared with one fluvial and three lacustrine stocks located upstream of a major dam. These studies provided important information to regional fisheries managers, but may not accurately describe Arctic Grayling structure in Alberta. Arctic Grayling are typically distributed throughout large, unobstructed river systems in Alberta, whereas the previous study areas included reservoirs or dams. Additionally, most Arctic Grayling populations in the province have not been altered by stocking, but this practise has been widely implemented in Montana (Peterson and Arden 2009).

Similar to population genetics, Arctic Grayling phylogeography has received relatively little attention. In North America, the species was likely founded by individuals belonging to the Pacific-basin lineage of Siberian Arctic Grayling (Stamford and Taylor 2004). Dispersal events across the Bering land bridge are thought to have occurred from the mid-late Pliocene to the mid-Pleistocene (Redenbach and Taylor 1999; Stamford and Taylor 2004). There is evidence that Arctic Grayling persisted in refugia located in the Nahanni River valley in the Northwest Territories, the Yukon River valley and the Brooks Mountain Range in Alaska, and in the Upper Missouri River valley in Montana during the Wisconsin glaciation (McPhail and Lindsey 1970; Redenbach and Taylor 1999; Stamford and Taylor 2004). The existence of these putative refugia is supported by relatively higher levels of genetic diversity in extant populations close to assumed refugia locations (Stamford and Taylor 2004), fossils of Arctic Grayling in southern Alberta

dating between 20,000 and 30,000 years ago (Burns 1991), and these locations have been proposed as refugia for other fish species as well (McPhail and Lindsey 1970).

Colonization from these refugia following glacial retreat resulted in three major phylogeographical lineages of Arctic Grayling in present-day North America (Stamford and Taylor 2004). The North Beringia lineage includes populations distributed throughout the northern Arctic, Saskatchewan, and Montana. The South Beringia lineage encompasses the Pacific coastline and interior of British Columbia. Lastly, populations composing the Nahanni lineage are confined to the Nahanni River valley. Previous studies have not included samples from Alberta, which may represent a major contact zone between all three lineages. Addressing this knowledge gap will elucidate possible dispersal routes for fish species colonizing North America in a southward direction post-glaciation and may have important implications for species conservation with regards to the appropriate delineation of Designatable Units as employed by the Committee on the Status of Endangered Wildlife in Canada.

Thesis goals and overview

The aim of this thesis is to characterize the genetic attributes of Arctic Grayling populations. In Chapter 2, I characterize variation at microsatellite DNA markers to elucidate broad- and fine-scale population structure and diversity across the Hay, Peace, and Athabasca River basins in Alberta. My findings may be used to infer post-glacial colonization routes. In Chapter 3, I investigate associations between landscape characteristics, human activities, and genetic diversity of Arctic Grayling. Specifically, I determine the best predictor(s) of allelic richness, and expected and observed heterozygosity from a suite of variables describing latitude, adult density, environmental factors limiting productivity, recreational angling pressure, spatial position in the stream network, and anthropogenic disturbance. Chapter 4 is a summary of the major findings of the data chapters and will provide recommendations for species management and future research.

The findings of this thesis will be valuable for delineating Management Units, identifying vulnerable populations (i.e., those exhibiting low genetic diversity that may indicate low abundance or limited connectivity), informing future stocking policies, and understanding the interactions among anthropogenic disturbance, sport fishing, and genetic variability. In a broader context, this study advances the field of landscape genetics by investigating if increased intensity of human disturbance and low habitat availability can significantly reduce genetic variability in wild populations continuously distributed across a landscape. Additionally, my findings will allow comparisons of genetic characteristics and demographic processes (i.e., frequency of homing to natal streams) among salmonid species, increasing the understanding of the biology of this commercially and recreationally valuable family of fish.

Chapter 2

Population Genetics of Arctic Grayling Distributed Across Large, Unobstructed River Systems

Introduction

Spatial patterns of genetic differentiation and diversity of wild species reflect historical events, reproductive behaviour, effective population size, and contemporary geography and connectivity (Frankham 1996; Costello et al. 2003; Taylor et al. 2003). Understanding the genetic characteristics of populations is a common component of effective fisheries management (Spangler et al. 1981). The management of an exploited fish species often involves dividing its range into smaller management units that contain distinct populations having restricted or no exchange of individuals with neighboring populations (Taylor and Dizon 1999; Palsbøll et al. 2006). Support for separate management units is provided when panmixia is statistically rejected or, ideally, when measures of genetic divergence between populations are higher than a threshold level defined by managers (Taylor and Dizon 1999; Palsbøll et al. 2006). Genetic data have also been used to prioritize populations for conservation and identify populations that could serve as a source of broodstock for supplementation programs (Coleman et al. 2013).

Many studies investigating fine scale population structure of freshwater fish occur within systems that contain probable or known man-made barriers to movement, such as hydroelectric dams, weirs, and culverts. These studies often conclude that barriers disrupt the natural patterns of gene flow and could result in small and/or isolated populations with reduced genetic diversity (e.g., Wofford et al. 2005; Neville et al. 2006). Ideally, a comparison of genetic differentiation and diversity before and after barrier installation should be conducted to reduce the influence of confounding factors and to better quantify changes to gene flow. However, opportunities to investigate the genetic characteristics of populations pre- and post- disturbance are rare. As an alternative, population structure could be compared between species inhabiting systems with and without barriers if

ecological conditions, historical colonization patterns, and life history of the species are similar. Arctic Grayling provide an opportunity to characterize genetic differentiation and diversity of a migratory freshwater fish species that is continuously distributed within large, unobstructed river systems.

Arctic Grayling are a salmonid species with a holarctic distribution ranging from central Asia to eastern Canada (Scott and Crossman 1973). In North America, the species is continuously found east from Alaska to Hudson Bay and south to the Athabasca River basin in Alberta (Nelson and Paetz 1992). There is also a disjunct population in Montana (Nelson and Paetz 1992). Mitochondrial DNA (mtDNA) analyses support three major phylogeographic lineages of Arctic Grayling in North America distributed within: 1) northern Alaska, Saskatchewan, and Montana; 2) southern Alaska and British Columbia; and 3) the Nahanni and Liard rivers (Stamford and Taylor 2004). Alberta represents a potential contact zone between these lineages.

Historical processes and contemporary isolation have likely influenced Arctic Grayling population structure. Homing in salmonid species can lead to reproductive isolation between populations and a high degree of differentiation within connected river systems and even within the same river (Taylor et al. 2003; Warnock et al. 2010). Conversely, straying between populations can act to homogenize allele frequencies at varying spatial scales (Mills and Allendorf 1996; Rogers and Curry 2004). Arctic Grayling fidelity to spawning areas has not been extensively investigated, although there have been observations of tagged fish returning to the same stream during the spawning period (Craig and Poulin 1975; Jessop and Lilley 1975; Merkowsky 1989). A similar species, the European Grayling (*Thymallus thymallus*), exhibits homing to spawning sites at variable levels (19%-92%) (Witkowski and Kowaleski 1988; Pavlov et al. 1998). Occasional straying of Arctic Grayling between spawning streams has also been documented (Jessop and Lilley 1975). Previous studies on Arctic Grayling population structure have found significant genetic differentiation at the sub-basin level and a strong isolation by distance pattern among populations in British Columbia (Hop and Gharrett 1989; Stamford and Taylor 2005; Peterson and Ardren 2009). The degree of genetic

differentiation among populations distributed throughout large river systems without hydroelectric dams or reservoirs has not been investigated.

My main objectives were to investigate the broad and fine scale spatial patterns of genetic differentiation and diversity of Arctic Grayling. I hypothesized that major river basins would contain highly divergent populations due to spatial isolation. Within major river basins, I expected moderate differentiation among populations within different sub-basins because populations may be linked by occasional gene flow. My findings will be compared with that reported for other Arctic Grayling populations and will be used to inform management plans for the species.

Methods

Sample sites and tissue collection

The study area was located in northern Alberta, Canada (Figure 2-1). Tissue samples from Arctic Grayling were collected at 40 sites, including 29 sites in the Athabasca River basin, eight sites in the Peace River basin, and two sites in the Hay River basin (Table 2-1). There are no known movement barriers between sites. Samples were also collected from Quarry Lake in southern Alberta, which is not hydrologically connected to the other sampled rivers and is stocked with hatchery- raised fish descended from Arctic Grayling captured in Freeman River. Study sites were selected based on archived sample availability, suspected presence of Arctic Grayling, and accessibility for sampling.

Tissue samples from 1,116 Arctic Grayling were collected in the field or were provided by the provincial government and volunteer organizations. All Arctic Grayling included in the study were collected by angling or electrofishing from May to September, 2007-2012. Ages of sampled fish were not estimated and sex was not distinguishable in the field. However, it is assumed that Arctic Grayling represent multiple age classes and both sexes based on the large number of individuals used in the study and their range in total length (94mm to 402mm). Tissue samples consisted of dried pelvic fin clips and scales

stored at room temperature, or of adipose and pelvic fin clips stored in 95% ethanol at -20^{o}C.

Microsatellite analysis

A Qiagen DNeasy^{TM} Blood and Tissue Kit DNA was used to extract DNA from a 1 mm^2 piece of fin tissue or scale following the manufacturer's protocol. Genetic variation was examined at nine microsatellite loci (Table 2-2). GTTT pigtails were added to reverse primers to promote adenylation during polymerase chain reaction (PCR) amplification (Brownstein et al. 1996). This reduced the potential for genotyping errors resulting from the occasional, non-templated addition of adenosine to the 3' end of PCR products (Brownstein et al. 1996). Loci were amplified in one individual and three multiplexed 10 μL reactions (Table 2-2) containing ~100 ng of genomic DNA, 1X PCR buffer (10 mM Tris pH 8.8, 0.1% Triton X-100, 50 mM KCl, 0.16 mg/mL BSA), 1.5 mM MgCl2, 200 μM each dNTP, optimized primer amounts (Table 2-2), and 1U Taq DNA polymerase. PCR cycling conditions consisted of an initial denaturing step at 94°C for 30 s, 38 amplification cycles of 94°C for 30 s (denaturation), 58°C for 30 s (annealing), and 72°C for 30 s (extension) followed by a final extension at step at 72°C for 7 min. These amplification reactions were loaded into three injections (Table 2-2) on an ABI 3730 DNA analyzer and genotyped using GeneMapper softwear (Applied Biosystems). Marker lengths were determined relative to GeneScan-500LIZ (Applied Biosystems). All loci were successfully genotyped for >95% of individuals and any individuals that displayed missing genotypes for more than two of the nine loci were excluded.

Genetic diversity

Each site was tested for Hardy–Weinberg equilibrium (HWE) using the exact test (Guo and Thompson 1992) implemented in GENEPOP 4.2 (Rousset 2008). Tests for linkage disequilibrium (LD) between all pairs of loci per site were conducted using a Markov chain method in GENEPOP 4.2 (Rousset 2008) and default parameter values. Non-sequential Bonferroni corrections (Rice 1989) were used to adjust the level of significance ($P < 0.05$) for the HWE and LD tests. Genotyping errors and the presence of null alleles were assessed using the program Micro-Checker v.2.2.3 (Oosterhout et al.

2004). The Excel Microsatellite Toolkit (Park 2001) was used to identify duplicated samples. Relatedness between individuals at each sample site was tested via the program ML-Relate (Kalinowski et al. 2006) using 99% confidence sets and 10,000 randomisations.

Genetic diversity was measured as gene diversity, allele richness, and private allele richness. The Excel Microsatellite Toolkit (Park 2001) was used to calculate allele frequencies and expected heterozygosity averaged across all loci per site. Allele and private allele richness were calculated and standardized for sample size (Kalinowski 2004) via the program HP-Rare 1.0 (Kalinowski 2005). A private allele was defined as an allele with a frequency of ≥ 0.01 that was observed at only one sample location. Diversity estimates for Hightower Creek, Athabasca River at Lynx Creek, Caribou River, and Lawrence River should be interpreted with caution as few individuals were analysed (i.e., <15 fish). I calculated Spearman's rank correlation coefficients (r_s) of allele richness and heterozygosity against ranked sample site latitude to detect significant spatial trends in genetic diversity. I excluded the stocked site, Qu, from the spatial analysis.

Population structure and isolation by distance

The level of genetic divergence (F_{ST}) was estimated using θ_{ST} (Weir and Cockerham 1984) calculated in FSTAT v.2.9 (Goudet 2001). Statistical significance of θ_{ST} was obtained using 6000 permutations. The significance level ($P < 0.05$) was adjusted using a non-sequential Bonforreni correction (Rice 1989). Reported θ_{ST} estimates should be considered as relative measures of differentiation because of population sub-sampling (Holsinger and Weir 2009). Hierarchical partitioning of genetic variation between major river basins and other potential regional divisions was evaluated using analysis of molecular variance (AMOVA; Excoffier et al. 1992) in Arlequin v.3.5 (Excoffier and Lischer 2010).

I tested for isolation by distance within the Peace River and Athabasca River basins using Mantel tests and spatial autocorrelation analyses. Simple Mantel tests (Mantel 1967) were implemented to assess the significance of correlations between pairwise genetic

divergence and river distance. River distances between sites were calculated using 1:20 000 National Topographic System map sheets (AltaLIS 2008) and tests were implemented in the program ZT (Van de Peer 2002). Spatial autocorrelation analyses were used to assess the geographic extent of non-random mating within three areas in the Athabasca River basin that had the greatest number of sample sites. I generated an individual-by-individual genetic distance matrix using the method of Smouse and Peakall (1999) implemented in GENALEX v.6.3 (Peakall and Smouse 2006). The distance matrix was composed of river distances between sites, with a value of zero assigned for pairs of individuals collected from the same site. I calculated autocorrelation coefficients (r) in GENALEX for 25 km distance size classes. Autocorrelation coefficients were considered significant when (1) the 95% error bar estimated by 1,000 bootstrap trials was greater than zero and (2) when the estimated mean value of r did not overlap with the 95% confidence interval about the null hypothesis of no spatial genetic structure generated using 100 permutations (Peakall et al. 2003; Neville et al. 2006).

Discriminant analysis of principle components (DAPC) is a multivariate method that maximizes between-group variation while minimizing within-group variation and requires no assumptions regarding the underlying genetic model (Jombart et al. 2010). I used DAPC to cluster genetically similar sites among and within major river basins, excluding the stocked site, Qu. First, data were transformed using Principle Component Analysis. I then retained the number of principle components sufficient to explain >90% of total variance of the data for use in the subsequent Discriminant Analysis. All analyses were performed using the adegenet package (Jombart 2008) in R version 2.15.2 (R Development Core Team 2012). Results were visualized in a scatterplot generated by the adegenet package (Jombart 2008).

The Bayesian clustering method implemented in STRUCTURE v.2.3.1 (Pritchard et al. 2000) was also used to characterize population structure within the Peace River and Athabasca River basins. In contrast to DAPC, this method clusters individuals by minimizing Hardy–Weinberg and gametic disequilibrium and does not require prior spatial information (Pritchard et al. 2000). All analyses in STRUCTURE used the

correlated allele frequencies model and allowed admixture. Ten independent runs were executed for *K*=1 to 7 (*K*= number of clusters) using sites located within the Peace River basin. For sites in the Athabasca River basin, including Qu, 10 independent runs were executed for *K*=1 to 9. I also conducted an additional 10 runs (*K*=1 to 9) excluding Qu to determine if the stocked site had substantial influence on the STRUCTURE results. All runs had a 250, 000 burn-in followed by 500, 000 iterations. Multiple runs per *K* were processed using Structure Harvester (Earl and vonHoldt 2012). The optimal alignment of all runs for each *K* was found using CLUMPP (Jakobsson and Rosenberg 2007) and visualized using DISTRUCT (Rosenburg 2004). I followed the recommendations of Evanno et al. (2005) during model selection because I assumed that gene flow among populations was not homogenous. The optimal *K* was chosen based on the highest rate of change in the log probability of the data (*DeltaK*). In cases where *DeltaK* was multimodal, I visualized the partitioning of individuals at each *K* to determine if individuals were assigned to additional clusters asymmetrically and if assignments were biologically meaningful (Pritchard et al. 2000).

Results

Microsatellite summary

Nine microsatellite loci used in this study displayed moderate to high polymorphism (Table 2-2). Alleles having a one base-pair deletion were observed in many individuals from the Hay River and Peace River basins at *Tar*108 and *Tar*110. I assumed that deletions were a result of mutation because they were consistently observed in replicated PCR amplification and genotyping trials. Genotypic frequencies were out of HWE for 3 of 360 tests. One locus was out of HWE at Qu (*Tar*104), Wt (*Tar*109), and Dv (*Tar*101). Significant LD was observed at 6 of 1440 total pairs of loci across sites, with no consistent pattern of linkage between any two loci. Evidence for null alleles was rare, with positive tests occurring at AtL (*Tar*115), Ed (*Tar*100), Wt (*Tar*109), Le (*Tar*110), and Dv (*Tar*101). No evidence of relatedness was found for the majority (>98%) of the possible pairs of fish at each sample site, indicating that relatedness had little effect on the results of subsequent analyses.

Broad scale population structure and diversity

The level of genetic diversity declined from north to south across major river basins (heterozygosity-latitude: $r_s = 0.345$, $P < 0.05$; allele richness-latitude: $r_s = 0.793$, $P < 0.05$; Table 1). The Athabasca River basin had the lowest average expected heterozygosity, allele richness, and private allele richness. Average expected heterozygosity was similar between the Peace River and Hay River basins. Allele and private allele richness was highest in the Hay River basin.

Major river basins were highly divergent (Figure 2-2A; Table 2-3, 2-4). Overall θ_{ST} was 0.13, reaching a low of 0 for many pairwise comparisons and a high of 0.37 between Ra and La. Mean θ_{ST} was 0.20 between sites in the Hay River and Peace River basins and between sites the Peace River and Athabasca River basins. Mean θ_{ST} was 0.24 between sites in the Hay River and Athabasca River basins. The greatest amount of regional variation was explained when sites were grouped based on their location within the Hay River basin, Athabasca River basin, and upper and lower sections of the Peace River basin (Table 2-5).

Diversity within major river basins

Genetic diversity within major river basins was variable (Table 2-1). Sites within the Peace River basin displayed moderate to high levels of genetic diversity when compared to the averages for all sites in my study. Expected heterozygosity averaged 0.72 and ranged from 0.66 in Na to 0.76 in Sm and Ca. Allele richness averaged 6.83 and ranged from 5.74 in Na to 8.54 in Ca. Private allele richness averaged 0.19. Diversity in the Athabasca River basin tended to be lower. Excluding Qu, expected heterozygosity at sites averaged 0.67 and ranged from 0.50 in Ra to 0.72 in Ed. Allele richness averaged 5.78 and ranged from 4.12 in Ra to 6.52 in In. Private allele richness averaged 0.05. Samples from the stocked population in Qu exhibited the lowest expected heterozygosity and allele richness.

Peace River basin population structure

Varying degrees of genetic differentiation were detected between sample sites in the Peace River basin (Figure 2-2B; Table 2-3, 2-4). Mean θ_{ST} was 0.08 and ranged from 0 to 0.16. The greatest differentiation was observed between sites situated in the Wapiti River sub-basin and the Caribou and Lawrence rivers. In contrast, θ_{ST} between many sites located in close proximity within the Simonette River sub-basin and within the Wapiti River sub-basin was low or not statistically significant (adjusted P=0.00006). A strong and significant pattern of isolation by distance was detected (Figure 2-3).

Evidence of hierarchical population structure was found using an individual based Bayesian clustering method. Two modes of *DeltaK* were identified in the STRUCTURE results (Figure 2-4) when following the recommendations of Evanno et al. (2005). The strongest signal occurred at K=2 (*DeltaK* = 96.1), which indicates the uppermost level of structure. The two genetic clusters identified under this model included individuals primarily from the Wapiti River sub-basin or primarily from Sm, Ca, and La. The second peak of *DeltaK* occurred at K=4 (*DeltaK*= 60.1). Under this model, the four clusters mostly included individuals from: (1) Lm, (2) Ca and La, (3) Na, Wa, and No, and (4) Si, Dv, and No. AMOVA indicated that greater regional variation was explained when No was grouped with other sites in the Wapiti River sub-basin rather than the Simonette River sub-basin (Table 2-5).

Athabasca River basin population structure

The Athabasca River basin also contained genetically differentiated groups of Arctic Grayling (Table 2-3, 2-4). Excluding Qu, mean θ_{ST} was 0.07 and ranged from 0 to 0.20. Sites in the Pembina River sub-basin and Ho were most differentiated. Many sites in the same sub-basin were not distinct (47 out of 435 pairwise θ_{ST} comparisons, adjusted P=0.00006). Genetically similar sites tended to be located within four broad geographic areas including: (1) all rivers upstream of the Freeman River, (2) the Freeman River sub-basin, (3) the Pembina River sub-basin, and (4) all other downstream sites (Figure 2-2C). Investigation of sub-structure revealed further divisions including the Lesser Slave Lake sub-basin, Upper Athabasca River sub-basin, Central McLeod River sub-basin, Upper

McLeod River sub-basin, Ho, Ra, and Di (Figure 2-2D-F). Fish from sites within the Freeman River sub-basin (i.e., Fc, Fr, and Mr) and Qu were moderately differentiated (mean $\theta_{ST} = 0.10$). Considering all sites within the basin, the greatest amount of regional variation was explained by grouping stocked and natural populations (AMOVA; Table 2-5).

Similar patterns of differentiation were found using individual-based clustering methods when Qu was included or excluded from the analysis. I identified three modes of *DeltaK* in STRUCTURE results (Figure 2-5). The strongest signal occurred at K=2 (*DeltaK* = 416.7). This model grouped individuals based on their capture location upstream or downstream of the Freeman River. Subsequent modes at K=4 (*DeltaK* = 262.7) and K=8 (*DeltaK* = 38.1) supported further sub-structuring within these reaches. At all hierarchical levels, sites that were geographically intermediate between clusters tended to contain large proportions of migrant or admixed individuals, who were either assigned, or were partially assigned, to neighbouring clusters. A slight increase in the percentage of explained regional variation occurred when sites were grouped based on the K=2, K=4, and K=8 STRUCTURE models, with the majority of remaining variation found within groups (Table 2-5). Explained regional variation was typically lower when Qu was excluded from analyses, but the resulting trends in variation were comparable (data not shown).

A significant isolation by distance pattern was detected between sites and individuals (Figure 2-3). Multilocus genotypes of individuals collected from the McLeod River and tributaries were positively and significantly correlated up to 50 km, with an x-intercept at 131 km. Significant negative coefficients were first detected at 150 km. In the Upper Athabasca River sub-basin, autocorrelation coefficients between individuals were positive and significant up to 25 km with an x-intercept at 116 km. Significantly negative coefficients were first detected at 150 km. Individuals collected from the Lesser Slave Lake sub-basin were positively and significantly correlated up to 50 km, with an x-intercept at 117 km; no significant negative coefficients were detected.

Discussion

Broad scale divergence and diversity

Significant differentiation was observed when Arctic Grayling were compared between the Hay River, Peace River, and Athabasca River basins. Although the major river basins are hydrologically connected, the lack of long distance dispersal between populations in the upper reaches of each basin has likely resulted in isolation and subsequent divergence. Similar patterns of broad scale population structure in European Grayling have also been attributed to low gene flow between distant populations (Swatdipong et al. 2009). It is unknown to what extent historical processes have shaped current population structure. Stamford and Taylor (2004) found evidence of three distinct lineages of Arctic Grayling in North America based on mtDNA and microsatellite marker variation. These authors postulated that the current range of Arctic Grayling is largely the result of southward dispersal from refugia in North Beringia. However, there is also evidence of limited dispersal from Nahanni and South Beringia refugia, potentially facilitated by post-glacial waterway connections such as Glacial Lake Agassiz (Stamford 2001). It is possible that Arctic Grayling populations in the major river basins in Alberta originated from different ancestral refugia, which would also contribute to the significant levels of genetic divergence I detected.

It is likely that historical processes have influenced the broad scale patterns of genetic diversity in Arctic Grayling. Serial founding effects or bottlenecks during post-glacial colonization can result in reduced genetic diversity at range peripheries of freshwater fish (Bernatchez and Wilson 1998). Under such scenarios, dispersal from northern refugia would result in a southward decline of diversity across major river basins, similar to what I documented in Arctic Grayling. This pattern is comparable to that reported for another Arctic species, Broad Whitefish (*Coregonus nasus*) (Harris and Taylor 2010) and contrasts with patterns for other sympatric salmonid species that likely survived in southern refugia (e.g., Tamkee et al. 2010, Costello et al. 2003). My current analyses do not enable me to determine if Arctic Grayling in the study area belong to a Nahanni, North Beringia, or South Beringia lineage because colonists from all three putative

refugia would have dispersed into the study area from the north. Similarly, sites with high genetic diversity could represent areas near putative refugia or contact zones between phylogeographic lineages. Further research investigating mtDNA variation of Arctic Grayling distributed across Alberta and near putative locations of glacial refugia are needed to elucidate how post-glacial dispersal influenced patterns of population structure and genetic diversity.

A southward decline in diversity may also reflect recent changes in population size and environmental factors. Substantial declines in Arctic Grayling abundance and range extent have been reported in the Athabasca River basin and, to a lesser degree, in the Peace River basin (ASRD 2005). For example, Arctic Grayling were described as a commonly encountered species in the Pembina River in the 1970s (Blackburn and Johnson 2004). However, an extensive sampling program in 2002-2003 (Blackburn and Johnson 2004) encountered low numbers of fish suggesting that the Pembina population had collapsed. Additionally, Arctic Grayling in the Athabasca River basin are at the southern limit of the species' continuous range where population size may be naturally limited by lower habitat suitability (Lesica and Allendorf 1995; ASRD 2005). Although the sizes of Arctic Grayling populations in Alberta are largely unknown, it is possible that populations in the Athabasca River basin may be relatively smaller than those in the north and less genetically diverse due to inbreeding and genetic drift.

Fine scale differentiation and diversity

Genetic diversity was similar across sample sites in the Peace River basin, but was variable in the Athabasca River basin. I sampled more sites in the Athabasca River basin spanning a broader range of environmental conditions that may affect population size and gene flow, and therefore, the level of genetic diversity. Previous studies have found significant correlations between genetic diversity of salmonid populations and drainage pattern and altitude (Angers et al. 1999; Costello et al. 2003).

Sub-basins tended to hold differentiated groups of Arctic Grayling. However, defining clear boundaries around genetically distinct populations was challenging because of gene

flow between neighbouring sites. In the Peace River basin, I found support for four populations situated within the Wapiti River sub-basin, Simonette River sub-basin, Little Smoky River, and Caribou and Lawrence rivers. In the Athabasca River basin, populations were identified in the House River and the Upper Athabasca River, Upper McLeod River, Central McLeod River, Lesser Slave Lake, Freeman River, and Pembina River sub-basins. In some cases, these populations may contain sub-populations that are, to some extent, genetically or demographically independent from each other (e.g., Rat and Dismal creeks). In general, the spatial extents of populations identified in this study were smaller than that found previously for Arctic Grayling inhabiting rivers that flow into Williston Reservoir, British Columbia (Stamford and Taylor 2005). It is unknown if this difference is solely due to the use of different and fewer microsatellite markers in the British Columbia study, or if it reflects natural variation in gene flow or recent, but substantial, changes to gene flow resulting from reservoir construction in British Columbia. My findings are more similar to patterns reported for Arctic Grayling persisting in the Big Hole River system in Montana, which were analyzed with a more comparable, albeit not identical, suite of microsatellite markers (Diggs and Ardren 2008; Peterson and Ardren 2009).

I found a strong isolation by distance pattern in the Peace River and Athabasca River basins. Genetic similarity of fish within adjacent rivers was supported by the results of fine-scale spatial autocorrelation analyses in the Athabasca River basin. This stepping stone pattern of demographic connectivity has been previously reported for Arctic Grayling (Stamford and Taylor 2005), and is not surprising given the species' continuous distribution, mobility, and possible fidelity to rivers for spawning (Scott and Crossman 1974; Jessop and Lilley 1975).

Genetic differentiation of Arctic Grayling populations occurred at an intermediate spatial scale when compared to other sympatric salmonid species. For example, Bull Trout (*Salvelinus confluentus*) populations tend to be highly differentiated among tributaries (Costello et al. 2003) and Mountain Whitefish (*Prosopium williamsoni*) exhibit low, or no, differentiation between sub-basins (Whiteley et al. 2004). Interspecific differences

could reflect species' demographic characteristics, such as population size and degree of fidelity to spawning sites (Whiteley et al. 2004). Primary driving forces behind population structure also appear to differ among these species. Unlike Arctic Grayling, patterns of isolation by distance were found to be weak or absent between Bull Trout and Mountain Whitefish populations studied at a similar geographic extent (Whiteley et al. 2004; Whiteley et al. 2006). For Mountain Whitefish, this observation was attributed to relatively high gene flow and large population size (Whiteley et al. 2006). In contrast, variable differentiation between Bull Trout populations separated by similar river distances may indicate that dispersal of this species is more influenced by landscape heterogeneity than Arctic Grayling or Mountain Whitefish (Costello et al. 2003; Whiteley et al. 2004).

Conservation implications

My findings on Arctic Grayling population structure and genetic diversity have important implications for the conservation of this species. In Alberta, Arctic Grayling have experienced severe declines in abundance within many river systems and are now provincially classified as a Species of Special Concern (ASRD 2001). The moderate to high levels of genetic differentiation detected in this study suggest that some rivers hold demographically independent units of Arctic Grayling. Consequently, immigration from neighbouring rivers is unlikely to bolster declining populations or result in re-population of extirpated areas over short time scales (i.e., several generations). If supplementation is considered necessary to conserve declining stocks, my data will be useful when selecting donor populations based on genetic similarity and devising collection protocols for broodstock (Miller and Kapuscinski 2003; Coleman et al. 2013). For example, Quarry Lake demonstrates how the propagation of relatively small numbers of Arctic Grayling can result in low genetic diversity and differentiation of stocked and donor populations over a period of approximately 16 years. Loss of genetic diversity due to hatchery propagation has corresponded to fitness declines in other fish species (Araki and Schmid 2010).

To further resolve fine scale population structure, I recommend future studies that investigate genetic variation within groups of actively spawning Arctic Grayling. A previous telemetry study found that adult Arctic Grayling often move into different rivers to feed after spawning, resulting in moderate to high levels of mixing between spawning aggregates (Blackman 2002). Grouping individuals collected from summer feeding habitats may have impaired my ability to delineate discrete populations. However, my study still offers a useful preliminary investigation of population structure that can be used to guide future sampling protocols (see Diniz-Filho and De Campos Telles 2002).

Chapter 2 Tables and Figures

Table 2-1. Site location and genetic diversity of Arctic Grayling captured in the Hay River, Peace River, and Athabasca River basins in Alberta, Canada. Shown are the number of individuals genotyped (*N*), expected heterozygosity (H_{exp}), and rarefied estimates of allele richness (Ar) and private allele richness (Pa).

Watercourse	Site Code	Latitude (Dec. Deg.)	Longitude (Dec. Deg.)	*N*	H_{exp}	Ar	Pa
Athabasca River basin							
Berland River	Be	53.998	-117.74	43	0.64	5.59	0.05
Hightower Creek	Hi	53.789	-117.93	11	0.67	5.85	0.01
Pinto Creek	Pi	53.727	-117.84	41	0.63	4.98	0.00
Wildhay River[b]	WiUp	53.715	-117.72	33	0.67	5.43	0.02
Wildhay River[a]	WiDo	53.940	-117.34	34	0.67	5.33	0.00
Athabasca River	AtL	53.911	-117.08	11	0.67	5.12	0.00
Athabasca River	AtN	53.980	-116.93	26	0.70	6.00	0.02
Windfall Creek	Wi	54.218	-116.22	30	0.70	6.44	0.04
McLeod River	Mc	53.534	-116.92	31	0.68	5.92	0.01
Sundance Creek	Su	53.555	-116.59	30	0.71	6.12	0.09
Edson River	Ed	53.644	-116.36	30	0.72	6.05	0.01
Wolf Creek	Wo	53.341	-116.09	30	0.69	5.52	0.02
Unnamed Tributary	Wt	53.348	-116.13	35	0.70	5.58	0.08
Lendum Creek	Le	53.183	-116.59	30	0.67	5.39	0.00
Erith River	Er	53.325	-116.65	29	0.70	5.58	0.00
Embarras River	Em	53.304	-116.89	32	0.68	5.64	0.00
Freeman Creek	Fc	54.677	-115.48	31	0.70	6.09	0.02
Morse River	Mr	54.505	-115.07	31	0.67	5.77	0.00
Freeman River	Fr	54.588	-115.57	31	0.71	6.31	0.00
Quarry Lake	Qu	51.075	-115.37	29	0.55	3.62	0.00
Marten Creek	Mn	55.534	-114.82	32	0.70	6.12	0.01
Sawridge Creek	Sa	55.177	-114.95	33	0.71	6.21	0.04
Otauwau River	Ot	55.148	-114.55	33	0.67	6.47	0.17
Driftpile River	Dr	55.078	-115.74	31	0.67	5.63	0.05
Inverness River	In	55.034	-115.39	31	0.70	6.52	0.04
Swan River	Sw	54.800	-115.52	27	0.70	6.46	0.05
Moosehorn River	Mo	54.873	-115.47	29	0.67	6.19	0.03
Rat Creek	Ra	53.207	-115.57	32	0.50	4.16	0.01
Dismal Creek	Di	53.108	-115.68	32	0.59	4.78	0.18
House River	Ho	55.642	-112.15	31	0.65	6.37	0.47
Peace River basin							
Narraway River	Na	54.536	-119.82	22	0.66	5.74	0.04
Wapiti River	Wa	54.737	-120.00	29	0.71	6.05	0.06
Nose Creek	No	54.453	-119.56	21	0.74	6.15	0.18
Simonette River	Si	54.378	-118.16	27	0.71	7.01	0.06
Deep Valley Creek	Dv	54.457	-117.72	15	0.73	6.77	0.04
Little Smoky River	Sm	54.185	-117.50	29	0.76	7.13	0.04
Caribou River	Ca	58.775	-115.89	13	0.76	8.54	0.66
Lawrence River	La	58.763	-115.28	14	0.71	7.25	0.45
Hay River basin							
James Creek	Ja	59.364	-116.30	22	0.73	7.67	0.84
Dizzy Creek	Dz	59.222	-116.14	15	0.70	7.17	0.61

[a]All samples from fish collected downstream of the Pinto Creek confluence.
[b]All samples from fish collected upstream of the Pinto Creek confluence.

Table 2-2. Polymerase chain reaction (PCR) conditions and diversity of nine microsatellite loci used to genotype Arctic Grayling. The number of alleles (*N*) and size range (in base pairs, bp), are given for each locus. Average expected heterozygosity (H_{exp}) per locus is an average value from all 40 sample sites.

Locus	Multiplex PCR and loading[c]	Primer concentration (μM)	*N*	Size range (bp)	H_{exp}
*Tar*100[a]	A,1	0.5	16	254 - 318	0.73
*Tar*101[a]	A,1	0.5	22	263 - 347	0.68
*Tar*104[a]	B,2	0.5	28	133 - 257	0.70
*Tar*106[a]	C,3	0.5	26	246 - 350	0.85
*Tar*108[a]	D,2	1	48	196 - 398	0.79
*Tar*109[a]	B,2	0.5	12	276 - 356	0.34
*Tar*110[a]	C,3	0.5	45	286 - 386	0.88
*Tar*115[a]	C,3	0.5	31	186 - 314	0.88
BFRO 004[b]	C,3	0.2	6	166 - 178	0.30

[a] Primer sequences in Diggs and Ardren (2008)

[b] Primer sequences in Snoj et al. (1998)

[c] Loci with the same letter were included in the same multiplex PCR. Loci with the same number were co-loaded on the ABI 3730 DNA analyzer.

Table 2-3. Pairwise F_{ST} estimates (θ_{ST}, Weir and Cockerham 1984) among sample sites (see Table 2-1 for site code definitions). Bold and underlined values are not significantly different from panmixia following Bonferroni correction ($P < 0.00006$).

Site code	Be	Hi	Pi	WiUp	WiDo	AtL	AtN	Wi	Mc	Su	Ed	Wo	Wt
Hi	**0.02**												
Pi	0.02	**0.01**											
WiUp	**0.01**	**0.01**	**0.00**										
WiDo	**0.00**	**0.01**	**0.01**	**0.00**									
AtL	**0.00**	**0.01**	**0.01**	**0.00**	**0.00**								
AtN	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.00**							
Wi	0.04	**0.04**	0.05	0.03	0.04	**0.02**	0.02						
Mc	0.06	0.07	0.09	0.06	0.06	0.05	0.04	0.02					
Su	0.05	0.05	0.07	0.05	0.04	0.04	0.03	0.03	**0.02**				
Ed	0.06	**0.04**	0.08	0.06	0.05	0.04	0.04	0.03	0.02	**0.01**			
Wo	0.07	0.07	0.10	0.07	0.07	0.06	0.04	0.04	0.02	**0.01**	**0.01**		
Wt	0.06	0.06	0.07	0.05	0.05	0.05	0.04	0.03	0.03	**0.01**	**0.02**	**0.02**	
Le	0.09	0.06	0.10	0.07	0.08	0.07	0.07	0.04	0.03	0.05	0.04	0.06	0.06
Er	0.08	0.06	0.09	0.07	0.07	0.07	0.06	0.04	**0.01**	0.03	0.03	0.04	0.03
Em	0.10	0.10	0.11	0.09	0.10	0.08	0.08	0.04	**0.01**	0.04	0.04	0.05	0.05
Fc	0.09	0.05	0.09	0.07	0.08	0.07	0.07	0.03	0.07	0.06	0.04	0.07	0.07
Mr	0.09	0.05	0.10	0.08	0.09	0.07	0.06	0.03	0.07	0.07	0.04	0.07	0.08
Fr	0.07	0.04	0.07	0.05	0.06	0.05	0.04	0.02	0.05	0.05	0.03	0.06	0.06
Qu	0.16	0.10	0.15	0.14	0.16	0.15	0.14	0.13	0.18	0.18	0.14	0.18	0.19
Mn	0.09	0.09	0.12	0.09	0.10	0.08	0.08	0.03	0.05	0.05	0.05	0.06	0.07
Sa	0.09	0.09	0.11	0.09	0.09	0.07	0.07	0.03	0.04	0.06	0.04	0.05	0.07
Ot	0.09	0.07	0.11	0.09	0.10	0.08	0.07	0.04	0.06	0.07	0.05	0.07	0.09
Dr	0.09	0.08	0.12	0.09	0.10	0.09	0.08	0.04	0.06	0.07	0.06	0.08	0.09
In	0.07	0.07	0.09	0.07	0.08	0.05	0.05	0.02	0.04	0.04	0.04	0.05	0.06
Sw	0.07	0.07	0.09	0.07	0.08	0.05	0.05	0.02	0.04	0.04	0.04	0.04	0.06
Mo	0.08	0.08	0.10	0.08	0.08	0.05	0.06	0.03	0.04	0.06	0.05	0.05	0.07
Ra	0.17	0.16	0.19	0.18	0.19	0.18	0.17	0.14	0.17	0.18	0.15	0.16	0.18
Di	0.12	0.09	0.13	0.11	0.13	0.11	0.10	0.07	0.10	0.11	0.08	0.10	0.12
Ho	0.13	0.12	0.15	0.13	0.13	0.11	0.11	0.09	0.11	0.12	0.10	0.12	0.14
Na	0.18	0.19	0.18	0.17	0.17	0.18	0.15	0.17	0.19	0.18	0.17	0.19	0.18
Wa	0.17	0.17	0.17	0.15	0.16	0.16	0.14	0.16	0.17	0.16	0.16	0.18	0.17
No	0.15	0.14	0.15	0.13	0.14	0.13	0.12	0.12	0.14	0.13	0.13	0.15	0.14
Si	0.21	0.19	0.20	0.19	0.20	0.20	0.18	0.19	0.22	0.20	0.19	0.22	0.21
Dv	0.19	0.17	0.19	0.17	0.18	0.18	0.17	0.17	0.20	0.18	0.17	0.20	0.19
Sm	0.16	0.14	0.15	0.14	0.15	0.15	0.14	0.16	0.19	0.17	0.16	0.19	0.17
Ca	0.24	0.21	0.23	0.22	0.22	0.22	0.20	0.22	0.24	0.21	0.21	0.24	0.22
La	0.27	0.24	0.27	0.25	0.25	0.25	0.24	0.24	0.25	0.23	0.23	0.25	0.24
Ja	0.28	0.25	0.29	0.26	0.26	0.25	0.24	0.22	0.23	0.22	0.21	0.23	0.23
Dz	0.29	0.26	0.30	0.27	0.27	0.26	0.25	0.23	0.23	0.23	0.21	0.23	0.23

Table 2-3 continued.

Site code	Le	Er	Em	Fc	Mr	Fr	Qu	Mn	Sa	Ot	Dr	In	Sw
Er	**0.02**												
Em	**0.02**	**0.01**											
Fc	0.04	0.06	0.08										
Mr	0.05	0.07	0.08	**0.01**									
Fr	0.05	0.05	0.07	**0.01**	**0.01**								
Qu	0.16	0.18	0.20	0.10	0.09	0.11							
Mn	0.06	0.06	0.06	0.06	0.06	0.05	0.16						
Sa	0.07	0.06	0.06	0.06	0.06	0.05	0.15	**0.01**					
Ot	0.07	0.07	0.08	0.05	0.05	0.04	0.13	0.02	**0.02**				
Dr	0.07	0.07	0.08	0.05	0.05	0.04	0.16	0.03	0.03	0.02			
In	0.05	0.05	0.06	0.05	0.05	0.04	0.16	**0.02**	**0.01**	0.02	0.02		
Sw	0.06	0.05	0.06	0.05	0.05	0.04	0.16	**0.02**	**0.01**	0.03	0.02	**0.00**	
Mo	0.06	0.06	0.06	0.06	0.05	0.05	0.17	0.03	0.02	0.04	0.04	**0.00**	**0.00**
Ra	0.15	0.17	0.20	0.12	0.12	0.13	0.16	0.15	0.14	0.11	0.13	0.14	0.15
Di	0.07	0.10	0.11	0.05	0.05	0.06	0.16	0.09	0.08	0.07	0.07	0.06	0.07
Ho	0.15	0.13	0.14	0.10	0.09	0.09	0.16	0.09	0.07	0.07	0.08	0.08	0.07
Na	0.23	0.20	0.21	0.21	0.21	0.18	0.28	0.19	0.19	0.21	0.20	0.18	0.18
Wa	0.21	0.18	0.19	0.19	0.19	0.17	0.26	0.18	0.17	0.19	0.19	0.17	0.17
No	0.17	0.15	0.16	0.15	0.16	0.13	0.24	0.15	0.14	0.16	0.16	0.14	0.13
Si	0.23	0.21	0.23	0.20	0.22	0.19	0.27	0.20	0.20	0.22	0.22	0.20	0.21
Dv	0.21	0.19	0.20	0.18	0.19	0.17	0.26	0.18	0.18	0.19	0.18	0.18	0.18
Sm	0.19	0.17	0.19	0.16	0.18	0.15	0.22	0.17	0.17	0.18	0.17	0.17	0.17
Ca	0.25	0.23	0.25	0.22	0.24	0.21	0.30	0.23	0.22	0.25	0.24	0.22	0.23
La	0.26	0.24	0.25	0.23	0.25	0.23	0.32	0.23	0.23	0.26	0.25	0.23	0.23
Ja	0.23	0.22	0.24	0.22	0.24	0.22	0.32	0.22	0.21	0.23	0.23	0.22	0.22
Dz	0.23	0.22	0.23	0.22	0.24	0.22	0.33	0.22	0.20	0.23	0.24	0.22	0.22

Table 2-3 continued.

Site code	Mo	Ra	Di	Ho	Na	Wa	No	Si	Dv	Sm	Ca	La	Ja
Ra	0.14												
Di	0.07	0.08											
Ho	0.09	0.20	0.15										
Na	0.20	0.33	0.26	0.19									
Wa	0.19	0.31	0.25	0.18	**0.00**								
No	0.15	0.27	0.20	0.16	0.03	**0.02**							
Si	0.23	0.32	0.27	0.21	0.05	0.04	0.03						
Dv	0.21	0.32	0.25	0.19	0.08	0.05	0.04	**0.01**					
Sm	0.19	0.27	0.23	0.18	0.08	0.06	0.07	0.04	0.03				
Ca	0.24	0.35	0.29	0.24	0.16	0.13	0.12	0.10	0.10	0.07			
La	0.25	0.37	0.31	0.27	0.16	0.15	0.15	0.12	0.14	0.11	0.07		
Ja	0.22	0.32	0.27	0.25	0.25	0.23	0.19	0.22	0.21	0.18	0.14	0.14	
Dz	0.23	0.33	0.27	0.25	0.27	0.25	0.20	0.23	0.23	0.19	0.16	0.16	**0.01**

Table 2-4. *P* values associated with pairwise F_{ST} estimates (θ_{ST}, Weir and Cockerham 1984; Table 3) among sample sites (see Table 2-1 for site code definitions). Asterisks indicate significant F_{ST} estimates following Bonferroni correction ($P < 0.00006$).

Site code	Be	Hi	Pi	WiUp	WiDo	AtL	AtN	Wi	Mc	Su	Ed	Wo	Wt
Hi	0.010												
Pi	*	0.282											
WiUp	0.000	0.381	0.125										
WiDo	0.145	0.043	0.000	0.463									
AtL	0.382	0.462	0.028	0.313	0.337								
AtN	0.003	0.390	0.000	0.033	0.005	0.545							
Wi	*	0.000	*	*	*	0.003	*						
Mc	*	*	*	*	*	*	*	*					
Su	*	*	*	*	*	*	*	*	0.002				
Ed	*	0.000	*	*	*	*	*	*	*	0.018			
Wo	*	*	*	*	*	*	*	*	*	0.025	0.006		
Wt	*	*	*	*	*	*	*	*	*	0.009	0.000	0.002	
Le	*	*	*	*	*	*	*	*	*	*	*	*	*
Er	*	*	*	*	*	*	*	*	0.045	*	*	*	*
Em	*	*	*	*	*	*	*	*	0.179	*	*	*	*
Fc	*	*	*	*	*	*	*	*	*	*	*	*	*
Mr	*	*	*	*	*	*	*	*	*	*	*	*	*
Fr	*	*	*	*	*	*	*	*	*	*	*	*	*
Qu	*	*	*	*	*	*	*	*	*	*	*	*	*
Mn	*	*	*	*	*	*	*	*	*	*	*	*	*
Sa	*	*	*	*	*	*	*	*	*	*	*	*	*
Ot	*	*	*	*	*	*	*	*	*	*	*	*	*
Dr	*	*	*	*	*	*	*	*	*	*	*	*	*
In	*	*	*	*	*	*	*	*	*	*	*	*	*
Sw	*	*	*	*	*	*	*	*	*	*	*	*	*
Mo	*	*	*	*	*	*	*	*	*	*	*	*	*
Ra	*	*	*	*	*	*	*	*	*	*	*	*	*
Di	*	*	*	*	*	*	*	*	*	*	*	*	*
Ho	*	*	*	*	*	*	*	*	*	*	*	*	*
Na	*	*	*	*	*	*	*	*	*	*	*	*	*
Wa	*	*	*	*	*	*	*	*	*	*	*	*	*
No	*	*	*	*	*	*	*	*	*	*	*	*	*
Si	*	*	*	*	*	*	*	*	*	*	*	*	*
Dv	*	*	*	*	*	*	*	*	*	*	*	*	*
Sm	*	*	*	*	*	*	*	*	*	*	*	*	*
Ca	*	*	*	*	*	*	*	*	*	*	*	*	*
La	*	*	*	*	*	*	*	*	*	*	*	*	*
Ja	*	*	*	*	*	*	*	*	*	*	*	*	*
Dz	*	*	*	*	*	*	*	*	*	*	*	*	*

Table 2-4 continued.

Site code	Le	Er	Em	Fc	Mr	Fr	Qu	Mn	Sa	Ot	Dr	In	Sw
Er	0.015												
Em	0.004	0.076											
Fc	*	*	*										
Mr	*	*	*	0.000									
Fr	*	*	*	0.007	0.002								
Qu	*	*	*	*	*	*							
Mn	*	*	*	*	*	*	*						
Sa	*	*	*	*	*	*	*	0.005					
Ot	*	*	*	*	*	*	*	*	0.000				
Dr	*	*	*	*	*	*	*	*	*	*			
In	*	*	*	*	*	*	*	0.001	0.001	*	*		
Sw	*	*	*	*	*	*	*	0.000	0.001	*	*	0.778	
Mo	*	*	*	*	*	*	*	*	*	*	*	0.078	0.159
Ra	*	*	*	*	*	*	*	*	*	*	*	*	*
Di	*	*	*	*	*	*	*	*	*	*	*	*	*
Ho	*	*	*	*	*	*	*	*	*	*	*	*	*
Na	*	*	*	*	*	*	*	*	*	*	*	*	*
Wa	*	*	*	*	*	*	*	*	*	*	*	*	*
No	*	*	*	*	*	*	*	*	*	*	*	*	*
Si	*	*	*	*	*	*	*	*	*	*	*	*	*
Dv	*	*	*	*	*	*	*	*	*	*	*	*	*
Sm	*	*	*	*	*	*	*	*	*	*	*	*	*
Ca	*	*	*	*	*	*	*	*	*	*	*	*	*
La	*	*	*	*	*	*	*	*	*	*	*	*	*
Ja	*	*	*	*	*	*	*	*	*	*	*	*	*
Dz	*	*	*	*	*	*	*	*	*	*	*	*	*

Table 2-4 continued.

Site code	Mo	Ra	Di	Ho	Na	Wa	No	Si	Dv	Sm	Ca	La	Ja
Ra	*												
Di	*	*											
Ho	*	*	*										
Na	*	*	*	*									
Wa	*	*	*	*	0.216								
No	*	*	*	*	*	0.001							
Si	*	*	*	*	*	*	*						
Dv	*	*	*	*	*	*	*	0.014					
Sm	*	*	*	*	*	*	*	*	*				
Ca	*	*	*	*	*	*	*	*	*	*			
La	*	*	*	*	*	*	*	*	*	*	*		
Ja	*	*	*	*	*	*	*	*	*	*	*	*	
Dz	*	*	*	*	*	*	*	*	*	*	*	*	0.060

Table 2-5. Genetic variance among regions (V_{AR}), among groups (V_{AG}), and within groups (V_{WG}) partitioned by analysis of molecular variance (AMOVA; Excoffier et al. 2005). All values were significant ($P < 0.001$). See Table 2-1 for site code and sub-basin definitions.

Region Composition			V_{AR}	V_{AG}	V_{WG}
Major River Basins					
Test 1	Region 1:	Hay River basin	15.5	5.8	78.7
	Region 2:	Athabasca River basin			
	Region 3:	Upper Peace River basin (No, Na, Wa, Si, Dv, Sm)			
	Region 4:	Lower Peace River basin (Ca, La)			
Test 2	Region 1:	Hay River basin	15.0	6.1	78.9
	Region 2:	Athabasca River basin			
	Region 3:	Peace River basin			
Athabasca River Basin					
Test 1	Region 1:	Athabasca River basin sites, excluding Quarry Lake	7.9	6.3	85.8
	Region 2:	Stocked site, Quarry Lake			
Test 2	Region 1:	Upper Athabasca River sub-basin	5.2	2.3	92.5
	Region 2:	Central McLeod River sub-basin			
	Region 3:	Upper McLeod River sub-basin			
	Region 4:	Freeman River sub-basin			
	Region 5:	Lesser Slave Lake sub-basin			
	Region 6:	Pembina River sub-basin			
	Region 7	House River			
Test 3	Region 1:	Upper Athabasca River sub-basin	4.2	3.5	92.3
	Region 2:	McLeod River and tributaries			
	Region 3:	Freeman River and Pembina River sub-basins			
	Region 4:	Lesser Slave Lake sub-basin and House River			
Test 4	Region 1:	Upper Athabasca River, Upper McLeod River, and Central	3.1	5.1	91.8
	Region 2:	Freeman River, Pembina River, and Lesser Slave Lake sub-			
Peace River Basin					
Test 1	Region 1:	Little Smoky River and Wapiti River, Simonette River sub-	7.7	4.3	88.0
	Region 2:	Lower Peace River basin (Ca, La)			
Test 2	Region 1:	Wapiti River sub-basin including Nose Creek	5.6	2.4	92.0
	Region 2:	Simonette River sub-basin			
	Region 3:	Little Smoky River			
	Region 4:	Lower Peace River basin (Ca, La)			
Test 3	Region 1:	Wapiti River sub-basin	5.0	3.5	91.5
	Region 2:	Simonette River sub-basin including Nose Creek			
	Region 3:	Little Smoky River			
	Region 4:	Lower Peace River basin (Ca, La)			

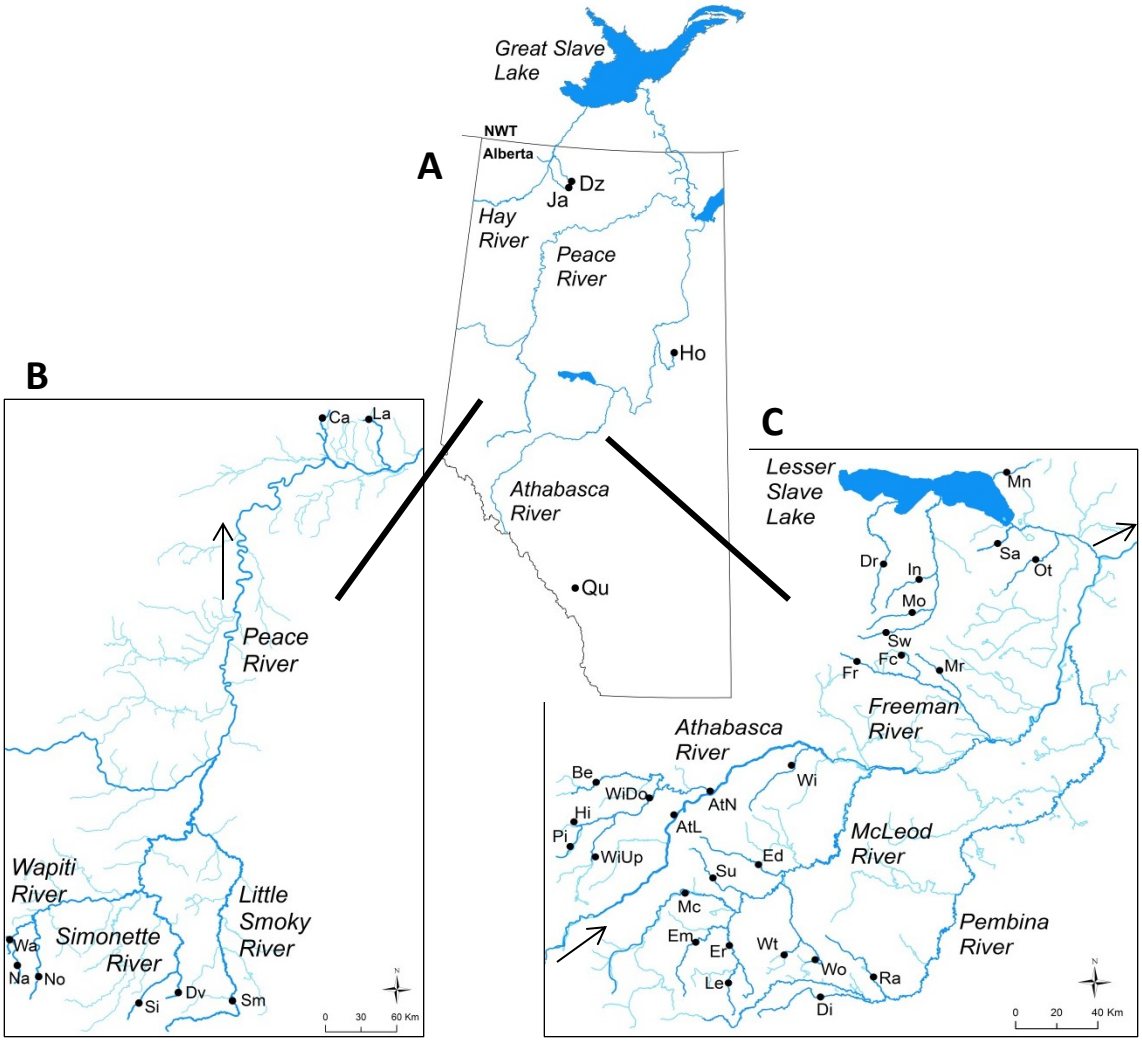

Figure 2-1. Sample sites for Arctic Grayling in Alberta, Canada. **(A)** Map of the study area showing the Peace River, Athabasca River, and two sites in the Hay River basin. **(B)** Peace River basin sample sites. **(C)** Athabasca River basin sample sites. Note the location of House River (Ho) and Quarry Lake (Qu) on map (A). Quarry Lake is an isolated waterbody and not connected to the Athabasca River. Flow directions are denoted by arrows.

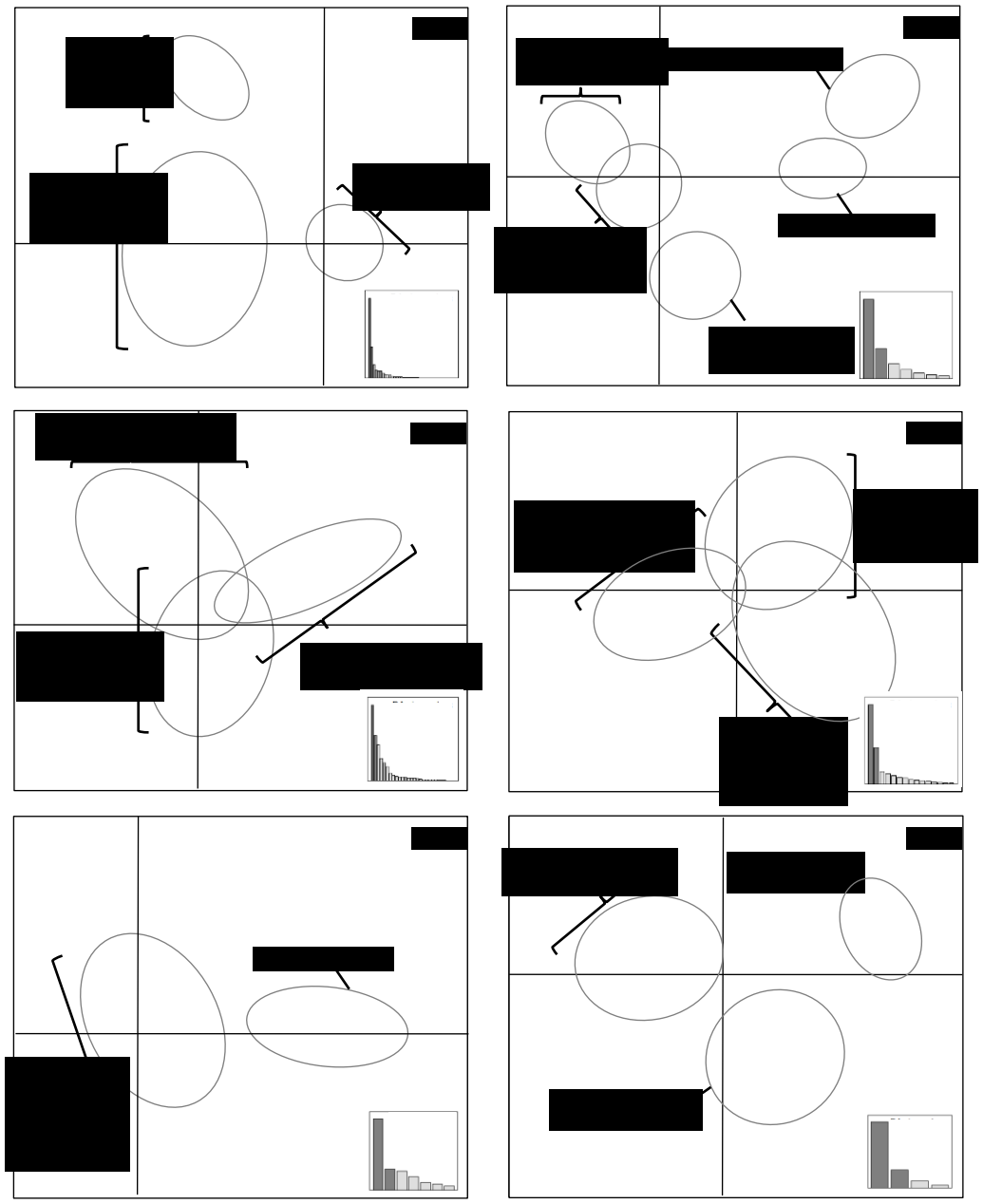

Figure 2-2. Scatterplots of DAPC of multilocus genotypes of Arctic Grayling at sites in the **(A)** Hay River, Peace River, and Athabasca River basins, **(B)** Peace River basin, **(C)** Athabasca River basin, **(D)** upstream of the Freeman River sub-basin, **(E)** downstream of the Freeman River sub-basin, and **(F)** Freeman River and Pembina River sub-basins. Major regional groupings and sites are shown by ellipses and the bottom-right inset shows the relative magnitude of discriminant analysis eigenvalues. The highest eigenvalue reflects the largest between/within site variance ratio achievable based on the retained principle components. Individual points were removed to aid interpretation of patterns. See Table 2-1 for sites included in each sub-basin.

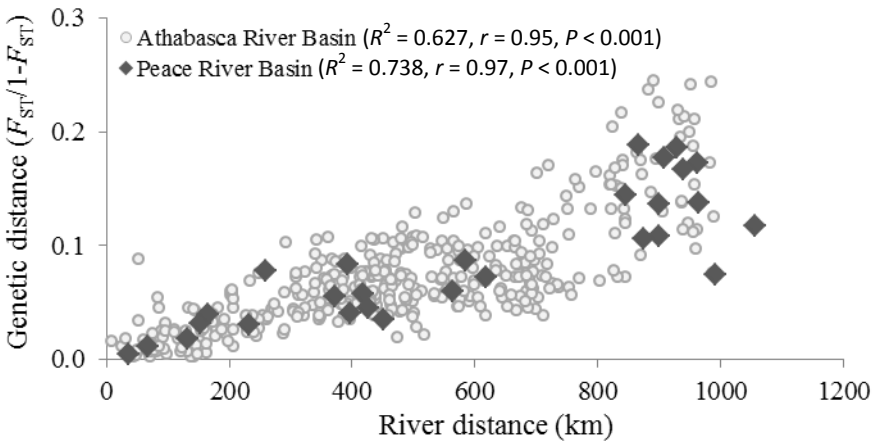

Figure 2-3. Pairwise genetic distances for Arctic Grayling between sites within the Athabasca and Peace River basins plotted against their pairwise river distances.

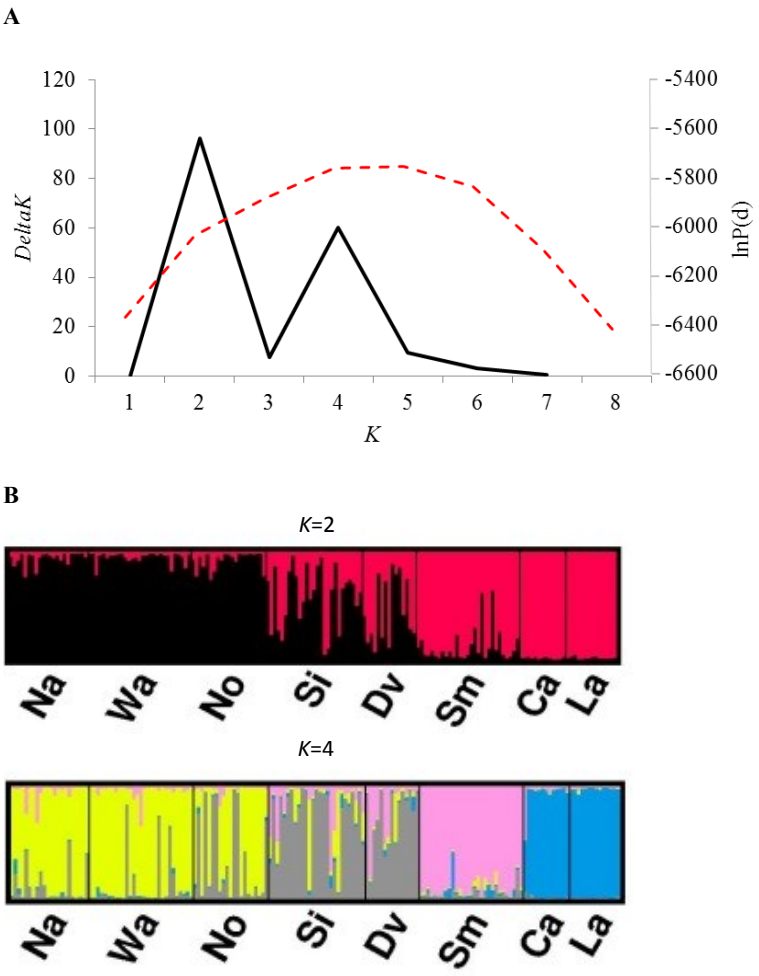

Figure 2-4. STRUCTURE results for Arctic Grayling from the Peace River basin. **(A)**: Multi-modal distribution of *DeltaK* values based on Evanno et al. (2005) (solid line) with lnP(d) values as proposed by Pritchard et al. (2000) (dashed line). **(B)** Admixture plots showing individual genotype membership to *K* clusters for *K*=2 and *K*=4. Each cluster is represented by a different colour, thin black lines denote separate sampling sites, and vertical bars represent individuals. Na, Narraway River; Wa, Wapiti River; No, Nose Creek; Si, Simonette River; Dv, Deep Valley Creek; Sm, Little Smoky River; Ca, Caribou River; La, Lawrence River.

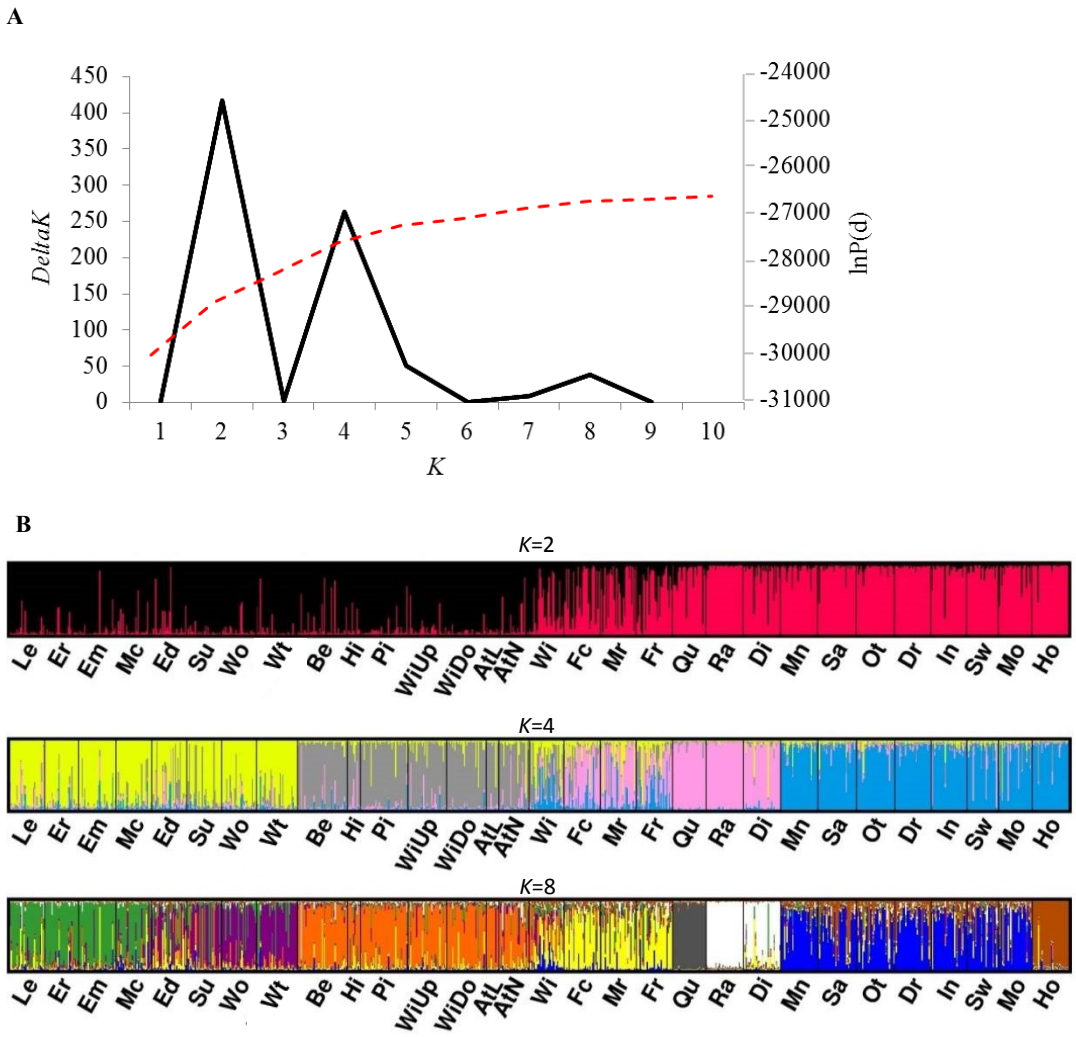

Figure 2-5. STRUCTURE results for Arctic Grayling from Athabasca River basin. **(A)**: Multi-modal distribution of *DeltaK* values based on Evanno et al. (2005) (solid line) with lnP(d) values as proposed by Pritchard et al. (2000) (dashed line). **(B)** Admixture plots showing individual genotype membership to *K* clusters for *K*=2, *K*=4, and *K*=8. Each cluster is represented by a different colour, thin black lines denote separate sampling sites, and vertical bars represent individuals. Le, Lendrum Creek; Er, Erith River; Em, Embarras River; Mc, McLeod River; Ed, Edson River; Su, Sundance Creek; Wo, Wolf Creek; Wt, Unnamed Tributary; Be, Berland River; Hi, Hightower Creek; Pi, Pinto Creek; WiUp, Wildhay River; WiDo, Wildhay River; AtL, Athabasca River; AtN, Athabasca River; Wi, Windfall Creek; Fc, Freeman Creek; Mr, Morse River; Fr, Freeman River; Qu, Quarry Lake; Ra, Rat Creek; Di, Dismal Creek; Mn, Marten Creek; Sa, Sawridge Creek; Ot, Otauwau River; Dr, Driftpile River; In, Inverness River; Sw, Swan River; Mo, Moosehorn River; Ho, House River.

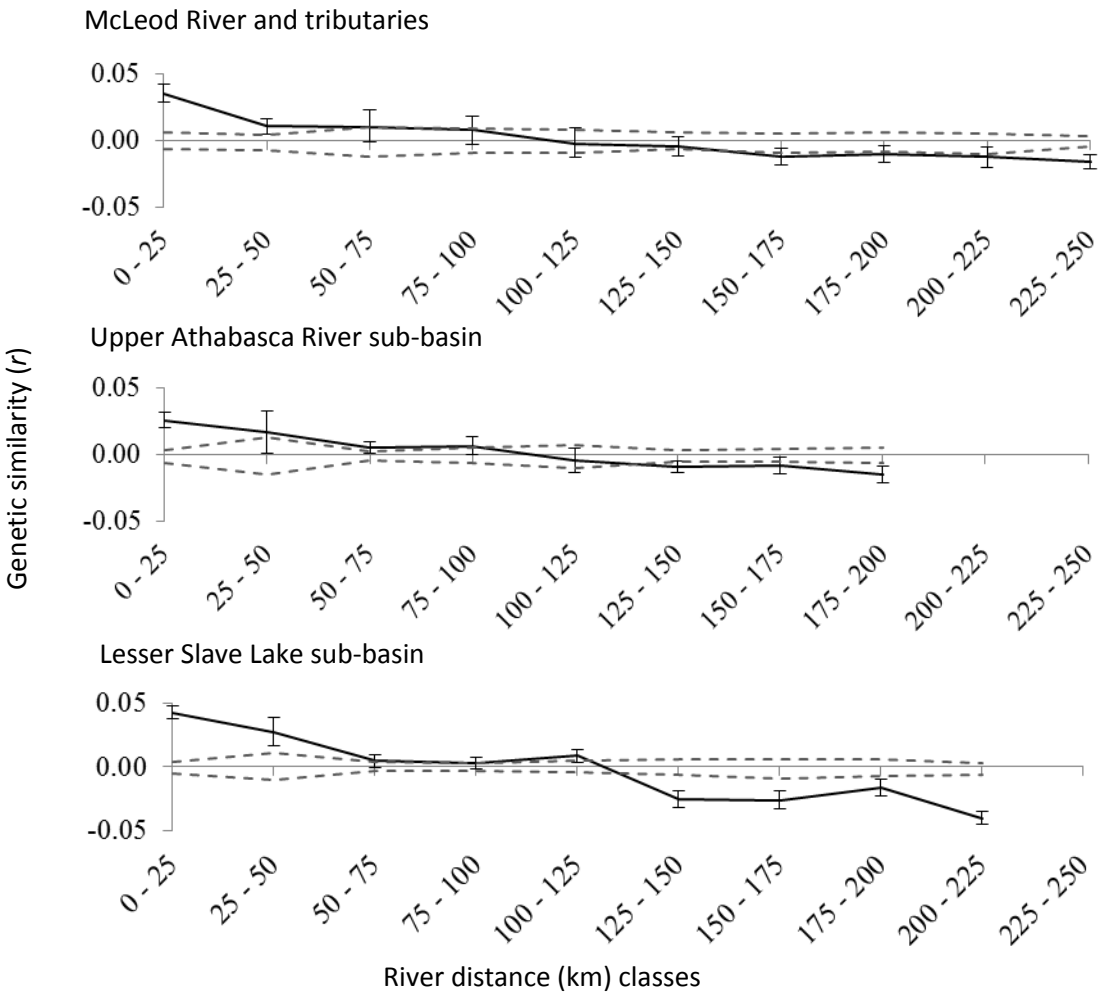

Figure 2-6. Correlograms of genetic and river distance for individual Arctic Grayling from the Athabasca River basin. Grey dotted lines represent upper and lower 95% confidence intervals for the null hypothesis. Error bars represent 95% confidence intervals of *r*.

Chapter 3

Predictors of Intra-population Genetic Diversity of a Stream-Dwelling Salmonid, the Arctic Grayling

Introduction

Evolutionary concepts are among the foundations of conservation biology (Fox and Carroll 2008) and essential components of management plans designed to conserve natural processes and patterns (Bowen 1999). Genetic diversity is a major level of biodiversity (Angermeier and Karr 1994) and its maintenance may lead to greater resilience of populations existing within changing environments, enhanced potential for adaption (Toro and Caballero 2005; Reusch et al. 2005), and higher fitness of individuals (Wang et al. 2002; Reed and Frankham 2003). The importance of genetic diversity to population persistence is acknowledged internationally within laws and resource management strategies (e.g., Canada National Parks Act, ASRD 2006).

Genetic diversity can be characterized as adaptive (i.e., genes that directly influence fitness) or non-adaptive (Holderegger et al. 2006). Non-adaptive diversity is influenced by contemporary and historical processes and can be measured at microsatellite loci presumed to be neutral, not affecting traits that influence fitness. The total neutrality of these markers is a contentious issue; microsattelites have been found within protein-coding genes (Li et al. 2004). However, a recent meta-analysis demonstrated that natural selection is not a significant force shaping diversity of microsatellite loci of freshwater fish (McCusker and Bentzen 2010). Therefore, the expected polymorphism for a neutral microsatellite locus at mutation-drift equilibrium is proportional to the effective population size (N_e; number of spawning individuals within a population) (Frankham 1996). However, deviations from this relationship occur because of population genetic bottlenecks, differential gene flow between populations, and serial founding effects during colonization of freshwater systems (Bernatchez and Wilson 1998). Understanding the relative roles of these processes in shaping current patterns of diversity is useful for identifying fish populations that may be at risk (i.e., populations experiencing reductions in population size and/or connectivity), and for predicting how populations will be affected by future landscape

changes (e.g., Pujolar et al. 2011). This information is particularly relevant to the conservation of sensitive species, such as Arctic Grayling.

In Alberta, Arctic Grayling abundance has been steadily declining since the 1950s and many populations have been functionally extirpated (ASRD 2005). It is unknown if contemporary reductions in abundance have resulted in lower levels of genetic diversity, either adaptive or non-adaptive. Examining the genetic diversity of Arctic Grayling populations will expand current understanding of trade-offs between human activities and sustainability of populations of this species.

A simple conceptual model illustrates the factors potentially influencing Arctic Grayling non-adaptive genetic diversity (Figure 3-1). Broad-scale patterns of diversity often reflect the direction of post-glacial colonization; genetic diversity is expected to decrease with distance from ancestral refugia (Bernatchez and Wilson 1998). Previous studies on Arctic Grayling in North America have found evidence of multiple northern refugia and a significant, southward decline of diversity (Stamford and Taylor 2004; see Chapter 2). N_e and gene flow may also influence genetic variation, assuming that larger, well-connected populations are more diverse because they are less influenced by the processes of inbreeding and genetic drift. Variables expected to influence N_e and gene flow for Arctic Grayling are quantifiable. In Alberta, these variables form four categories: environmental factors limiting productivity; anthropogenic disturbance; recreational angling; and spatial position within stream networks (Figure 3-1).

Freshwater fish populations are limited by environmental factors and anthropogenic activities that reduce habitat quantity and quality. Temperature, elevation, and surrogate measures of the amount of available habitat have been significantly correlated to abundance (Nate et al. 2000; Weigel and Sorensen 2001; Isaak and Hubert 2004) and genetic diversity of some fish species (Cena et al. 2006; Tamkee et al. 2010). In the case of Arctic Grayling, temperature may restrict Arctic Grayling population size (ASRD 2005); high summer water temperature has been linked to mass mortality of the species in Montana (Lohr et al. 1996). Anthropogenic disturbance can also reduce habitat quality, and consequently, abundance (Scrimgeour et al. 2008) and genetic

diversity (Blum et al. 2012) of fish populations through multiple mechanisms, such as sedimentation, nutrient enrichment, hydrologic alteration, riparian clearing, and loss of large woody debris (Allan 2004). For example, high road density has been associated with reductions in water quality due to increased sediment loads in run-off (Eaglin and Hubert 1993) and correlated with reduced abundance of sensitive fish species (Bradford and Irvine 2000; Stevens et al. 2010).

Recreational angling is suspected to have played a large role in the decline of some Arctic Grayling stocks due to indirect and direct mortality (ASRD 2005). Compared to other Canadian provinces, fishing pressure in Alberta is high because of the disproportionate ratio of licensed anglers to number of fish-bearing systems (Sullivan 2003). Many rivers containing Arctic Grayling are accessible to anglers due to extensive road networks required to support industrial development and forestry. Incidental mortality of Arctic Grayling may be substantial even under catch and release management because of their high catchability (ASRD 2005).

Lastly, spatial position in the stream network may also influence abundance and gene flow between fish populations. Greater distances and complexity of intervening habitats can reduce gene flow and restrict the distribution of alleles across the landscape (Whiteley et al. 2004; Whiteley et al. 2006). Conversely, immigration and high gene flow between populations may act to bolster declining populations and reduce the effects of bottlenecks (Neville et al. 2006). Position also affects the ability of individual fish to access large, relatively stable rivers to use as refuges during periods of unsuitable conditions within tributaries (Hitt and Angermeier 2008). Populations situated far from large rivers may have lower abundance, and consequently lower genetic diversity, because individuals cannot escape localized, extreme disturbance events (e.g., floods, high debris loading, low water conditions, etc.).

My objective was to explore the relationships between historical and contemporary processes and non-adaptive genetic variation in Arctic Grayling. Specifically, I expected post-glacial colonization patterns, environmental factors limiting productivity, and spatial position to be significant predictors of genetic diversity because of their influence on past and current N_e and

gene flow. Anthropogenic disturbance and recreational angling were expected to be less important because these processes have operated over shorter time periods and may not have reduced abundance to such an extent that gene flow cannot ameliorate allele loss. I also anticipated finding evidence of recent genetic bottlenecks because of anecdotal accounts of substantial declines in Arctic Grayling abundance (ASRD 2005). My findings will be compared with those of other lotic salmonid species and will be used to inform Arctic Grayling management plans.

Methods

Sample Collection and Microsatellite Analysis

The study area was located within the Athabasca River basin in Alberta, Canada (Figure 3-2). Tissue samples were collected from 785 Arctic Grayling captured at 26 sites (Table 3-1), between which there are no known barriers to movement. Genetic variation was examined at nine microsatellite loci. See Chapter 2 for more details regarding tissue collection methods and microsatellite analysis.

Genetic diversity at each site was measured using allelic richness (A_r), expected heterozygosity (H_{exp}), and observed heterozygosity (H_{obs}). The Excel Microsatellite Toolkit (Park 2001) was used to calculate H_{exp} and H_{obs} averaged across all loci per site. A_r at each site was calculated and standardized for sample size (Kalinowski 2004) via the program HP-Rare 1.0 (Kalinowski 2005).

I tested for heterozygosity excess and mode shifts in allele frequency distributions to detect recent genetic bottlenecks (Cornuet and Luikart 1997). Both tests were performed in the program BOTTLENECK 1.2.02 (Piry et al. 1999). To test for heterozygosity excess at each site, I used the two-phase model (95% single step mutations, 5% multi-step mutations, 12% variance) recommended by Piry et al. (1999) and determined statistical significance using a one-sided Wilcoxon's signed-rank test ($\alpha = 0.05$) based on 1000 simulation iterations. Under the mode shift method, a bottleneck was considered to have occurred if the proportion of low frequency alleles was lower than the proportion of alleles having intermediate frequencies (Piry et al. 1999).

Model Predictor Variables

Provincial fisheries biologists provided estimates of adult Arctic Grayling abundance based on the most recent catch rates, population estimates, and anecdotal information. Adult abundance was ranked on a five-point scale, ranging from 1 (very low density) to 5 (very high density). This index of abundance was assumed to be positively correlated with N_e.

In addition to the index of adult abundance, I quantified 23 predictor variables to describe environmental factors limiting productivity, anthropogenic disturbance, recreational angling, spatial position, and post-glacial colonization (Table 3-2). Derived data were used when field measurements were unavailable. Preliminary data exploration was conducted for all variables following the protocol outlined by Zuur et al. (2010).

Environmental factors may influence Arctic Grayling abundance and genetic diversity. Potential available habitat was represented by the area of the contributing watershed upstream of each site and stream order (Strahler 1957). The local thermal environment was used as a proxy for habitat quality and described using elevation and growing degree days (GDD). Significant correlative relationships have been found between maximum stream temperature and elevation (Isaak and Hubert 2001) and between air and stream surface temperature during ice-free periods (Webb et al. 2003). Thus, I used elevation and GDD as surrogates for stream temperature. Elevation data were retrieved from a Digital Elevation Model of Alberta (Natural Resources Canada 2003). GDD per site for the time period 2001 to 2010 was calculated as the accumulated temperature sum (^{o}C) per day that the mean temperature was >18^{o}C. The 18^{o}C threshold was chosen based on evidence that adult Arctic Grayling become physiologically stressed around this temperature (Wojcik 1955). GDD was determined using the program ClimateWNA© (Hamann & Wang 2005; Wang *et al*. 2006).

The total area of disturbed land and area of linear (i.e., roads, seismic lines, power lines, pipelines, railway lines) and non-linear features (i.e., cut-blocks, well pads, mine sites, cultivated land) was calculated to assess the effects of anthropogenic disturbance on genetic diversity. I determined the density of disturbance (i.e., extent of disturbed area within total area) within two

spatial extents: 1) within the contributing watershed; and 2) within the contributing, 100 m wide stream corridor. Disturbance densities were calculated in ArcGIS 10.1 using data from the Alberta Biodiversity Monitoring Index Human Footprint Layers (Version 3).

Fishing pressure varies with angling effort, which is a function of the number of anglers, cost (i.e., travel time) of visiting a site, and accessibility (Lewin et al. 2006). Therefore, potential angling effort was described using road-way distance to the nearest urban center and the number of urban centers and campgrounds within circular buffers of different spatial extents centered at each site. I also calculated road density within 100m and 500m corridors extending 5km up and downstream of each site, with the assumption that higher road density corresponds to greater accessibility (e.g., Sullivan 1988).

I used five metrics to characterize the spatial position of sites and distance from putative ancestral refugia. Downstream link (D-link) was used to describe the channel magnitude below the nearest downstream confluence from each site (Osborne and Wiley 1992). Channel magnitude is equivalent to the number of 1st order streams upstream of a given point (Shreve 1966; Osborne and Wiley 1992). I used confluence difference (CD) to describe disparity in stream order between the tributary that each site was located on and its receiving river (Mattingly and Galat 2002). For example, Freeman River had a CD of 3 because it is a 3rd order tributary that flows into the Athabasca River, which is a 6th order river at the confluence. CD differs from D-link because it captures both upstream and downstream influences at a particular site. I also calculated river-way distance to the Athabasca River and to the nearest major river (i.e., $\geq$4th order). Lastly, latitude was used to capture potential effects of post-glacial colonization.

Statistical Analysis

Multiple linear regression models were used to explore alternative hypotheses concerning the drivers of genetic diversity. The low number of observations (i.e., 26 sites located in the Athabasca River basin) required the selection of a subset of predictor variables because considering all possible models can lead to spurious effects when sample sizes are low (Burnham and Anderson 2002). All variables, excluding latitude and the index of adult abundance, were

grouped by category and then explored using principal component analysis (PCA) to investigate the relationships among variables within each category and to determine which described the most variability among sites. Data were scaled such that all variables had zero mean and unit variance. Significant axes for each PCA were considered as those that contributed the most to explained variance, up to threshold of 70% cumulative variance. Uncorrelated variables (i.e., Pearson coefficient <0.70) that had the greatest loadings on significant axes were carried forward in modelling. This approach limited my ability to explore all possible relationships, but was successful in terms of reducing the number of variables.

Following PCA, differences between groups of sites representative of sub-populations were investigated using multi-response permutation procedures (MRPP). MRPP is a non-parametric method that determines if the average dispersion within groups is significantly different than the average dispersion generated by random assignment of individuals to groups (McCune and Grace 2002). Sub-populations of Arctic Grayling were identified using individual clustering analyses (see Chapter 2) and were generally located within the upper Athabasca River basin, McLeod River basin, Lesser Slave Lake area, and Freeman River basin (Table 3-1). MRPP was performed on the group of variables included in the candidate linear regression models. Matrices were based on Euclidean distances and 1000 permutations were used for each comparison. Significant tests ($\alpha = 0.05$) were considered an indication that geography confounded the ability to detect or understand relationships between genetic diversity and environmental variables. Sites were retained for modelling purposes if they were included within sub-populations that exhibited similar within-group dispersion (i.e., geography did not confound interpretation of model results). Analyses were performed in the vegan package (Oksanen et al. 2005) within R version 2.15.2 (R Development Core Team 2012).

Candidate models were then developed using an iterative process in which non-significant variables ($P>0.05$) were excluded in succession (Zuur et al. 2009). Models were validated following the approach of Zuur et al. (2009). Statistical support (R^2) for each model was calculated with ordinary least squares regression. Model selection was based on corrected information theoretic values (AICc) (Akaike 1973; Hurvich and Tsai 1989; Burnham and

Anderson 2002). We considered the optimal model as having the lowest AIC value and calculated ΔAICc to determine the amount of support for each model relative to the optimum (i.e., acceptable strong support Δ <3; Burnham and Anderson 2002). All analyses were performed in R version 2.15.2 (R Core Development Team 2012) using the stats (R Core Development Team 2012), graphics (R Core Development Team 2012), AICcmodavg (Mazerolle 2011), and ape (Paradis et al. 2004) packages.

Several methods were employed to address concerns over the non-independent nature of genetic diversity and potential spatial autocorrelation of the predictor variables. The global Moran's I (α = 0.05) for each model was calculated using inverted river-way distances between sites as weights and visual checks for spatial patterns in residuals were conducted using boxplots and by mapping residuals based on site coordinates. I also developed linear mixed-effects models, using sub-population as a random effect to account for potential spatial autocorrelation and non-independence of diversity (Blair et al. 2013). The results of this analysis are not reported here because the amount of between-sub-population variability (σ) was too low to warrant adopting a mixed-model approach (σ ranged from 0.00 to 0.07 for candidate models) (Bates 2010).

Results

Genetic diversity

Genetic diversity varied among sites (Table 3-1). Ar averaged 5.86, and ranged from 4.98 in Pinto Creek to 6.52 in the Inverness River. Ar was normally distributed (Shapiro-wilk P=0.46) with no outliers. H_{exp} averaged 0.683, and ranged from 0.629 in Pinto Creek to 0.721 in the Edson River, whereas H_{obs} averaged 0.680, and ranged from 0.624 in the Berland River to 0.751 in Sundance Creek. H_{obs} was normally distributed (Shapiro-wilk P=0.38), but H_{exp} was not (Shapiro-wilk P<0.05) even after applying logarithmic and square root transformations. Non-normality was likely due to the limited range of H_{exp} values encountered. Nevertheless, H_{exp} was still used as a measure of genetic diversity within candidate models for the purposes of data exploration, but results should be interpreted with caution. I did not detect evidence of genetic bottlenecks within the study area.

Variable Selection

Ten predictor variables were selected for modelling purposes (Table 3-2). In addition to latitude and adult density, two variables per category were chosen based on the highest correlative values with the first two PCA axes (Figure 3-3). PCA for recreational angling variables yielded a first axis that explained 52.6 % (DistCTVH, r= -0.44) of the total variance and a second axis that explained 21.4 % (C50, r= -0.58). For environmental limiting factors, the first axis explained 48.4 % (Elev, r=-0.69), and the second axis explained 42.5 % (WshedArea, r=-0.70) of the total variance. The first two axes of the PCA of land disturbance variables explained 64.0 % (WhsedTotal, r=0.49), and 29.6 % (CorLin, r=0.71) of the total variance, respectively. Analysis of spatial position variables yielded a first PCA axis that explained 55.2 % (DistAtha, r=0.59) of the total variance and a second axis that explained 23.6 % (CD, r=-0.96). No significant correlative relationships were found between the 10 selected variables using a threshold of 0.70 (Pearson's correlation coefficient range: -0.68 – 0.66). However, the two anthropogenic disturbance variables, WhsedTotal and CorLin, are inherently non-independent and so were tested in separate model sets.

MRPP and Regression Analysis

Average dispersion within sub-populations significantly differed when all sites were considered during MRPP (observed $\delta = 1.7 \times 10^9$, expected $\delta = 2.1 \times 10^9$, effect size A = 0.19, $P = 0.02$). Pairwise analysis of sub-populations indicated that this difference was being driven by the Athabasca River sub-population. The two sites situated directly on the Athabasca River mainstem, AtL and AtN, represent occasional outliers when environmental conditions were compared to other sites (i.e., AtL and AtN have larger watershed areas, Figure 3-3A). After these sites were excluded from the analysis, the average dispersion was not found to significantly differ (observed $\delta = 5.8 \times 10^8$, expected $\delta = 6.6 \times 10^8$, effect size A = 0.12, $P = 0.05$). Consequently, AtL and AtN were not included in the linear regression models.

Genetic diversity measures displayed inconsistent relationships with predictor variables. Ar was significantly and positively influenced by latitude, CD, and CorLin (multivariate stepwise regression, $P<0.05$; Figure 3-4). I tested five models containing different combinations of these

variables (Table 3-3). All model residuals were normal, randomly distributed, and did not display significant spatial autocorrelation based on global Moran's I value (α=0.05). The optimal model included all three variables (AICc=11.5) and explained 68% of the variation of Ar (Table 3-3). Latitude appeared to have a relatively larger influence than the other variables (Figure 3-4). In contrast, H_{exp} was only significantly influenced by DistCTVH (H_{exp} = 0.70 (0.007 SE) -0.0005 (0.0.0001 SE) DistCTVH, P<0.002, R^2=0.38). However, I considered this model invalid because residuals displayed a homoscedastic pattern and were not normally distributed, likely because of the non-normality of H_{exp}. No significant relationships were found between predictor variables and H_{obs}.

Discussion

The objective of this study was to investigate how historical and contemporary processes have influenced genetic diversity of Arctic Grayling. Allelic richness was the only measure of genetic diversity that could be predicted from the suite of candidate models explored in this study. Assuming no selective differences between alleles, Ar is a more sensitive indicator of short-term or historical bottlenecks than H_{exp} and H_{obs}, particularly for species that have a high number of alleles per locus (Allendorf 1986; Amos and Balmford 2001) like the Arctic Grayling. Therefore, Ar may have better captured subtle differences in genetic variation across sample sites. The best supported model predicting Ar included variables describing post-glacial colonization and spatial position in the stream network. Linear disturbance in the stream corridor was also found to be a significant factor, but to a lesser extent and exhibiting a relationship with diversity that differed from a priori expectations.

Allelic richness exhibited a significant, positive relationship with latitude (N), which was included in the top four models (Table 3-3). Latitude likely reflects the sequential founder events and population bottlenecks during colonization at the end of the Wisconsin Glaciation (Bernatchez and Wilson 1998). The persistence of a strong genetic signature reflecting historical biogeographic and demographic events has been reported for other salmonid species in North America (e.g., Costello et al. 2003, Harris and Taylor 2010, Tamkee et al. 2010). The southward

decline in genetic diversity that we detected provides additional evidence that Arctic Grayling persisted in northern refugia (Stamford and Taylor 2004).

In addition to latitude, the two best-performing models predicting allelic richness included confluence difference (CD). Arctic Grayling from small streams flowing into large rivers tended to display greater genetic diversity than fish from sites where contributing and receiving rivers were similar in size. Connections with large rivers could facilitate immigration, increase or stabilize population size and promote admixture between straying and local fish (Neville et al. 2006). Sites with high confluence difference values situated on Windfall, Freeman, and Otauwau rivers contained a moderate to high proportion of admixed individuals compared to many other sites in the basin (see Chapter 2).

Extreme disturbance events are more likely to completely eradicate refuges for fish in small streams, versus large rivers (Sedell et al. 1990). Hitt and Angermeier (2008) found evidence of opportunistic use of riverine habitats during disturbances by fish residing in connected tributaries. If Arctic Grayling exhibit this behaviour, it could result in greater population stability, size, and genetic diversity within mainstem tributaries compared to stocks from headwater streams. Floods and periods of low water are natural events within lotic systems in Alberta. However, recent changes in land-cover and effects of climate change may have altered the magnitude and frequency of these events (Schindler et al. 1996; Alila et al. 2009), increasing the reliance on and relative importance of large river refugia.

The optimal model also contained density of linear disturbance within the stream corridor (CorLin), which exhibited an unexpected, positive relationship with genetic diversity. This echoes the results of a previous study, wherein Arctic Grayling occurrence and density increased with greater percent disturbance within two boreal forest watersheds (Scrimgeour et al. 2008). Scrimgeour et al. (2008) postulated that this relationship may have arisen due to an increase in invertebrate prey and/or a reduction of predator abundance (i.e., Bull Trout [*Salvelinus confluentus*]) following forest harvesting. This explanation may not hold for my study area; it is unknown if abundance of invertebrate prey is a limiting factor for Arctic Grayling and the

relationship between disturbance and the abundance of local predatory fish (e.g., Northern Pike [*Esox lucius*]) has not been investigated. The likelihood of local land disturbance may be related coincidentally to stream features (e.g., topography, vegetation cover) that inherently favor abundant Arctic Grayling populations. This finding could also be an artefact of spatial scale; linear disturbance was found to be positively associated with genetic diversity at the small, site-specific level but this relationship may change when considering genetic diversity and disturbance within the entire sub-basin.

Recreational angling variables and adult density estimates were not found to be significant predictors of genetic diversity. The lack of evidence of genetic bottlenecks indicates that reductions in abundance, potentially due to angling pressure, may be too recent or not severe enough to have resulted in detectable genetic consequences. This is in agreement with a previous study in which the occurrence of sport fishing was not a significant predictor of intra-population genetic diversity of Brook Trout (*Salvelinus fontinalis*) (Angers et al. 1999). Additionally, loss of genetic diversity has likely been minimized or delayed by gene flow among sites and sub-populations of Arctic Grayling (see Chapter 2). Relatively high ratios between genetic diversity and N_e within salmonid populations have been previously attributed to gene flow resulting from source-sink meta-population dynamics (Consuegra et al. 2005).

The absence of a relationship between genetic diversity and most predictor variables may also be the result of parameter oversimplification. For example, using watershed area as a surrogate measure of available habitat ignores habitat suitability, which could vary substantially across the landscape and for different life stages of Arctic Grayling. The index of adult abundance may lack the resolution needed to reflect N_e accurately and does not adequately capture the variance between adult abundance and N_e within Arctic Grayling populations arising from differences in sex ratio, family size, and frequency and magnitude of abundance fluctuations (Frankham 1995). Lastly, temporal aspects of variables were not incorporated into models (e.g., establishment date of campgrounds, magnitude and intensity of land-use over time), which may have obscured relationships with genetic diversity.

My study adds to the growing body of literature investigating landscape correlates of intra-population genetic diversity of river-dwelling salmonids. Position in the stream network has been consistently reported to have significant, substantial relationship with the observed level of diversity. For example, drainage pattern was a powerful descriptor of genetic variation of Bull Trout (Costello et al. 2003) and Brook Trout (Angers et al. 1999) and higher connectivity among populations of Rainbow Trout (*Oncorhynchus mykiss*) (Narum et al. 2008; Tamkee et al. 2010), and Cutthroat Trout (*Oncorhynchus clarkii*) (Neville et al. 2006) was positively correlated with genetic diversity. Similarly, I found that latitude and CD, both indices of spatial position, had significant and positive relationships with allele richness. Elevation has also been associated with diversity of Rainbow Trout and Brook Trout (Castric et al. 2001; Narum et al. 2008; Tamkee et al. 2010). Fish populations at higher elevations may be partially or completely isolated due to increased distance from major rivers and barriers (e.g., waterfalls and high stream gradients) (Castric et al. 2001; Narum et al. 2008; Tamkee et al. 2010). In contrast, elevation was not selected as a predictor by my analyses, which may indicate that Arctic Grayling dispersal is less restricted by elevation than other studied salmonids. Alternatively, the lack of a significant relationship could be the consequence of only including highly-connected sites that were in the species "optimal" elevational range. My finding of a significant and positive relationship with linear disturbance in the stream corridor also contrasts with previous studies of salmonid genetic diversity patterns (e.g, Brook Trout (Angers et al. 1999) and Bull Trout (Costello et al. 2003)). This discrepancy may reflect alternative population-level responses of Arctic Grayling to environmental change or different impacts of land-clearing, roads, etc. on streams embedded in different landscapes.

Conservation implications

Disentangling the interplay between contemporary human-activities and fundamental patterns of Arctic Grayling genetic diversity was challenging. In Alberta, density of anthropogenic disturbance has been shown to have an unexplained, positive relationship with both abundance (Scrimgeour et al. 2008) and genetic diversity of Arctic Grayling. Understanding the mechanisms behind these relationships will require further research that explores potential

covariates and investigates complex, ecosystem-scale changes that could temporarily or permanently benefit Arctic Grayling. Further, I did not detect relationships between diversity and variables estimating fishing pressure. In some cases, angling may have severely reduced Arctic Grayling abundance to such a degree to cause demographic changes, but have no genetic affects (ASRD 2005). Alternatively, the genetic consequences of severe declines in population size may be offset by gene flow or difficult to detect because of the strong and persistent influence of historic, demographic events during post-glacial colonization.

The role of gene flow in the maintenance of genetic diversity may become increasingly important if population declines continue. Consequently, natural patterns of dispersal between stocks should be maintained through the use of appropriate water-crossing structures, fish ladders, etc., that do not impede fish movement. Stocks that have low abundance and are isolated because of migration barriers or spatial gaps in distribution resulting from over-fishing or environmental change are more vulnerable to the negative effects of inbreeding and genetic drift. Regardless of industrial practises, stocks using headwater habitats may be at higher risk because of naturally lower gene flow and reduced opportunity to access large river refugia. These sources of vulnerability should be considered when devising Arctic Grayling harvest policies and land-use plans.

Chapter 3 Tables and Figures

Table 3-1. Site location and genetic diversity of Arctic Grayling captured in the Athabasca River basin in Alberta, Canada. Sites are grouped based on the general location of sub-populations identified using individual clustering analysis (see Chapter 2). Shown are the number of individuals genotyped (N), rarefied estimates of allele richness (Ar), expected heterozygosity (H_{exp}), and observed heterozygosity (H_{obs}).

Watercourse	Site	Latitude	Longitude	*N*	Ar	H_{exp}	H_{obs}
Athabasca River							
Berland River	Be	53.998	-117.74	43	5.59	0.637	0.624
Hightower Creek	Hi	53.789	-117.93	11	5.85	0.674	0.687
Pinto Creek	Pi	53.727	-117.84	41	4.98	0.629	0.649
Wildhay River[a]	WiUp	53.715	-117.72	33	5.43	0.668	0.692
Wildhay River[b]	WiDo	53.94	-117.34	34	5.33	0.667	0.703
Athabasca River[c]	AtL	53.911	-117.08	11	5.12	0.668	0.657
Athabasca River[d]	AtN	53.98	-116.93	26	6	0.695	0.683
Windfall Creek	Wi	54.218	-116.22	30	6.44	0.698	0.692
McLeod River							
McLeod River	Mc	53.534	-116.92	31	5.92	0.684	0.685
Sundance Creek	Su	53.555	-116.59	30	6.12	0.712	0.751
Edson River	Ed	53.644	-116.36	30	6.05	0.721	0.665
Wolf Creek	Wo	53.341	-116.09	30	5.52	0.686	0.654
Unnamed Tributary	Wt	53.348	-116.13	35	5.58	0.701	0.693
Lendum Creek	Le	53.183	-116.59	30	5.39	0.670	0.639
Erith River	Er	53.325	-116.65	29	5.58	0.699	0.697
Embarras River	Em	53.304	-116.89	32	5.64	0.676	0.667
Freeman River							
Freeman Creek	Fc	54.677	-115.48	31	6.09	0.698	0.699
Morse River	Mr	54.505	-115.07	31	5.77	0.666	0.681
Freeman River	Fr	54.588	-115.57	31	6.31	0.706	0.697
Lesser Slave Lake							
Marten Creek	Mn	55.534	-114.82	32	6.12	0.697	0.734
Sawridge Creek	Sa	55.177	-114.95	33	6.21	0.708	0.699
Otauwau River	Ot	55.148	-114.55	33	6.47	0.666	0.643
Driftpile River	Dr	55.078	-115.74	31	5.63	0.666	0.676
Inverness River	In	55.034	-115.39	31	6.52	0.700	0.683
Swan River	Sw	54.8	-115.52	27	6.46	0.696	0.688
Moosehorn River	Mo	54.873	-115.47	29	6.19	0.671	0.655

[a]All samples collected upstream of the Pinto Creek confluence.
[b]All samples collected downstream of the Pinto Creek confluence.
[c]All samples collected near the Lynx Creek confluence.
[d]All samples collected near the Nosehill Creek confluence.

Table 3-2. Summary statistics of potential predictive variables of Arctic Grayling genetic diversity. Asterisks denote variables retained for modelling purposes.

Variable	Abbreviation	Mean	Min	Max	SD
Population size					
*Adult density	Adult	2	1	3	1
Environmental limiting factors					
*Watershed area (m^2)	WshedArea	1330	113	11019	2806
Stream order	Order	3	2	5	1
*Elevation (m)	Elev	915	631	1176	144
Growing degree days (^oC)	GDD	234	121	358	61
Anthropogenic disturbance					
Non-linear disturbance - 100 m corridor (m^2/m^2)	CorNonlin	0.115	0.000	0.494	0.118
Non-linear disturbance - watershed (m^2/m^2)	WshedNonlin	0.204	0.033	0.630	0.152
*Linear disturbance - 100 m corridor (m^2/m^2)	CorLin	0.028	0.004	0.081	0.017
Linear disturbance - watershed (m^2/m^2)	WshedLin	0.028	0.005	0.053	0.015
Total disturbance - 100 m corridor (m^2/m^2)	CorTot	0.137	0.004	0.512	0.119
*Total disturbance - watershed (m^2/m^2)	WshedTot	0.241	0.044	0.654	0.153
Recreational angling					
Campground count - 10km buffer	Cp10	1	0	3	1
Campground count - 25km buffer	Cp25	5	0	17	4
*Campground count - 50km buffer	Cp50	22	3	48	12
Road density - 100 m corridor (m^2/m^2)	Rd100	0.010	0.000	0.055	0.013
Road density - 500 m corridor (m^2/m^2)	Rd500	0.011	0.000	0.034	0.010
Distance to city or town (km)	DistCT	50.8	11.6	110.0	27.7
*Distance to city, town, village, hamlet (km)	DistCTVH	40.2	10.0	110.0	25.0
City, town, village, hamlet count - 50 km buffer	CTVH50	3	0	8	3
Spatial position					
Downstream link	Dlink	71	3	550	128
*Confluence difference	CD	1	0	3	1
*Distance to Athabasca River (km)	DistAtha	180.1	0.0	558.8	134.7
Distance to a major river (>4th order) (km)	DistOrder4	76.1	0.0	198.1	65.4
Post-glacial colonization					
*Latitude (Decimal Degrees)	Lat	54.15	53.18	55.53	0.70

Table 3-3. Linear regression models predicting Arctic Grayling allelic richness (Ar) from combinations of latitude, site spatial position, and linear disturbance in the stream corridor in the Athabasca River basin, Alberta.

Model Variables	Equation[a]	AICc	ΔAICc	R^2
N+CD+CorLin	Ar = -20.25 (4.63) + 0.47 (0.08)**N** + 0.18 (0.05)**CD** + 8.95 (3.54)**CorLin**	11.5	-	0.68
N+CD	Ar = -14.17 (4.43) + 0.37 (0.08)**N** + 0.19 (0.06)**CD**	14.9	3.4	0.59
N+CorLin	Ar = -20.00 (5.65) + 0.47 (0.10)**N** + 9.78 (4.31)**CorLin**	19.0	7.5	0.50
N	Ar = -13.27 (5.26) + 0.35 (0.10)**N**	21.4	9.9	0.38
CD	Ar = 5.73 (0.11) + 0.18 (0.08)**CD**	28.1	16.6	0.17

[a]Values in parenthesis are standard errors

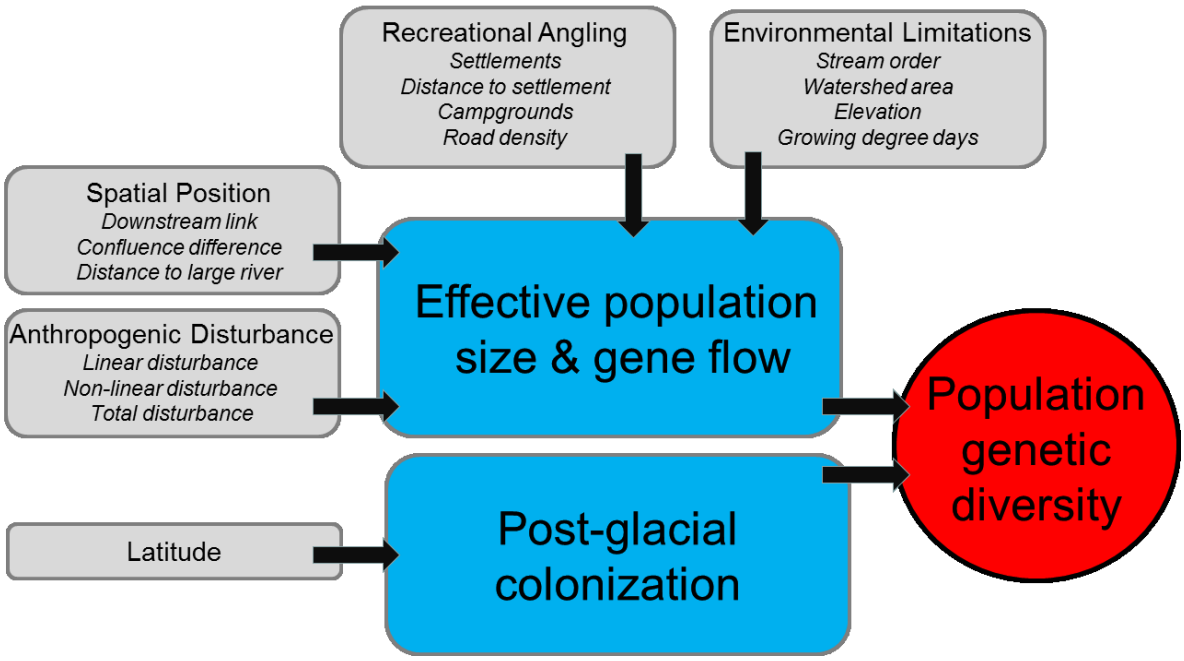

Figure 3-1. Conceptual model of how geographic, environmental, and anthropogenic factors (gray boxes) potentially influence neutral intra-population genetic diversity of Arctic Grayling in Alberta, Canada.

Figure 3-2. Sample site locations of Arctic Grayling in the Athabasca River basin within Alberta, Canada.

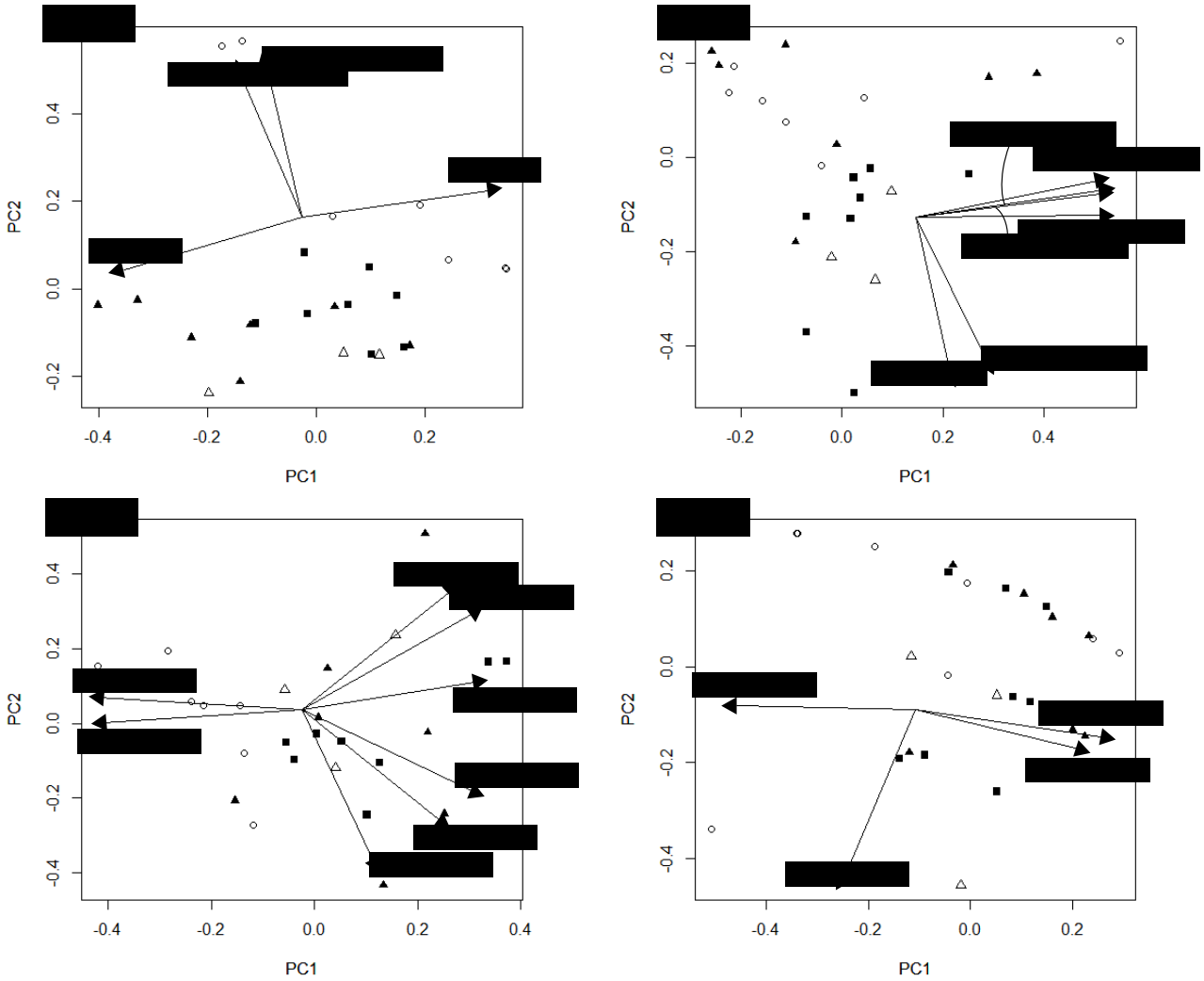

Figure 3-3. Biplots from Principal Component Analyses of potential variables influencing genetic diversity grouped by category, including: (A) environmental factors limiting productivity; (B) anthropogenic disturbance; (C) recreational angling; and (D) spatial position in the stream network. Asterisks denote variables retained for modelling purposes. Each point represents a sample site and is symbolized based on location within either the Athabasca River watershed (open circles), Mcleod River watershed (solid squares), Lesser Slave Lake area (solid triangles), or Freeman River watershed (open triangles). Variables include: WshedArea, Watershed area; Order, Stream order; Elev, Elevation; GDD, Growing Degree Days; CorNonlin, Non-linear disturbance - 100 m corridor; WshedNonlin, Non-linear disturbance – watershed; CorLin, Linear disturbance - 100 m corridor; WshedLin, Linear disturbance – watershed; CorTot, Total disturbance - 100 m corridor; WshedTot, Total disturbance – watershed; Cp10, Campground count - 10km buffer; Cp25, Campground count - 25km buffer; Cp50, Campground count - 50km buffer; Rd100, Road density - 100 m corridor; Rd500, Road density - 500 m corridor; DistCT, Distance to city or town; DistCTVH, Distance to city, town, village, hamlet; CTVH50, City, town, village, hamlet count - 50 km buffer; Dlink, Downstream link; CD, Confluence difference; DistAtha, Distance to Athabasca River; DistOrder4, Distance to a major river (>4th order).

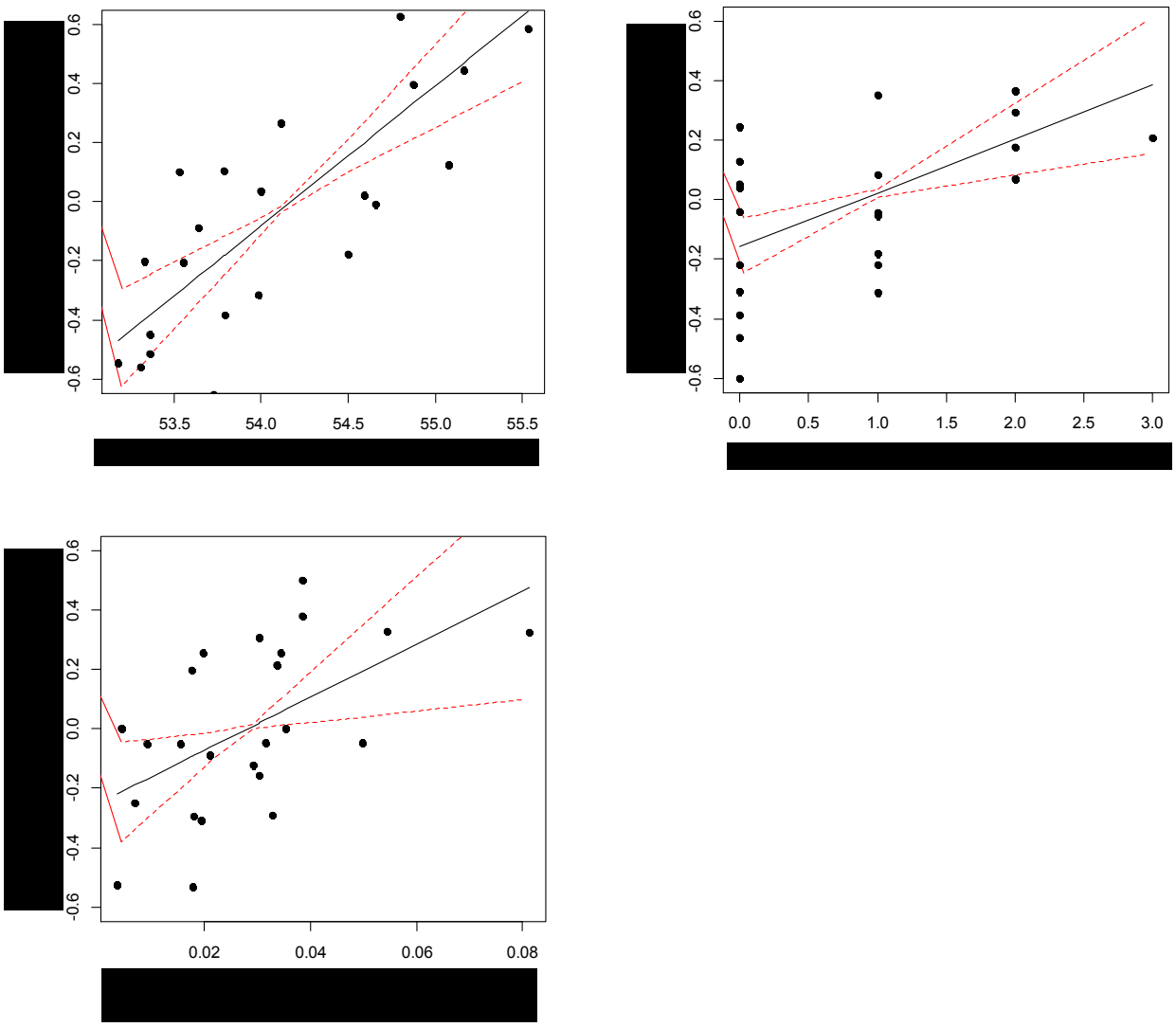

Figure 3-4. Partial residual plots showing the relationships between allelic richness and (A) latitude; (B) confluence difference; and (C) density of linear features within the stream corridor when controlling for the contributions of the other predictor variables. The plots are based on the most parsimonious model in Table 3-3. Solid, black lines are best fit lines and red dashed lines denote standard errors.

Chapter 4

Summary of Major Findings

The objective of this thesis was to use genetic techniques to characterize population structure and genetic diversity of Arctic Grayling, as well as to identify predictors of genetic diversity and infer post-glacial colonization routes. This research was conducted within the Athabasca River, Peace River, and Hay River basins of Alberta, with a specific focus on Arctic Grayling residing in unobstructed rivers within the Athabasca River basin. In Chapter 2, broad- and fine-scale patterns of genetic differentiation and diversity across and within river basins were examined using variation at microsatellite markers and a suite of analyses including traditional measures of differentiation (F_{ST}), individual and group clustering techniques, and spatial autocorrelation. In Chapter 3, genetic diversity measures (i.e., allelic richness and expected and observed heterozygosity) were related to a set of variables thought to influence or describe effective population size, amount of gene flow, and historical colonization events. Excluding latitude and the index of adult abundance, these variables were grouped into four categories, including: 1) environmental factors limiting productivity; 2) anthropogenic disturbance; 3) recreational angling; and 4) spatial position. A linear modelling approach was used to identify which variables were significantly related to genetic diversity.

Chapter 2 result summary: Population structure and genetic diversity

Arctic Grayling exhibited hierarchical population genetic structure among major river basins and sub-basins. At the coarsest scale, Arctic Grayling in the Hay River, Peace River, and Athabasca River basins were significantly differentiated likely because of isolation resulting from limited dispersal between the basins. Fine-scale structure was characterized by indiscrete populations linked by moderate to high gene flow, which followed an isolation by distance pattern. Sub-basins tended to represent the spatial extent of differentiated groups of Arctic Grayling. This extent is intermediate to that of Bull Trout and Mountain Whitefish, which is likely a result of interspecific differences with regards to population sizes and frequency of straying between spawning aggregates (Whiteley et al. 2004). My findings were similar to that reported for Arctic

Grayling in the Big Hole River system, Montana (Peterson and Ardren 2009), and may represent Arctic Grayling population structure in other river systems free of movement barriers, such as waterfalls, dams and weirs.

I detected a significant decline in genetic diversity in a southward direction across the Athabasca River and Peace River basins. This pattern is most likely attributable to serial founding effects and bottlenecks experienced during post-glacial colonization. I was unable to determine the major refugium of Arctic Grayling in Alberta because dispersal into the province from all putative refugia could have occurred in a north to south direction. Further, high genetic diversity at some sites could be indicative of areas near putative refugia or contact zones between lineages.

Chapter 3 result summary: Predictors of genetic diversity

Allelic richness was the only genetic diversity measure that displayed significant relationships with predictor variables examined in this thesis. The best supported model included latitude, confluence difference, and density of linear disturbance in the contributing stream corridor. The strong, positive relationship with latitude supports the findings of Chapter 2, in that the spatial pattern of genetic diversity is largely the product of historical, demographic events. Confluence difference was also found to have a significant, positive relationship with allelic richness. This variable describes the position of each sampled site/tributary in the stream network by comparing the stream order of the tributary to its receiving river. There are several possible mechanisms behind this observation. Diversity may be greater at sites situated on tributaries directly connected to a relatively larger river because of higher rates of immigration and admixture (Neville et al. 2006). Additionally, proximity to large rivers may provide fish an opportunity to escape temporary, unsuitable local conditions such as intense flooding (Hitt and Angermeier 2008). This could have a positive effect on population size and stability, and subsequently, genetic diversity.

Allelic richness and the density of linear disturbance in the stream corridor were also positively related. This was unexpected, because increased human activity on the landscape (e.g., road

development) was predicted to affect population size and genetic diversity negatively due to decreased habitat quality and increased exploitation. The causal factor(s) behind this relationship remains unclear; increased diversity could be the result of ecosystem-based changes post-disturbance or other incidental environmental covariates of disturbance and abundance/diversity.

Conservation and management implications

Arctic Grayling is considered a sensitive species and has experienced declines in many areas, particularly along the southern margin of its range in Alberta (ASRD 2005). Severe reductions in abundance have been attributed to fragmentation, overharvest, and the cumulative effects of anthropogenic disturbance (ASRD 2005). The genetic data provided in my thesis may be used to guide more effective management of this vulnerable species within unobstructed river systems within and outside of Alberta.

Arctic Grayling are mobile and gene flow between neighbouring rivers appears to occur frequently and over relatively recent timeframes. This has generally acted to homogenize genetic diversity within sub-basins. Occasionally, the degree of differentiation within sub-basins suggests that demographically independent units of Arctic Grayling may occur at an even finer spatial scale.

My results summarized above can be used to inform, but not dictate, Management Unit (MU) boundaries for Arctic Grayling. From an evolutionary perspective, an exchange of several individuals per year between fish stocks has significant effects and can vastly reduce genetic differentiation (Mills and Allendorf 1996). However, fisheries managers are typically concerned with other demographic attributes of stocks (e.g., will a stock persist at a level of abundance that will permit harvest over the next 100 years?) (Sass and Allen 2014), which are rarely influenced by such low rates of exchange. Thus, relying strictly on genetic data may result in the delineation of MUs that contain several demographically disconnected populations, which should not be managed as a whole because they react independently to exploitation and environmental change (Carvalho and Hauser 1994). This erroneous grouping of populations can lead to unexpected reductions in abundance and even extirpation if, for example, harvest levels are set using data

collected from the relatively abundant population within the MU (Taylor and Dizon 1999, Taylor et al. 2000). Rather, the first step should be to determine the dispersal rate at which stocks are demographically correlated through experimental or theoretical research (e.g., Hastings 1993) (Palsboll et al. 2007). Genetic data can then be used to determine what spatial groupings of Arctic Grayling yield that critical dispersal rate, and essentially, where MUs should be delineated (Taylor and Dizon 1999, Palsboll et al. 2007).

Supplementation activities may be considered by fisheries managers to bolster declining stocks of Arctic Grayling. The findings of my thesis will be valuable if the supplementation approach includes translocations or rearing of fish in hatcheries. In both cases, managers can refer to the patterns of population structure I identified when selecting a genetically similar donor stock for a particular river. This approach is essential in terms of reducing the risk of negative fitness effects from outbreeding depression (Miller and Kapuscinski 2003), which has been noted in other wild populations of salmonids following hybridization of distinct stocks (Gilk et al. 2004). In the absence of additional genetic or movement data, I would generally recommend collecting individuals or broodstock from either the same river that is being supplemented or from neighbouring rivers situated within the same sub-basin. Additionally, my documentation of low diversity in a stocked population (Quarry Lake) should caution managers against the release of propagated individuals into natural populations in case this reduction corresponds to a loss of fitness. The multi-generational use of wild-caught fish as broodstock has resulted in significant declines of reproductive fitness in some populations of salmonids (Araki 2008; Araki and Schmid 2010).

I attempted to address potential interactions between sport fishing, anthropogenic disturbance, and genetic variability of Arctic Grayling. Recreational fishing pressure did not appear to have had genetic impacts, potentially because 1) adequate numbers of Arctic Grayling persist and the effects of genetic drift and inbreeding are negligible; 2) high gene flow is replenishing diversity; 3) the prominent and persistent influence of post-glacial colonization masks contemporary reductions in abundance or connectivity; or 4) I neglected to include important predictor variables, or predictor variables lacked resolution/detail. Anthropogenic disturbance had an unexpected positive association with diversity which, without further research, is difficult to

explain. It is possible that density of disturbance is correlated with landscape elements that benefit Arctic Grayling.

In light of the many unknowns, I recommend applying a precautionary approach towards Arctic Grayling management. Gene flow is likely key in maintaining levels of genetic diversity when stock abundance is reduced (although there is a risk of eroding or hindering local adaptation if gene flow is high). Collapsed and isolated stocks, such as those in the Pembina River (Blackburn and Johnson 2004), are more vulnerable to a loss of genetic diversity through inbreeding and genetic drift. Reduced heterozygosity often results in negative effects on population fitness (Reed and Frankham 2003; England et al. 2003; Spielman et al. 2004), although there are some exceptions where populations and species thrive despite extremely low genetic diversity (e.g., Milot et al. 2007). A conservative approach, due to the paucity of data on adaptive genetic variation and fitness of Arctic Grayling, is to assume that low genetic diversity impacts the sustainability of stocks and that some have the potential to enter an extinction vortex, wherein a feedback loop of negative genetic effects can, theoretically, result in reduced fitness and eventual extirpation (Gilpin and Soule 1986). To avoid this worst-case scenario, alternative management or land-use policies (e.g., bait bans, complete river closures, improved sediment control measures at stream crossings, etc.) may need to be employed to conserve remaining stocks. Detailed recommendations are outside the scope of this thesis.

Future directions

Much remains unknown about the genetic aspects of Arctic Grayling populations. For example, research using mitochondrial DNA variation of Arctic Grayling is needed to elucidate post-glacial dispersal patterns. Additionally, there have been no studies examining the nature of adaptive variation in Arctic Grayling. Exploring relationships between the environment, genotype, and phenotype (e.g., propsensity to home, thermal tolerance) may help predict how stocks will respond to environmental change and assist in selecting genetically-similar donor stocks should supplementation occur (Leaniz et al. 2007).

I employed an opportunistic sampling approach due to the extremely low abundance of some stocks and lack of available knowledge regarding the location of spawning areas. Future studies of Arctic Grayling population genetics should aim to include young-of-the-year or actively spawning fish. The resulting data would likely increase resolution among genetically differentiated groups and would facilitate Mixed Stock Analysis more effectively if paired with genetic data collected from individuals in summer feeding habitats (e.g., Warnock et al. 2011).

Characterizing the genetic variation of an entire spawning run of Arctic Grayling would also expand basic knowledge of this species and provide an opportunity to investigate the relationship between effective and census population size (N_e and N_c). The species' reproductive strategy could be elucidated by determining if different age-classes or the sexes stray at dissimilar rates. Quantifying the N_e:N_c ratio would enable managers to make conclusions regarding stock size and status based on genetic data (e.g., Rieman and Allendorf 2001). In times of limited resources for management, this could permit the collection of new or additional information on relatively unstudied stocks, assuming that a genetic study is adequately accurate/precise, less costly and time-consuming than a population estimate using traditional techniques (i.e., mark-recapture). Beyond providing current, baseline knowledge on stock size, genetic data could also be used to monitor changes in stock status over time if conditions in a watershed improve or degrade. Such a monitoring program could address some of the uncertainties raised in Chapter 3 regarding the trade-offs between human activities and the sustainability of Arctic Grayling stocks in Alberta.

Conclusion

This research on the population and landscape genetics of Arctic Grayling contributes to our basic understanding of this species and can be applied during the development of management strategies within Alberta and in other regions encompassing similar habitats. Including genetic methods in the suite of sampling tools used by resource managers improves decisions regarding delineation of management units, supplementation procedures, conservation priorities (i.e., protecting small and/or isolated stocks), and land-use planning. The soundness of these decisions is vital to the conservation of Arctic Grayling and will become increasingly important in light of

the expanding industrial sector and human population within the species' range in North America.

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