Characterizing adaptive morphological features and resource selection of Rocky Mountain Sculpin (*Cottus* sp.), a species at risk in Canada

by

Tyana Ashlie Rudolfsen

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

Master of Science

in

CONSERVATION BIOLOGY

Department of Renewable Resources
University of Alberta

`

Abstract

Freshwater biodiversity is presently one of the world's largest conservation concerns. Both direct and indirect human activities contributing to waterway modifications, climate change, and habitat alteration are causing major declines in freshwater fish species richness and abundance. While these impacts are well studied for pelagic fishes, little is known about how to best direct management efforts toward benthic, dispersal-limited fishes. The Rocky Mountain Sculpin (*Cottus* sp.), a benthic, sedentary, and federally listed species at risk in Canada, was used to address the following objectives: 1) to identify its susceptibility to varying flow regime, and whether or not it displays morphological adaptation to flow, and 2) to characterize hybrid zones and the driving environmental factors that lead to their persistence. Using geometric morphometrics and meristic counts of fin rays/spines and sensory pores, phenotype was compared across the four river populations in Canada. Systems with higher flow regimes generally had Rocky Mountain Sculpin that were more dorso-ventrally compressed and had meristic features better suited to positioning themselves among cobble substrate and detecting prey in fast moving water. Biogeographic isolation contributed to phenotypic variation, indicating that the Rocky Mountain Sculpin might not be able to quickly adapt to human-induced flow alterations. To achieve the second objective, hybrid zones between the westslope Rocky Mountain Sculpin population (Flathead River drainage, BC) and the Slimy Sculpin (*Cottus cognatus*) were studied. Using 731 genetic samples and 10 polymorphic microsatellite loci, two hybrid zones were identified more upstream than expected from previous studies. A logistic mixed-effects model revealed that habitat features relating to climate change and water quality were driving an upstream range expansion of Rocky Mountain Sculpin and movement of hybrid zones. While the Rocky Mountain Sculpin, despite their sedentary life history, appears to have

`

more tolerance to anthropogenic habitat alterations that originally expected, their tolerance level likely has limits that can be widely detrimental to the species if tested. Further, given their high levels of phenotypic variation across populations, management efforts involving dispersal-limited species should be directed at the population level. In the event of hybrid zones, which are indicators of more localized environmental factors driving species presence, a more localized scale of management might be required.

`

Preface

This dissertation is the original work of Tyana A. Rudolfsen, with the support and contributions of professionals in the field of fisheries ecology and molecular biology. I conducted the field work logistics, data collection, and assisted in modifications to study design. Dr. Mark Poesch was my primary supervisor, developing a study design that would fill key deficits in our understanding of Rocky Mountain Sculpin ecology. He provided assistance in the statistical analysis of data, as well as the finalization of manuscripts. Doug Watkinson was an important contributor to both chapters of this thesis, assisting in sample design and collection, guidance on data analysis, and manuscript draft critiques.

Several people were involved in the genetic component and revision of Chapter 3. Dr. Corey Davis processed genetic samples and contributed to the methodology of genotyping. Dr. Eric Taylor provided reference genotypes to Rocky Mountain and Slimy Sculpin in the Flathead River basin. Dr. Jonathan Ruppert conducted statistical genotype analysis to identify hybrids.

Approval for the collection and holding of sculpin was approved by the Canadian Council of Animal Care Approval (AUP 00000759), Alberta Fish Import License FIL2015-0074, Federal Introductions and Transfers License VI16-236322, Alberta Fish Research Licenses 14-2415AFR and 16-0101FR, and British Columbia Fish Collection Permits CB14-149123 and CB15-171090.

`

Acknowledgements

This thesis was funded by Fisheries and Oceans Canada, Species at Risk Program (SARCEP) (Doug Watkinson, Mark Poesch), and NSERC Discovery Grant (Mark Poesch). Supplementary scholarship funding was provided by the Graduate Scholarship (Government of Alberta) and the Queen Elizabeth II Scholarship (Government of Alberta).

I thank Dr. Mark Poesch who provided the guidance and support to be successful in the completion of this dissertation. His progressive approach to science and continued dedication to fisheries research not only enabled me to be part of this remarkable study, but also inspired me to continue making a difference in aquatic conservation.

I also thank my second supervisor, Doug Watkinson, whose interest and contributions to researching Rocky Mountain Sculpin has made him an integral constituent in their conservation. He was an ongoing guide in the study design, sample collection, and editorial phases of this thesis. He has come to be a highly valued mentor of mine.

John Acorn kindly agreed to review my thesis as chair member of my defense committee.

This project was made possible only through the assistance of many from the Poesch Lab. Primarily, thank you to Dr. Jonathan Ruppert for directing the genetic analysis component of Chapter 3. I also express profound appreciation to my two primary crew members Kenton Neufeld and Christopher Smith for their hard work helping collect sculpin in isolated, unpredictable conditions. I thank Marie Veillard for her encouragement and ongoing advice on many facets of the analysis and writing phase of this dissertation. Other notable and valued contributors include Elashia Young, Denyse Dawe, Kyle Hamilton, Caitlin Good, and Bryan Maitland.

`

The collaborative effort of research teams beyond the Poesch Lab were critical to the success of this thesis. Doug Leroux, Paul Fafard, and Colin Kovachik from the Fisheries and Oceans Canada took part in collecting sculpin. Thank you also to Dr. Corey Davis (Univrsity of Alberta) for processing the genetic samples and Dr. Eric Taylor (University of British Columbia) for his expertise on genotyping the sculpin genetics. Knowledge of the Flathead River area and field safety support was provided by Dr. Garth Mowat from the Ministry of Forests, Lands and Natural Resource Operations, BC, and Inspector Joe Caravetta from the Conservation Officer Service of the Ministry of Environment, BC.

My friends and family helped make my involvement in this study possible. Words cannot express my gratitude to my parents, Brent and Shannon Rudolfsen, for their unwavering faith in my capabilities and goals. Thank you to my Aunt and Uncle, Beverly and Stefan Betkowski, who opened their home to me, allowing me to remain focused on my studies. Hedin Nelson-Chorney not only provided invaluable revisions of my manuscript drafts, but was also an unconditional source of positivity and encouragement throughout this process. My best friend Dana Stromberg was a constant support system, and someone I could look to for perspective.

Finally, I wish to thank Dr. Simon Landhäusser, who originally introduced me to environmental sciences and mentored me over several years as an intern and research assistant. The opportunities and support he provided helped me explore multiple facets of environmental research and ultimately find my passion for fisheries and aquatic conservation.

`

Table of Contents

`

List of Tables

`

Table 3.2. Genetic diversity measures for the 10 microsatellite loci that were typed for all individuals, Slimy Sculpin and Rocky Mountain Sculpin from the Flathead River. The number of individuals typed at each loci (N), number of alleles (N_a), observed heterozygosity (H_o), unbiased expected heterozygosity (UH_e) and fixation rate (F) are indicated. All values were calculated using GenAIEx 6.5. Bold values indicate loci out of Hardy-Weinberg equilibrium.

Table 3.3. Mixed-effects model using backward selection depicts the habitat factors important in determining hybridization zones between Rocky Mountain Sculpin and Slimy Sculpin in the Flathead drainage. Standardized z-scores of the variables were used in to allow comparison across measurement units. The k column represents the number of variables including the response variable (q-value) used in the habitat model. The optimal model (in bold font) was selected based corrected Akaike Information Criteria (AICc*i*), the difference between $AICc_i$ values and the smallest $AICc_i$ value (AICc Δ_i), and the weightings of each model showing the probability of a given model being the most optimal (AICc w_i).

Table 3.4. Estimates and significance levels describing the key covariates that determine q-values of sculpin in the Flathead River drainage, BC. Values are based on the selected logistic mixed-effects model (model #4). A * next to P-values indicate covariate significance (≤0.05).

A3.3. Results from submerged temperature loggers relative to the presence or absence of Rocky Mountain Sculpin (RMS), Slimy Sculpin (SSC), and hybrid zones (HYB). Table **(A)** represents the results from the repeated measures Analysis of Variance (ANOVA). Table **(B)** shows the pairwise comparison of thermal regime between groups using the Tukey HSD test with Holm *P*-value adjustment. An asterix represents significant P-values.

`

List of Figures

`

A2.1. Landmarks used in geometric morphometric comparisons across Rocky Mountain Sculpin river populations. Figure **(A)** shows landmarks used in the dorsal perspective, and **(B)** shows the landmarks used in the lateral perspective.

A2.2. Dorsal outline of the mean shape of each river population. Dorsal landmark placements were amplified 4.5 times to make fine-scale differences visible across populations. Landmarks were superimposed to visually show the differences across populations. The X and Y axes represent the vector scores once standardized using General Procrustes Analysis.

A2.3. Lateral shape outline of each river population based on mean landmarks. Lateral landmark placements were amplified 3 times to visualize fine-scale differences across populations. Landmarks were superimposed to visually show the differences across populations. The X and Y axes represent the vector scores once standardized using General Procrustes Analysis.

Figure 3.1. (A) The Canadian distribution of Rocky Mountain Sculpin (top). The Slimy Sculpin distribution overlaps with Rocky Mountain Sculpin in the Flathead Drainage in southeastern BC, outlined in red (ranges modified from Scott and Crossman (1973) and COSEWIC (2010)). **(B)** Sample sites (n=95) in the Flathead River Drainage (bottom) were chosen based on areas that would possibly have hybrid zones based on findings by Hughes and Peden (1984). **(C)** Map of the Flathead Drainage showing the areas where hybrids and pure populations are found. Pies represent the proportion of parental and hybrid populations present in a given area of each lotic system.

Figure 3.2. Frequency distribution of q-values based on microsatellite analyses. Q-values represent the proportion of Rocky Mountain Sculpin microsatellites present in the genetic fin

`

clips. Q-values of 0-10% (black) were Slimy Sculpin, 11-90% (gray) were hybrids, and 91-100% were Rocky Mountain Sculpin.

Figure 3.3. Effects plots showing the influence of each significant variable on q-value based on the environmental conditions each sculpin (points) was collected in. A fitted regression line and 95% confidence intervals (dashed lines) show the general trends of each environmental variable

A3.1. Results from the STRUCTURE model including the (**A**) mean ln probability and (**B**) delta K value. Shown are values for K between 1 and 5.

A3.2. Average water temperature trends in the Flathead drainage where Rocky Mountain Sculpin, Slimy Sculpin, and hybrid zones were found. Plot includes means ± standard errors across July and August of 2015 and 2016.

`

Chapter 1: General Introduction

Anthropogenic activities are a major cause of extinctions globally and represent a prominent conservational challenge. Human populations and urbanized areas are rapidly growing (Cohen 2003), which can have negative implications to biodiversity. Species richness is declining at an alarming rate because of habitat loss from urbanization (McKinney 2002, McKee et al. 2004). For freshwater organisms, water pollution (ie. siltation and nutrient loading), habitat fragmentation, and hydrology alterations are especially detrimental (Richter et al. 1997). This, augmented by human-induced climate change and pollution can inadvertently extricate fish from aquatic systems.

Freshwater conservation is a crucial objective for the sustainability of human populations and overall biodiversity. Freshwater fish are currently declining faster than terrestrial or marine organisms (Sala et al. 2000, Dudgeon et al. 2006). They are typically in isolated drainages and are thereby unable to evade direct and indirect pressures, making environmental pressures more impactful (Ricciardi and Rasmussen 1999, Allan et al. 2005). This is of concern because freshwater species comprise 45% of known fish species, yet are restricted to freshwater systems that total only 1% of the global surfaces (Levêque et al. 2008). Additional barriers to movement such as dams, canals and habitat fragmentation leave freshwater fishes extremely vulnerable (Fagan et al. 2002). Water diversion and removal for irrigation or urban consumption is a particularly prevalent issue. These activities alter flow regimes, thereby destabilizing river banks and increasing sediment deposition (Poff et al. 1997).

Flow regime is an important habitat factor to fish species and can have detrimental consequences to populations if altered. Natural and seasonal flows are crucial to maintain

`

suitable habitat for resident fishes, therefore alterations in flow can result in a shift toward uninhabitable conditions (Poff et al. 1997). Stream flow is consistently changing due to irrigation, water diversion, and climate change. Both increased and decreased water flow as a result of these alterations can cause a decline in fish species richness and abundance, resulting in a need to balance human water demands with maintaining aquatic biodiversity (Pringle et al. 2000, Poff and Zimmerman 2010). This need can be met by determining species tolerance levels and minimizing habitat alterations. In some cases, pelagic fish can morphologically adapt to varying flow regimes and so can better cope with human-induced changes in flow (Langerhans 2008), but the ability for benthic fish to successfully adapt has not been intensively explored. Benthic fish tend to have more sedentary life histories making them less capable of moving away from negative impacts (Facey and Grossman 1990). Therefore the impacts of flow regime can cause the rapid loss of benthic species leading to the need to understand their ability to adapt to a range of flow variation.

Habitat alteration or loss from land use and climate change is a primary conservation issue for freshwater fishes. Siltation, and nutrient loading from human activities alter habitats and decrease overall biodiversity (Jenkins 2003, Postel and Richter 2012). Conversely, climate change is a more gradual phenomenon. As temperatures slowly increase, species can adapt by shifting their distribution ranges (Parmesan et al. 1999, Parmesan 2006). If these shifts cause similar species to converge, hybridization can occur along with the possible genetic dilution of previously pure populations (Levin et al. 1996, Muhlfeld et al. 2009, Garroway et al. 2010). Despite current understanding that climate change might be affecting the level of hybridization among fish species, a more comprehensive study of how habitat factors can be assisting in range shifts is required.

`

The Rocky Mountain Sculpin (*Cottus* sp.; a.k.a *Cottus* n.sp.nr.*bairdii*) is a small, benthic fish found in only a few southern watersheds in Alberta and British Columbia. It reaches upwards of 114 mm in total length, inhabiting cold, clear river systems with cobble substrate and shallow to moderate water depths (RL&L Environmental Services Ltd. 2002). Sculpin are a major part of community structure in cold and small river systems, maintaining biodiversity and occupying important parts of ecological food webs (Quinn and Mundahl 1994, Gray et al. 2005, Cheever and Simon 2009). They are particularly important as a food source to resident trout species and other piscivorous fishes (Dineen 1951, Beauchamp 1990, Tabor et al. 2007). Additionally, their sensitivity to human impacts makes them valuable as an indicator species to their river systems (Fisheries and Oceans Canada 2012). Despite their importance however, we lack relevant knowledge of the Rocky Mountain Sculpin's life history. Therefore, current management objectives are based on inferences made from other sculpin species (Fisheries and Oceans Canada 2012).

While Rocky Mountain Sculpin populations do not appear to be declining, their restricted range and sedentary behavior makes them highly susceptible to negative environmental impacts, leading to their listing as a species at risk in Canada (COSEWIC 2010a, Ruppert et al. 2017). The Rocky Mountain Sculpin is divided into two designatable units (DU's), the eastslope (Alberta) and westslope (British Columbia). The eastslope DU was listed as Threatened under the Species at Risk Act (SARA) in 2006 and the provincial *Wildlife* Act in 2007 (COSEWIC 2005, Fisheries and Oceans Canada 2012). The eastslope DU is particularly susceptible to habitat loss and fragmentation from the synergistic effects of water removal for irrigation and drought events from climate change (Fisheries and Oceans Canada 2012). In contrast, the westslope DU (Flathead River drainage) is more exposed to forestry road construction and ATV use resulting in

`

increased water turbid (COSEWIC 2010b). While these activities are not currently major impacts, there is concern that increased development may lead to population declines. There have also been proposals to mine the Flathead Valley for gold and coal that would add to development of the Flathead drainage (Hughes and Peden 1984). These potential threats to Rocky Mountain Sculpin in the westslope DU is why fish in this DU are listed as Special Concern (COSEWIC 2010b).

The Rocky Mountain Sculpin is an ideal species to study the impacts of changing environmental factors on freshwater fish species that are rare or imperiled. They inhabit four river systems that are relatively close to one another but exhibit different habitat characteristics including flow regime, anthropogenic pressures, and surrounding terrestrial environment. Further, they have a sedentary life history, which allows for the characterization of biogeographical influences as well as local impacts such as land use and interspecific hybridization.

This thesis has two major study objectives that are directed toward filling major knowledge gaps in the understanding of how Rocky Mountain Sculpin can cope with changing habitat conditions. First, I characterize morphological variation of Rocky Mountain Sculpin across their four river populations in Canada in relation to varying flow regimes and biogeography (Chapter 2). The prevalence of water diversion and removal in Alberta drives a need to understand how well Rocky Mountain Sculpin can adapt to flow alterations. This will aid in our understanding of sedentary fish species in isolated drainages and their susceptibility to the impacts of human-induced water flow alterations. The second objective of this thesis is to describe the nature of hybridization between Rocky Mountain Sculpin and Slimy Sculpin (*Cottus cognatus*) in the Flathead River drainage, BC, in order to determine the underlying habitat features that cause these hybrid zones to develop (Chapter 3). Results from this study will contribute to the understanding of whether Rocky Mountain Sculpin are likely to coexist or compete with closely related species. It will also further our knowledge of what environmental factors are likely to drive hybridization between fish species. The overall applicability of this thesis is two-fold. Firstly, it will fill major knowledge gaps in the Rocky Mountain Sculpin's ecology, thereby improving the ability to make management decisions regarding their conservation. Secondly, I expect these findings to guide conservation objectives for similar freshwater fish species by mitigating detrimental human impacts on habitat features.

1.1 Literature Cited

Allan, J. D., R. Abell, Z. Hogan, C. Revenga, B. W. Taylor, R. L. Welcomme, and K. Winemiller. 2005. Overfishing of Inland Waters. BioScience **55**:1041-1051.

Beauchamp, D. A. 1990. Seasonal and diel food habits of rainbow trout stocked as juveniles in Lake Washington. Transactions of the American Fisheries Society **119**:475-482.

Cheever, B., and K. Simon. 2009. Seasonal influence of brook trout and mottled sculpin on lower trophic levels in an Appalachian stream. Freshwater Biology **54**:524-535.

Cohen, J. E. 2003. Human population: the next half century. Science **302**:1172-1175.

COSEWIC. 2005. COSEWIC assessment and status report on the "eastslope" sculpin (St. Mary and Milk River Population) *Cottus* sp. in Canada. Ottawa.

COSEWIC. 2010. COSEWIC assessment and status report on the Rocky Mountain Sculpin *Cottus* sp., westslope populations in Canada. Ottawa.

Dineen, C. F. 1951. A comparative study of the food habits of *Cottus bairdii* and associated species of Salmonidae. The American Midland Naturalist **46**:640-645.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, and M. L. Stiassny. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological reviews **81**:163-182.

`

Facey, D. E., and G. D. Grossman. 1990. The metabolic cost of maintaining position for four North American stream fishes: effects of season and velocity. Physiological Zoology **63**:757-776.

Fagan, W. F., P. J. Unmack, C. Burgess, and W. Minckley. 2002. Rarity, fragmentation, and extinction risk in desert fishes. Ecology **83**:3250-3256.

Fisheries and Oceans Canada. 2012. Recovery Strategy for the Rocky Mountain Sculpin (*Cottus* sp.), eastslope populations, in Canada. . Ottawa.

Garroway, C. J., J. Bowman, T. J. Cascaden, G. L. Holloway, C. G. Mahan, J. R. Malcolm, M. A. Steele, G. Turner, and P. J. Wilson. 2010. Climate change induced hybridization in flying squirrels. Global Change Biology **16**:113-121.

Gray, M. A., R. A. Curry, and K. R. Munkittrick. 2005. Impacts of nonpoint inputs from potato farming on populations of slimy sculpin (*Cottus cognatus*). Environmental Toxicology and Chemistry **24**:2291-2298.

Hughes, G. W., and A. E. Peden. 1984. Life history and status of the shorthead sculpin (*Cottus confusus*: Pisces, Cottidae) in Canada and the sympatric relationship to the slimy sculpin (*Cottus cognatus*). Canadian Journal of Zoology **62**:306-311.

Jenkins, M. 2003. Prospects for biodiversity. Science **302**:1175-1177.

Langerhans, R. B. 2008. Predictability of phenotypic differentiation across flow regimes in fishes. Integrative and Comparative Biology **48**:750-768.

Levêque, C., T. Oberdorff, D. Paugy, M. Stiassny, and P. Tedesco. 2008. Global diversity of fish (Pisces) in freshwater. Hydrobiologia **595**:545-567.

Levin, D. A., J. Francisco-Ortega, and R. K. Jansen. 1996. Hybridization and the extinction of rare plant species. Conservation Biology **10**:10-16.

McKee, J. K., P. W. Sciulli, C. D. Fooce, and T. A. Waite. 2004. Forecasting global biodiversity threats associated with human population growth. Biological Conservation **115**:161-164.

McKinney, M. L. 2002. Urbanization, biodiversity, and conservation: the impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. BioScience **52**:883-890.

Muhlfeld, C. C., S. T. Kalinowski, T. E. McMahon, M. L. Taper, S. Painter, R. F. Leary, and F. W. Allendorf. 2009. Hybridization rapidly reduces fitness of a native trout in the wild. Biology Letters:rsbl. 2009.0033.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annu. Rev. Ecol. Evol. Syst. **37**:637-669.

`

Parmesan, C., N. Ryrholm, C. Stefanescu, and J. K. Hill. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature **399**:579.

Poff, N. L., J. D. Allan, M. B. Bain, J. R. Karr, K. L. Prestegaard, B. D. Richter, R. E. Sparks, and J. C. Stromberg. 1997. The natural flow regime. BioScience **47**:769-784.

Poff, N. L., and J. K. Zimmerman. 2010. Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. Freshwater Biology **55**:194-205.

Postel, S., and B. Richter. 2012. Rivers for life: managing water for people and nature. Island Press.

Pringle, C. M., M. C. Freeman, and B. J. Freeman. 2000. Regional Effects of Hydrologic Alterations on Riverine Macrobiota in the New World: Tropical–Temperate Comparisons. BioScience **50**:807-823.

Quinn, J. W., and N. Mundahl. 1994. Effects of trout stream habitat rehabilitation projects on Nongame fish communities in five southeastern Minnesota streams. Report to the Conservation Biology Research Grants Program, Division of Ecological Services, Minnesota Department of Natural Resources, St. Paul.

Ricciardi, A., and J. B. Rasmussen. 1999. Extinction rates of North American freshwater fauna. Conservation Biology **13**:1220-1222.

Richter, B. D., D. P. Braun, M. A. Mendelson, and L. L. Master. 1997. Threats to imperiled freshwater fauna. Conservation Biology **11**:1081-1093.

RL&L Environmental Services Ltd. 2002. Fish species at risk in the Milk and St. Mary drainages. Edmonton, AB.

Ruppert, J. L. W., P. M. A. James, E. B. Taylor, T. Rudolfsen, M. Veillard, C. S. Davis, D. Watkinson, and M. S. Poesch. 2017. Riverscape genetic structure of a threatened and dispersal limited freshwater species, the Rocky Mountain Sculpin (*Cottus* sp.). Conservation Genetics:1-13.

Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, and A. Kinzig. 2000. Global biodiversity scenarios for the year 2100. Science **287**:1770-1774.

Tabor, R. A., E. J. Warner, K. L. Fresh, B. A. Footen, and J. R. Chan. 2007. Ontogenetic diet shifts of prickly sculpin in the Lake Washington basin, Washington. Transactions of the American Fisheries Society **136**:1801-1813.

`

Chapter 2: Morphological divergence of Rocky Mountain Sculpin (*Cottus* sp.) attributed to biogeography and flow regime

2.1 Executive Summary

Stream hydrology is considered the primary factor in structuring freshwater fish communities, and influencing stream habitats, food resources and fish life history characteristics. Changes in stream hydrology, from climate change and anthropogenic sources (e.g. dams, irrigation channels), are thought to negatively impact many freshwater species. The Rocky Mountain Sculpin (*Cottus* sp.) is a threatened species in Canada. Here, I study the phenotypic response of Rocky Mountain Sculpin across a gradient of four streams differing in stream hydrology. I hypothesized that Rocky Mountain Sculpin are phenotypically diverse, adapting through selection to high-flow environments with a body form that minimizes drag and increases their ability to position themselves against bottom substrate. Using geometric morphometrics and meristic counts, I compared the body shape, fin rays, and sensory pores. High-flow river systems produced sculpin with more dorso-ventrally compressed, slender body shapes that minimized resistance to flow ($P<0.001$). They had more pectoral fin rays than populations in low-flow systems potentially allowing them to increase friction when positioning against substrate ($P<0.001$), and more anteriorly and dorsally located head pores to better detect floating prey ($P<0.001$). Biogeographic isolation is likely the basis of these variations, augmented by flow regime. This study emphasizes the importance of considering phenotypic variation when evaluating how anthropogenic stressors on flow can impact a sculpin species at risk.

`

2.2 Introduction

Alteration of stream hydrology from climate and anthropogenic sources is predicted to have a major impact to freshwater fishes through the gradual or sudden loss of habitat (Dudgeon et al. 2006). Stream hydrology is important for the consistency and maintenance of fish habitat, especially for resident or sedentary species, as they are unable to move from suddenly changing environments like increased or decreased river discharge. Alterations to stream hydrology, if severe enough, can reduce biodiversity (Pringle et al. 2000). Determining how stream hydrology affects freshwater fishes is important to determine how to mitigate the impacts of stream hydrology and provide appropriate conservation measures.

It is well known that local adaptations to various environments can lead to morphological and genetic differences within the same species through selection (Endler 1986, Kawecki and Ebert 2004, Collin and Fumagalli 2011). Even among similar flow gradients, morphological divergence can occur (McLaughlin and Grant 1994, Pakkasmaa and Piironen 2000). In freshwater systems, the presence/absence of flow has been recognized as a driving force behind intraspecific morphological adaptation in fish species (Brinsmead and Fox 2002, Langerhans et al. 2003, Langerhans 2008). In these studies, pelagic fish tend to have more slender, fusiform bodies and deeper caudal peduncles when exposed to faster flowing waters (Webb 1984, McLaughlin and Grant 1994, Collin and Fumagalli 2011). These morphological adaptations help minimize drag forces on the body, thereby optimizing energetic expenditures (Webb 1984, Sagnes and Statzner 2009). Few studies account for benthic fish however, which have different life histories (Facey and Grossman 1990).

`

The Rocky Mountain Sculpin (*Cottus* sp.) is a threatened species in Canada and is listed under the federal Species at Risk Act. The main threat to Rocky Mountain Sculpin is thought to be changes in stream hydrology. Rocky Mountain Sculpin live in a range of lotic systems that differ hydrologically between one another by more than one order of magnitude (from 8 and 9 m^3/s to 61 m^3/s to 125 m^3/s; data supplied by Water Survey of Canada (2016)). Flow regimes in the St. Mary and Flathead River exhibit a similar degree of seasonal variation. These differences in stream hydrology are due in large part to stream alterations to improve irrigation for agriculture. For example, the St. Mary River was diverted in northern Montana to the Milk River watershed to improve irrigation. In recent years, this has changed the annual flow in the North Milk River from about 7.5 m^3/s during natural conditions to upwards of $17m^3/s$ during augmentation (RL&L Environmental Services Ltd. 2002). Given the alteration of the natural hydrology of systems occupied by Rocky Mountain Sculpin and the difference in magnitude of hydrologic regimes, this is a potential model species in understanding impacts of stream hydrology to population level differences in freshwater fishes.

Bottom-dwelling fish like Rocky Mountain Sculpin exhibit intraspecific morphological differences due to varying habitat characteristics including substrate colour (Whiteley et al. 2009), thermal conditions (Koumoundouros et al. 2009), and flow regime (Natsumeda et al. 2014). Sculpin are sedentary fish with relatively deep bodies and a bulky caudal peduncle (Webb 1984). Their robust torso allows for short, strong bursts of forward movement when necessary, but prolonged swimming is energetically costly. Sculpin maintain their position in running water by relying heavily on their large, rigid pectoral fins to hold themselves in place among the substrate (Facey and Grossman 1990). In highly turbulent environments, sculpin exhibit prominent pectoral fins forming more robust insertion points where the fin meets the body (Kane and Higham 2012).

Benthic fishes such as catfish, suckers, and sculpin all must morphologically reduce drag against their body in riverine systems to hold their position (Koehl 1984). Because Rocky Mountain Sculpin live in varying hydrological regimes, if these flow regimes correspond to differences in water velocity, Rocky Mountain Sculpin should have more dorso-ventrally streamlined body shape to reduce drag and/or stronger pectoral fins to position themselves against cobble substrate in the higher flow systems. Larger heads may allow for increased anterior tissue density, helping sculpin anchor themselves to the bottom (Webb 1990). Higher velocities may also select for differences in pore and fin ray counts to account for different demands on flow compensation and prey detection rates. The objective of this study is to compare the body morphology and fin ray and pore counts of Rocky Mountain Sculpin across four populations in Canada that vary in stream hydrology and biogeography. Specifically, I explored the following questions: 1) do Rocky Mountain Sculpin exhibit morphological adaptations to stream flow across their four prominent river systems in Alberta and British Columbia, Canada, and 2) is there a difference in meristic counts between Rocky Mountain Sculpin across populations. This information will build upon the known ecology of this species and help in identifying if populations from different watersheds and biogeography are in unique in terms of their meristics and morphometrics.

2.3 Methods

2.3.1 Study Area

Rocky Mountain Sculpin occur across a wide range of stream hydrology (Fig 2.1). Of the four study systems, the Flathead River has the highest average peak discharge of 125 m^3/s. The

`

St. Mary River has the highest average peak discharge for streams occupied on the eastern slopes of the Rocky Mountains with a mean of 61 m^3/s. The next highest discharge occurs in Lee Creek with average peak of 9 m^3/s. North Milk River has the lowest discharge, 8 m^3/s. Much of this flow is through augmentation from the St. Mary's River. Built in 1917, the St. Mary canal diverts water from the St. Mary River to the Milk River watershed during the growing season to supplement flows for irrigation of crops in the Milk River basin.

2.3.2 Sculpin Collection

A minimum of 40 Rocky Mountain Sculpin were collected from each of the four rivers. Sculpin were electro-shocked using a Smith-Root LR-24 backpack electrofisher. Sculpin were left overnight in a flow-through bin, which reduced their stress levels before transport. In total, 263 live fish were moved to holding tanks in the Aquatics Research Facility at the University of Alberta, a level-three bio-secure aquatic holding facility.

2.3.3 Sculpin Data Preparation

Rocky Mountain Sculpin collected from each river were euthanized and positioned on a wax board with splayed fins using dissection pins. Images were taken with a Nikon D3100 digital single-lens reflex camera equipped with a Nikon DX AF-S Nikkor 18-55 mm zoom lens set at 35 mm, 225 ISO, 5.3 f-stop, and 1/60 second shutter speed. Torsos were placed at a level plane (perpendicular) to the camera from head to caudal fin to prevent a warped, disproportionate representation of shape in the digital photos. Digital photos of the dorsal and lateral perspectives of the fish were taken in RAW (.NREF) form (A2.1). Each photograph included a reference scaling factor to standardize fish size across photographs. Meristic measurements, such as fin rays, fin spines, and head/body pores, were subsequently determined for each individual from the photographs.

`

Landmarks were marked using tpsDIG software (Rohlf 2005), and their location was translated into X and Y coordinates in a .TPS file. Landmark criteria, as described by Dryden and Mardia (2016), were reference points on the sculpin's body, that could be accurately found and marked across a large number of specimens. These points included fin insertion points, eye placement, and caudal peduncle locations.

2.3.4 Differences in Body Structure

Differences in body morphology were tested using geometric morphometric analysis with the *geomorph* in the R programming language (Adams et al. 2014, R Core Team 2016). A General Procrustes Analysis (GPA) was conducted on the coordinates. The GPA optimally superimposed landmarks by rotating, sizing and centralizing them without compromising the overall shape from the coordinates (Rohlf and Marcus 1993, Rohlf 1999, Slice 2001). This process produced useable X-Y Procrustes residual coordinates that could be used for a variety of multivariate analyses.The Euclidean distances between the Procrustes landmarks of each population were measured and compared. Euclidean Distance Matrix Analysis (EDMA) was used to identify variance across the mean landmark positions between groups (Lele 1993).

To determine if there were differences in overall shape across populations, a distance-based Procrustes Analysis of Variance (ANOVA) using 1000 permutations was used to test between GPA residuals of dorsal and lateral landmarks. The Procrustes ANOVA allowed for a simultaneous analysis of sum-of-squared Procrustes distances across all the coordinates instead of considering one coordinate at a time (Goodall 1991). This allowed for the determination of significant differences in overall shape across populations using permutation analyses to generate reliable P-values (Goodall 1991, Sherratt 2014). Pairwise comparisons were conducted using permutational t-tests, with 1000 resampling iterations to determine which populations were significantly different using pairwiseD.test in *geomorph*. This test is specialized for comparing geometric shape variation across groups by using the Procrustes residual values that represent shape variation, and calculating P-values based on the Euclidean distances between the mean of each population (Adams et al. 2014, Collyer et al. 2015). To visualize the results of the pairwise comparisons, coordinate data for each sculpin was converted into a single warp score using *candisc* (Friendly and Fox 2010). Warp scores were generated based on the degree and location of morphometric variance relative to the other fish in the study (Webster and Sheets 2010, Zelditch et al. 2012). Means of the values in each population were calculated as well as the Euclidean distance between those means (Sherratt 2014). After 1000 permutations, a Canonical Variate Analysis (CVA) was used to determine differences between groups that were calculated in the pairwise comparison. As a form of discriminatory analysis, CVA was used because it can measure differences in overall shape between the four predefined vectors (Rohlf 1999, Zelditch et al. 2012). The vectors identified landmarks that were major drivers in population variation. This generated the data necessary for visualization and the *vegan* package (Oksanen et al. 2013) was used to help customize the visualization.

2.3.5 Meristic Differences in Body Form

Fin ray and pore counts were compared across populations using a permutational ANOVA. Permutational ANOVA is useful when datasets do not adhere to the assumptions of a traditional ANOVA (ie. independence, normality, and homogeneity of variances), which is common in natural and biological realms (Anderson 2001). To test significance across groups, I used a pairwise Wilcoxon Rank Sum Test and applied a Holm p-value adjustment to correct for Type I errors when multiple comparisons are made (Aickin and Gensler 1996).

`

2.4 Results

2.4.1 Differences in Body Structure

There was strong divergence in body shape of Rocky Mountain Sculpin across its Canadian range. The Flathead River population was the most visibly divergent among the four populations of Rocky Mountain Sculpin. From the dorsal perspective, the caudal peduncle and snout positions relative to pectoral fin insertion points were the primary features that discerned the westslope population from the eastslope groups ($P<0.001$; Fig. 2.2,2.4, A2.2). Alternatively, westslope Rocky Mountain Sculpin had stunted heads, creating a more antero-posteriorly compressed head shape. Additionally, the length of the torso beyond the pectoral fin insertion points was significantly narrower and longer. From the lateral perspective, the westslope population was significantly different from the eastslope populations ($P<0.001$; Table 2.1). Eye placement was oriented closer to the insertion point of the dorsal spines, contributing to a more dorsally flattened form (Fig. 2.4, A2.3). The placement of the isthmus showed significant differentiation in the Flathead River population (Fig.2.3). It was located closer to the snout, likely as the result of a more antero-posteriorly compressed head, as seen in the dorsal view. The westslope population also had the longest, narrowest, and flattest body shape of the four groups.

Differences in body morphology were strongly divergent not just across the continental divide, but also among the eastslope populations. All of the three eastslope populations showed a significant difference in shape ($P <0.001$; Table 2.1). The differences in the North Milk River population were pronounced in the pectoral fin insertion point locations (Fig. 2.2). The width between the anterior insertion points in the dorsal view showed that the North Milk River population had the widest overall shape (Fig. 2.4). The close proximity of the posterior and anterior pectoral fin insertion points led to the North Milk River population to have the smallest fin base (Fig. 2.3). Lee Creek sculpin had lower and posteriorly placed pelvic fin insertion points, leading to the most dorso-ventrally deep shape of the four populations (Fig. 2.4). St. Mary River sculpin eye placement was closest to the snout and the dorsal spine insertion point is almost level with the eyes, contributing to the most dorso-ventrally flattened eastslope population (Fig. 2.4).

2.4.2 Meristic Differences in Body Form

There were significant meristic differences in body form of Rocky Mountain Sculpin across its Canadian range. The average pore counts and fin ray/spine counts across populations were significantly different (permANOVA, $p<0.001$). Pore counts varied across populations by no more than five pores and fin ray/spine counts varied only by one in populations that were significantly different (Table 2.2).

The westslope population exhibited several significant meristic differences from the eastslope populations. They had significantly more infraorbital pores ($P<0.001$) and forehead pores ($P<0.001$), and fewer mandibular pores ($P<0.001$; Table 2.2, 2.3). Furthermore, the westslope population had more pectoral ($P<0.001$) and caudal fin rays ($P<0.001$) than the Lee Creek and North Milk River population ($P=0.03$; Table 2.2, 2.4).

There were some meristic variations among the eastslope populations. Lee Creek and North Milk River populations differed in three of the pore counts, where the Lee Creek population had significantly more mandibular pores ($P=0.005$), but fewer forehead ($P<0.001$) and lateral line pores ($P=0.01$; Table 2.2,2.3). Similar to Lee Creek, the St. Mary River population had a higher number of mandibular pores ($P=0.005$) than the North Milk River population, as well as fewer forehead ($P<0.001$) and lateral line pores ($P<0.001$). There were no differences in fin ray/spine counts across east slope populations. There were no meristic differences between the Lee Creek and St. Mary populations.

2.5 Discussion

Many species exhibit morphological divergence in relation to environmental gradients (Endler 1986, Kawecki and Ebert 2004). Rocky Mountain Sculpin exhibited strong morphological and meristic body differences across broad hydrologic gradients. Body morphology differed across both the lateral and dorsal perspectives. Specifically, we found higher flow rates were correlated with higher adaptive morphological divergence. For example, the westslope population exhibited the most divergence in meristics from the eastslope populations. Westslope populations had more caudal fin rays, which help provide thrust, and pectoral fin rays to increase friction against substrate in high flow environments (Webb 1984, Taft et al. 2008, Kane and Higham 2012). Further, westslope Rocky Mountain Sculpin had fewer mandibular pores and more forehead pores than the eastslope populations. In lotic systems, sculpin use their head pores to detect suspended prey and mandibular pores to find buried prey (Hoekstra and Janssen 1985). The increase in forehead pores helps to detect drifting prey in fast currents. The passive reduction in mandibular pores is an example of a feature that cannot be justified by flow regime, and is possibly the result of divergence based on prolonged biogeographic isolation from other populations.

Biogeographic isolation can play an important role in determining morphological divergence. The continental divide has undoubtedly influenced body morphology between westslope and eastslope Rocky Mountain Sculpin populations given the populations have likely been isolated ~10,000 years (Nelson and Paetz 1992). The westslope populations exhibited a

`

more compressed and elongated torso than the eastslope populations, likely due to the interplay between prolonged biogeographic isolation and different flow regimes (Langerhans et al. 2003). If flow regime was the primary source of variation, there would have been more similar morphologies between the St. Mary and Flathead River populations, and almost identical morphologies between the Lee Creek and North Milk River populations. Instead, the results revealed closer morphometric and meristic values between Lee Creek and St. Mary River populations. They are the closest geographically, as Lee Creek is a tributary of the St. Mary River. This theory is also validated by the large degree of phenotypic variation between the westslope population and the eastslope populations. Gene flow is a major influence on the success of local adaptation (Kawecki and Ebert 2004), therefore the disconnect from the eastslope populations undoubtedly contributes to the observed morphological divergence.

Whether local adaptation is genetically embedded is a prominent debate among scientific literature. The general consensus among evolutionary researchers is that phenotypic variation is required to optimize fitness (Taylor and McPhail 1985, McGuigan et al. 2003). However, the degree to which a phenotype becomes fixed throughout a fish’s lifespan is important in understanding adaptability to stochastic environmental events (Fraser and Bernatchez 2001). Since phenotypic diversity parallels genetic diversity in Rocky Mountain Sculpin (Ruppert et al. 2017), there is the possibility that persistent selection of a specific phenotype under selection from stream flow can become fixed, making it difficult to adapt to rapidly changing habitat conditions (Stearns and Kawecki 1994). Management efforts should be directed toward preserving genetic diversity at the population level of the species (Riffel and Schreiber 1998, Ruppert et al. 2017), with future research aimed at understanding how genotypic and phenotypic divergence change in relation to stream hydrology.

Understanding how biogeography and stream hydrology influences body morphology is important to improve species conservation. Rocky Mountain Sculpin are a threatened species in Canada and could be further imperiled if their dominant phenotypes are incapable of adapting to altered river conditions such as flood events and drought conditions (Lytle and Poff 2004). Their limited dispersal can lead to specialized morphologies and extreme events could eliminate a population. Therefore, preserving phenotypically and genetically different Rocky Mountain Sculpin populations should be considered a management goal for the preservation of the species. Almost all fisheries management programs are developed around preserving genetic variation, thereby protecting a species' ability to overcome unpredicted environmental circumstances (Fraser and Bernatchez 2001). Based on the results in this study, phenotypic variation is a valid indicator of genotype (see Ruppert et al. (2017)), and should be considered when managing this sedentary, benthic species.

2.6 Conclusion

Biogeography and flow regime appear to be driving morphological divergence between Rocky Mountain Sculpin populations. High-flow lotic systems have Rocky Mountain Sculpin populations that minimize body depth, but flow itself cannot account for all observed trends. Many of the phenotypic variations increase with biogeographical isolation between populations. Biogeographical isolation is likely the basis of morphological variation in Rocky Mountain Sculpin. While stream hydrology maybe driving local adaptation, the degree to which it is doing so remains unclear. Additional unmeasured environmental factors could have a significant or equal influence on phenotype.

`

Results from this study suggest that small physical differences within the range of a fish species impact the energetic ability for the Rocky Mountain Sculpin to exist in a wide range of lotic environments. To further understand population level differentiation of Rocky Mountain Sculpin, tolerance levels of human impacts such as increased turbidity in the systems must be determined. By understanding how Rocky Mountain Sculpin adapt to various environmental conditions, we can better predict its resiliency to both natural and anthropogenic alterations.

2.7 Acknowledgements

Funding for this project was provided by Fisheries and Oceans Canada, Species at Risk Program (SARCEP) (DW, MP), and NSERC Discovery Grant (MP). Thank you to Marie Veillard, Denyse Dawe, Christopher Smith, Elashia Young, Caitlin Good, Kenton Neufeld, and Bryan Maitland for assisting with field collection and laboratory work. The care and assistance importing fish to the University of Alberta Aquatics Facility was provided by Toni Bayans and Simmone Kerswell. Additional thanks to Dr. Jonathan Ruppert for his helpful advice on data analysis and Hedin Nelson-Chorney for his critiques on early manuscript drafts. The procedures for this project was conducted under an approved Canadian Council of Animal Care Approval (AUP 00000759), Alberta Fish Research Licences 14-2415AFR and 16-0101FR, Alberta Fish Import Licence FIL2015-0074, Federal Introductions and Transfers Licence VI16-236322, and British Columbia Fish Collection Permit CB15-171090.

2.8 Literature Cited

Adams, D., M. Collyer, and E. Sherratt. 2014. geomorph: Software for geometric morphometric analyses. R package version **2**.

Aickin, M., and H. Gensler. 1996. Adjusting for multiple testing when reporting research results: the Bonferroni vs Holm methods. American journal of public health **86**:726-728.

Anderson, M. J. 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian Journal of Fisheries and Aquatic Sciences **58**:626-639.

Brinsmead, J., and M. Fox. 2002. Morphological variation between lake-and stream-dwelling rock bass and pumpkinseed populations. Journal of Fish Biology **61**:1619-1638.

Collin, H., and L. Fumagalli. 2011. Evidence for morphological and adaptive genetic divergence between lake and stream habitats in European minnows (*Phoxinus phoxinus*, Cyprinidae). Molecular Ecology **20**:4490-4502.

Collyer, M., D. Sekora, and D. Adams. 2015. A method for analysis of phenotypic change for phenotypes described by high-dimensional data. Heredity **115**:357-365.

Dryden, I. L., and K. V. Mardia. 2016. Statistical Shape Analysis: With Applications in R. John Wiley & Sons.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, and M. L. Stiassny. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological reviews **81**:163-182.

Endler, J. A. 1986. Natural selection in the wild. Princeton University Press.

Facey, D. E., and G. D. Grossman. 1990. The metabolic cost of maintaining position for four North American stream fishes: effects of season and velocity. Physiological Zoology **63**:757-776.

Fraser, D. J., and L. Bernatchez. 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecular Ecology **10**:2741-2752.

Friendly, M., and J. Fox. 2010. Candisc: R package for canonical discriminant analysis. Accessed at: http://cran. r-project. org/web/packages/candisc.

Goodall, C. 1991. Procrustes methods in the statistical analysis of shape. Journal of the Royal Statistical Society. Series B (Methodological):285-339.

Hoekstra, D., and J. Janssen. 1985. Non-visual feeding behavior of the mottled sculpin, *Cottus bairdi*, in Lake Michigan. Environmental Biology of Fishes **12**:111-117.

Kane, E. A., and T. E. Higham. 2012. Life in the flow lane: differences in pectoral fin morphology suggest transitions in station-holding demand across species of marine sculpin. Zoology **115**:223-232.

Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. Ecology letters **7**:1225-1241.

Koehl, M. 1984. How do benthic organisms withstand moving water? American Zoologist **24**:57-70.

Koumoundouros, G., C. Ashton, D. Sfakianakis, P. Divanach, M. Kentouri, N. Anthwal, and N. Stickland. 2009. Thermally induced phenotypic plasticity of swimming performance in European sea bass *Dicentrarchus labrax* juveniles. Journal of Fish Biology **74**:1309-1322.

Langerhans, R. B. 2008. Predictability of phenotypic differentiation across flow regimes in fishes. Integrative and Comparative Biology **48**:750-768.

Langerhans, R. B., C. A. Layman, A. K. Langerhans, and T. J. Dewitt. 2003. Habitat-associated morphological divergence in two Neotropical fish species. Biological Journal of the Linnean Society **80**:689-698.

Lele, S. 1993. Euclidean distance matrix analysis (EDMA): estimation of mean form and mean form difference. Mathematical Geology **25**:573-602.

Lytle, D. A., and N. L. Poff. 2004. Adaptation to natural flow regimes. Trends in ecology & evolution **19**:94-100.

McGuigan, K., C. E. Franklin, C. Moritz, M. W. Blows, and P. Wainwright. 2003. Adaptation of rainbow fish to lake and stream habitats. Evolution **57**:104-118.

McLaughlin, R. L., and J. W. Grant. 1994. Morphological and behavioural differences among recently-emerged brook charr, *Salvelinus fontinalis*, foraging in slow-vs. fast-running water. Environmental Biology of Fishes **39**:289-300.

Natsumeda, T., T. Tsuruta, H. Takeshima, S. Awata, and K. i. Iguchi. 2014. Variation in morphological characteristics of Japanese fluvial sculpin related to different environmental conditions in a single river system in eastern Japan. Ecology of Freshwater Fish **23**:114-120.

Nelson, J. S., and M. J. Paetz. 1992. The fishes of Alberta. University of Alberta.

Oksanen, J., F. Blanchet, R. Kindt, P. Legendre, P. Minchin, R. O'Hara, G. Simpson, P. Solymos, M. Henry, and H. Stevens. 2013. Vegan: Community Ecology Package. 2013. R-package version 2.0-10. URL http//:CRAN.R-project. org/package= vegan.

Pakkasmaa, S., and J. Piironen. 2000. Water velocity shapes juvenile salmonids. Evolutionary Ecology **14**:721-730.

Pringle, C. M., M. C. Freeman, and B. J. Freeman. 2000. Regional Effects of Hydrologic Alterations on Riverine Macrobiota in the New World: Tropical–Temperate Comparisons. BioScience **50**:807-823.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2015. .

`

Riffel, M., and A. Schreiber. 1998. Morphometric differentiation in populations of the Central European sculpin *Cottus gobio L.*, a fish with deeply divergent genetic lineages. Canadian Journal of Zoology **76**:876-885.

RL&L Environmental Services Ltd. 2002. Fish species at risk in the Milk and St. Mary drainages. Edmonton, AB.

Rohlf, F. J. 1999. Shape statistics: Procrustes superimpositions and tangent spaces. Journal of Classification **16**:197-223.

Rohlf, F. J. 2005. tpsDig, digitize landmarks and outlines, version 2.05. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F. J., and L. F. Marcus. 1993. A revolution inmorphometrics. Trends in ecology & evolution **8**:129-132.

Ruppert, J. L. W., P. M. A. James, E. B. Taylor, T. Rudolfsen, M. Veillard, C. S. Davis, D. Watkinson, and M. S. Poesch. 2017. Riverscape genetic structure of a threatened and dispersal limited freshwater species, the Rocky Mountain Sculpin (*Cottus* sp.). Conservation Genetics:1-13.

Sagnes, P., and B. Statzner. 2009. Hydrodynamic abilities of riverine fish: a functional link between morphology and velocity use. Aquatic Living Resources **22**:79-91.

Sherratt, E. 2014. Quick guide to Geomorph v. 2.0.

Slice, D. E. 2001. Landmark coordinates aligned by Procrustes analysis do not lie in Kendall's shape space. Systematic biology **50**:141-149.

Stearns, S. C., and T. J. Kawecki. 1994. Fitness sensitivity and the canalization of life-history traits. Evolution:1438-1450.

Taft, N., G. Lauder, and P. Madden. 2008. Functional regionalization of the pectoral fin of the benthic longhorn sculpin during station holding and swimming. Journal of Zoology **276**:159-167.

Taylor, E. B., and J. McPhail. 1985. Variation in body morphology among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. Canadian Journal of Fisheries and Aquatic Sciences **42**:2020-2028.

Water Survey of Canada. 2016. Wateroffice: Historical Hydrometric Data. Environment Canada, Government of Canada.

Webb, P. W. 1984. Form and function in fish swimming. Scientific American.

Webb, P. W. 1990. How does benthic living affect body volume, tissue composition, and density of fishes? Canadian Journal of Zoology **68**:1250-1255.

`

Webster, M., and H. D. Sheets. 2010. A practical introduction to landmark-based geometric morphometrics. Quantitative Methods in Paleobiology **16**:168-188.

Whiteley, A. R., S. M. Gende, A. J. Gharrett, and D. A. Tallmon. 2009. Background matching and color-change plasticity in colonizing freshwater sculpin populations following rapid deglaciation. Evolution **63**:1519-1529.

Zelditch, M. L., D. L. Swiderski, and H. D. Sheets. 2012. Geometric morphometrics for biologists: a primer. Academic Press.

`

2.9 Tables

Table 2.5. Pairwise differences in morphometric landmarks of Rocky Mountain Sculpin (*Cottus* sp.) across populations including: the Flathead River, Lee Creek, North Milk River, and St. Mary River. Shown are permutated t-tests using Euclidean distances between the means of dorsal (top corner) and lateral (bottom corner) landmarks across each river. P-values are based on 1,000 permutations and are adjusted using a Holm correction. Asterisks indicate significant differences.

	Flathead	**Lee Creek**	**North Milk**	**St. Mary**
Flathead	-	0.001*	0.001*	0.001*
Lee Creek	0.001*	-	0.001*	0.13
North Milk	0.001*	0.001*	-	0.001*
St. Mary	0.001*	0.001*	0.001*	-

Table 2.6. Summary of meristic fin and pore counts of Rocky Mountain Sculpin (*Cottus* sp.) across its Canadian range, including: the Flathead River, Lee Creek North Milk River, and St. Mary River. Shown are means, ranges, and standard deviations.

	River Populations											
	Flathead			**Lee Creek**			**North Milk**			**St. Mary**		
Character	*Mean*	*Range*	*SD*	*Mean*	*Range*	*SD*	*Mean*	*Range*	*SD*	*Mean*	*Range*	*SD*
Pores												
Infraorbital	10	6-10	±1.5	10	8-10	±0.3	10	8-14	±1.1	10	6-10	±0.5
Preoperculomandibular	14	10-16	±1.3	14	11-18	±0.9	14	12-18	±1.1	14	12-16	±0.6
Mandibular	13	10-16	±1.4	17	12-18	±1.5	15	7-20	±2.9	17	10-18	±1.6
Posterior Orbital	17	12-22	±2.1	16	11-18	±1.7	17	11-22	±2.4	17	10-22	±2.6
Forehead	2	2	±0	1	1-3	±0.4	2	1-2	±0.5	1	1-2	±0.4
Lateral line	24	19-32	±2.7	24	15-30	±3.1	26	11-34	±4.2	23	14-36	±3.0
Fin Ray/Spine Counts												
Dorsal Spines	8	6-9	±0.7	8	7-9	±0.6	8	7-9	±0.6	8	7-8	±0.5
Dorsal Rays	17	13-19	±1.2	17	16-19	±0.9	17	15-20	±0.8	17	13-19	±1.3
Anal Rays	12	11-14	±0.8	12	11-13	±0.7	12	11-15	±0.7	12	10-13	±0.8
Pectoral Rays	13	11-14	±0.6	12	10-13	±1.1	12	9-13	±1.0	12	9-14	±1.0
Pelvic Rays	4	4	±0	4	4	±0	4	4	±0	4	4	±0
Caudal Rays	13	12-14	±0.5	12	10-13	±0.8	12	9-13	±0.9	12	11-15	±1.0
Opercular Spines	1	1	±0	1	1	±0	1	1	±0	1	1	±0
N	41			74			106			118		

`

Table 2.7. Pairwise differences in pore counts of Rocky Mountain Sculpin (*Cottus* sp.) across Canada, including: the Flathead River, Lee Creek, North Milk River, and St. Mary River. Pairwise Wilcoxon Rank Sum Test P-values are displayed with Holm corrected significance levels. Asterisks indicate significant results.

	Infraorbital Pore	**Mandibular Pore**	**Forehead Pore**	**Lateral Line Pore**
Flathead : Lee Creek	0.91	<0.001*	<0.001*	0.67
Flathead : North Milk	1.00	<0.001*	<0.001*	0.02
Flathead : St. Mary	0.81	<0.001*	<0.001*	0.06
Lee Creek : North Milk	0.22	0.002*	<0.001*	0.01
Lee Creek : St. Mary	1.00	0.48	1.00	0.21
North Milk : St. Mary	0.08	0.002*	<0.001*	<0.001*

Table 2.8. Pairwise differences in fin ray and spine counts of Rocky Mountain Sculpin (*Cottus* sp.) across Canada, including: the Flathead River, Lee Creek, North Milk River, and St. Mary River. Pairwise Wilcoxon Rank Sum Test P-values are displayed with Holm corrected significance levels. Asterisks indicate significant results.

	Dorsal Fin Spines	**Dorsal Fin Rays**	**Anal Fin Rays**	**Pectoral Fin Rays**	**Caudal Fin Rays**
Flathead : Lee Creek	0.64	0.99	1.00	<0.001*	<0.001**
Flathead : North Milk	0.07	1.00	1.00	<0.001*	<0.001**
Flathead : St. Mary	0.05	1.00	1.00	<0.001*	0.02
Lee Creek : North Milk	0.73	0.99	1.00	1.00	0.81
Lee Creek : St. Mary	0.73	1.00	1.00	1.00	0.81
North Milk : St. Mary	0.73	1.00	1.00	0.71	0.81

`

2.10 Figure Legends

Figure 2.1. Map of study locations (top) and associated hydrographs (bottom). Populations of Rocky Mountain Sculpin were collected from the Flathead River (blue), Lee Creek (orange), St. Mary River (red), and the North Milk River (green). Hydrographs were developed from 84 to 104 years of data collected by four representative gauging stations (Water Survey of Canada 2016).

Figure 2.2. Differences in dorsal Procrustes scores of Rocky Mountain Sculpin (*Cottus* sp.) grouped by river system. Using Canonical Variate analysis, the distance between centroids (crosshairs) of populations was maximized. Populations included the Flathead River (blue), Lee Creek (orange), St. Mary River (red), and North Milk River (green).

Figure 2.3. Differences in lateral Procrustes scores of Rocky Mountain Sculpin (*Cottus* sp.) grouped by river system. Using Canonical Variate analysis, the distance between centroids (crosshairs) of populations was maximized. Populations included the Flathead River (blue), Lee Creek (orange), St. Mary River (red), and North Milk River (green).

Figure 2.4. Dorsal (top) and lateral (bottom) landmarks of Rocky Mountain Sculpin (*Cottus* sp.) across its Canadian range, including: Flathead River (blue), Lee Creek (orange), St. Mary River (red), and North Milk River (green). Dorsal landmark placements were amplified 4.5 times to visually show the fine-scale differences across populations. Lateral landmark placements had to be amplified by only 3 times to visually show differences. The X and Y axes represent the vector scores once standardized using General Procrustes Analysis.

`

2.11 Figures

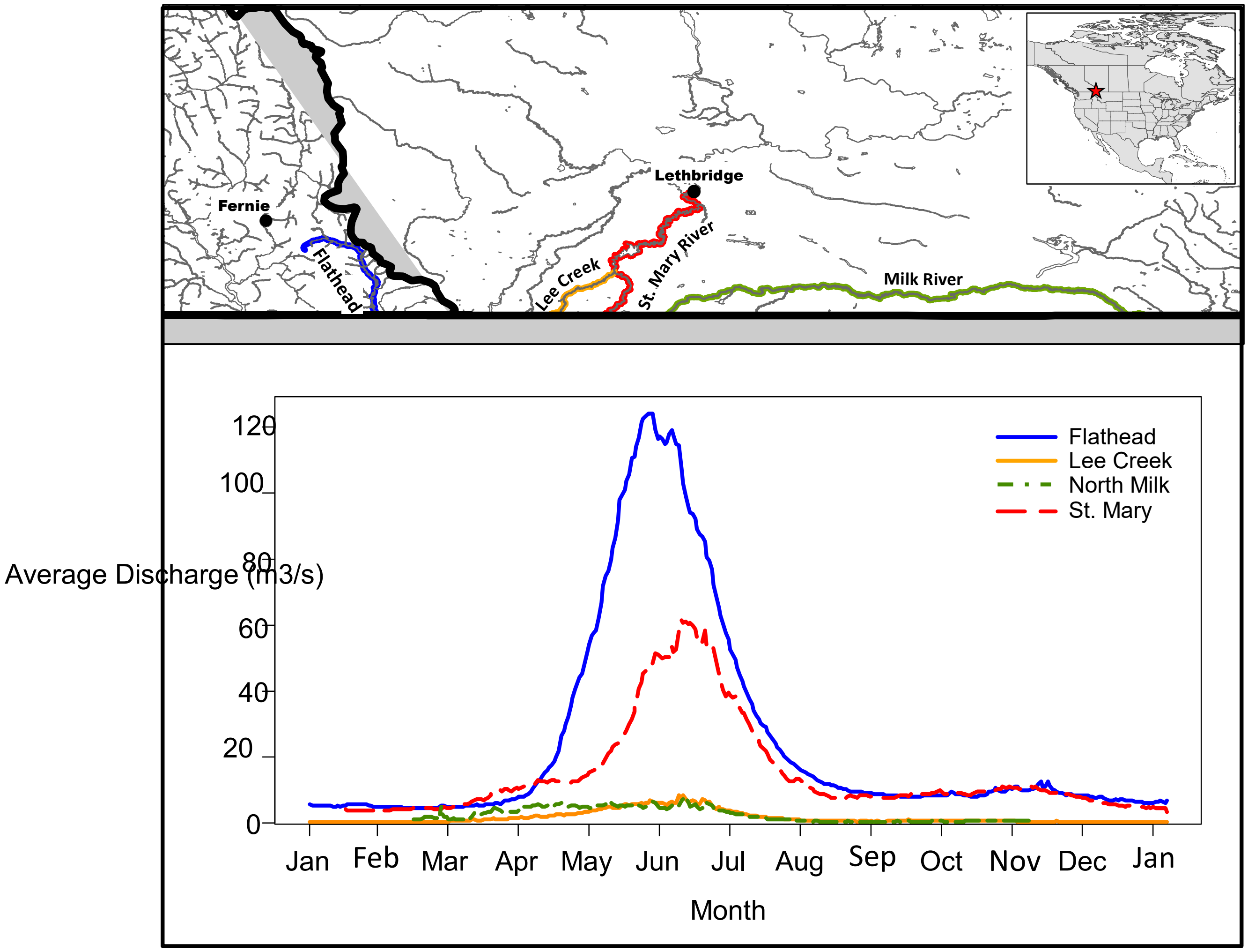
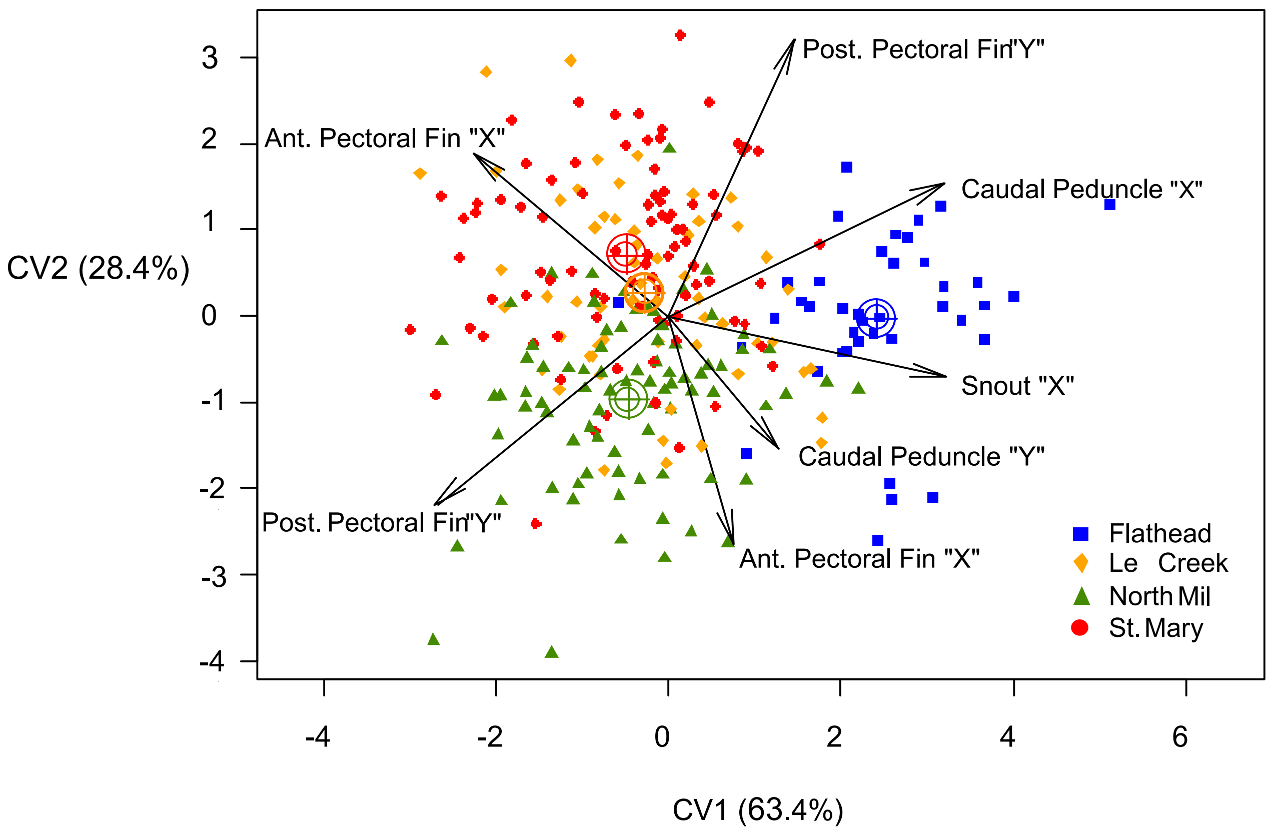


Figure 2.1

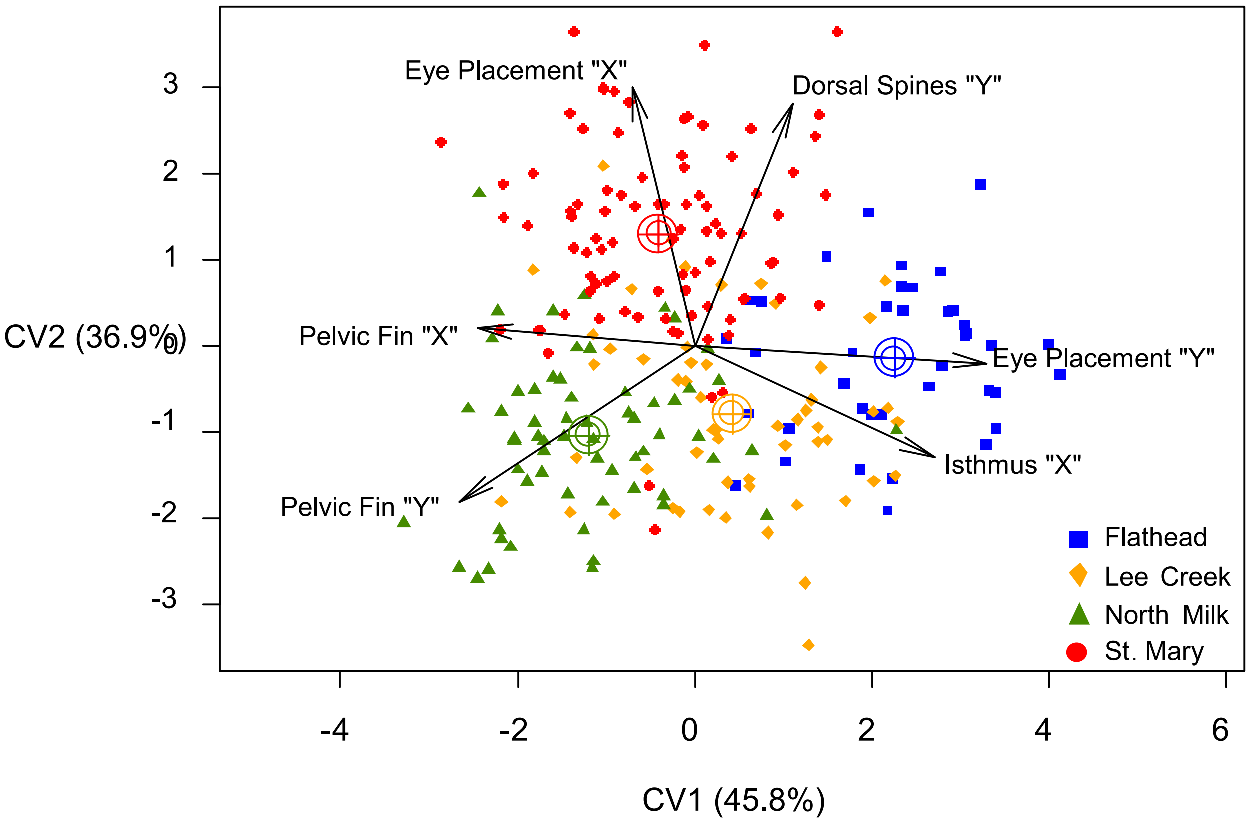
`



Post. Pectoral Fin"Y"
Ant. Pectoral Fin "X"
Caudal Peduncle "X"
Snout "X"
Caudal Peduncle "Y"
Post. Pectoral Fin"Y"
Ant. Pectoral Fin "X"
■ Flathead
◆ Le Creek
▲ North Mil
● St. Mary
CV2 (28.4%)
3
2
1
0
-1
-2
-3
-4
-4
-2
0
2
4
6
CV1 (63.4%)

Figure 2.2

`



Eye Placement "X"
Dorsal Spines "Y"
Pelvic Fin "X"
Eye Placement "Y"
Isthmus "X"
Pelvic Fin "Y"
CV2 (36.9%)
3
2
1
0
-1
-2
-3
-4
-2
0
2
4
6
CV1 (45.8%)
■ Flathead
◆ Lee Creek
▲ North Milk
● St. Mary

Figure 2.3

`

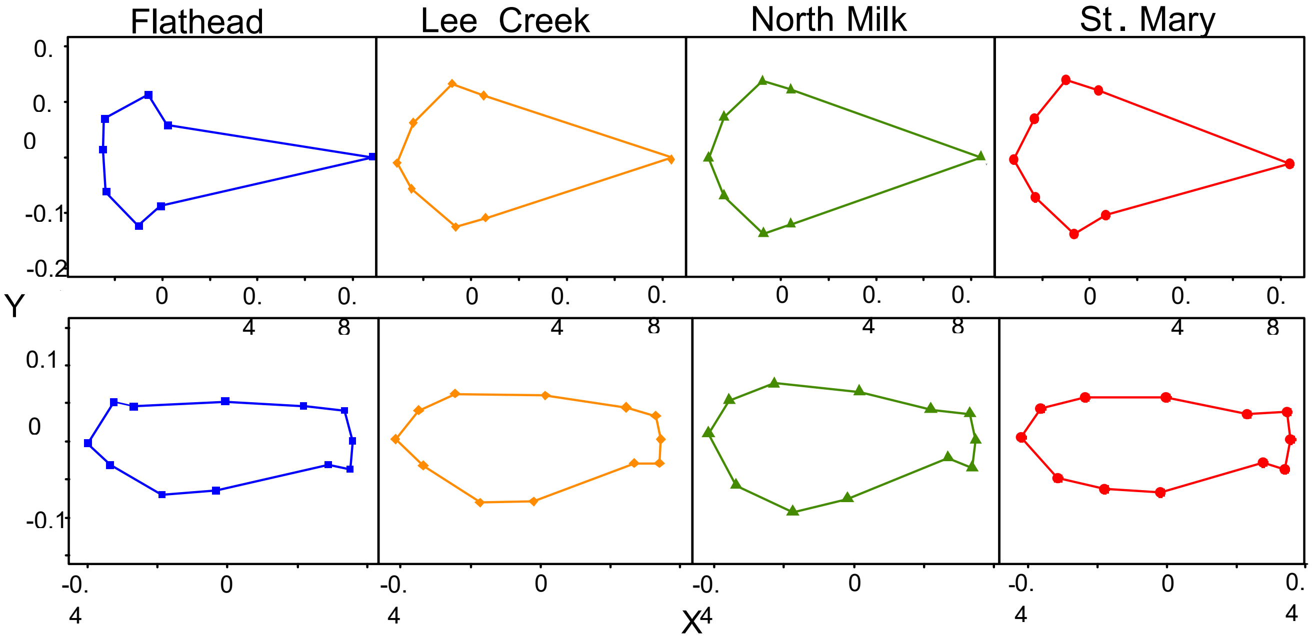

Flathead
Lee Creek
North Milk
St. Mary
Y
X
0.
0.
0
-0.1
-0.2
0
0.
4
0.
8
0.1
0
-0.1
-0.
4
0
0.
4

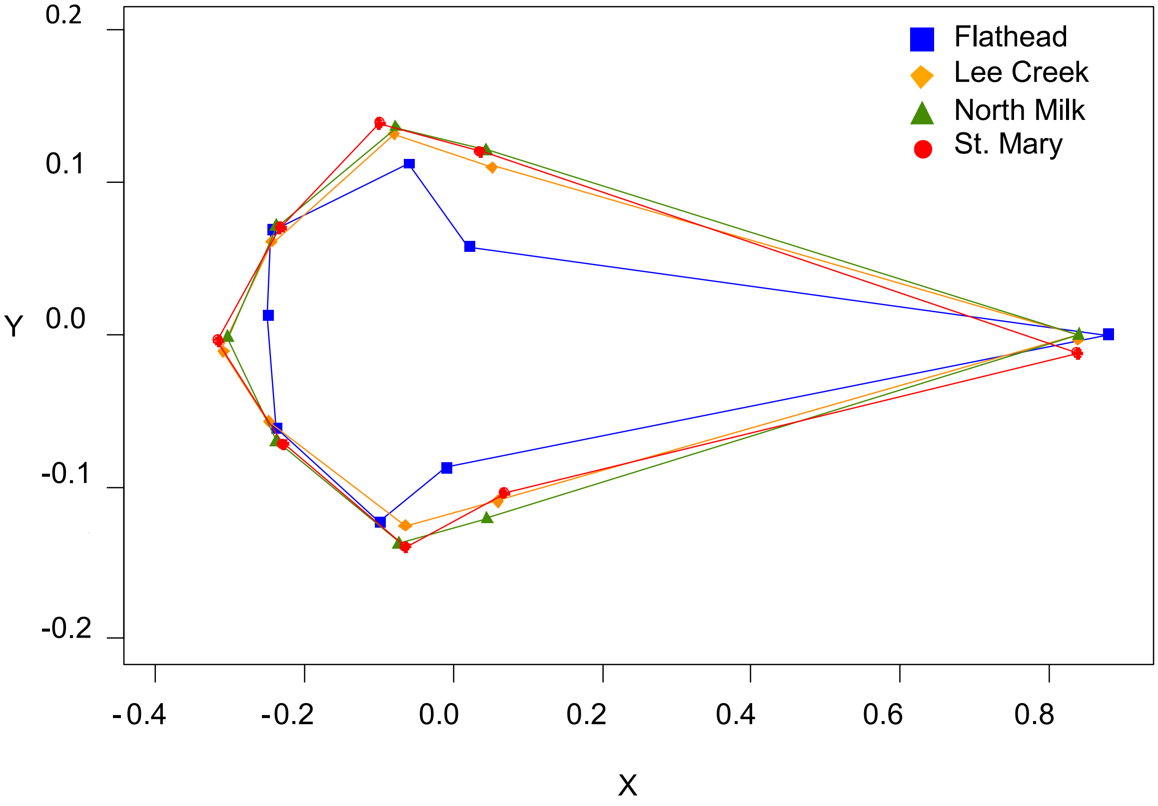
Figure 2.4

`

2.12 Appendix

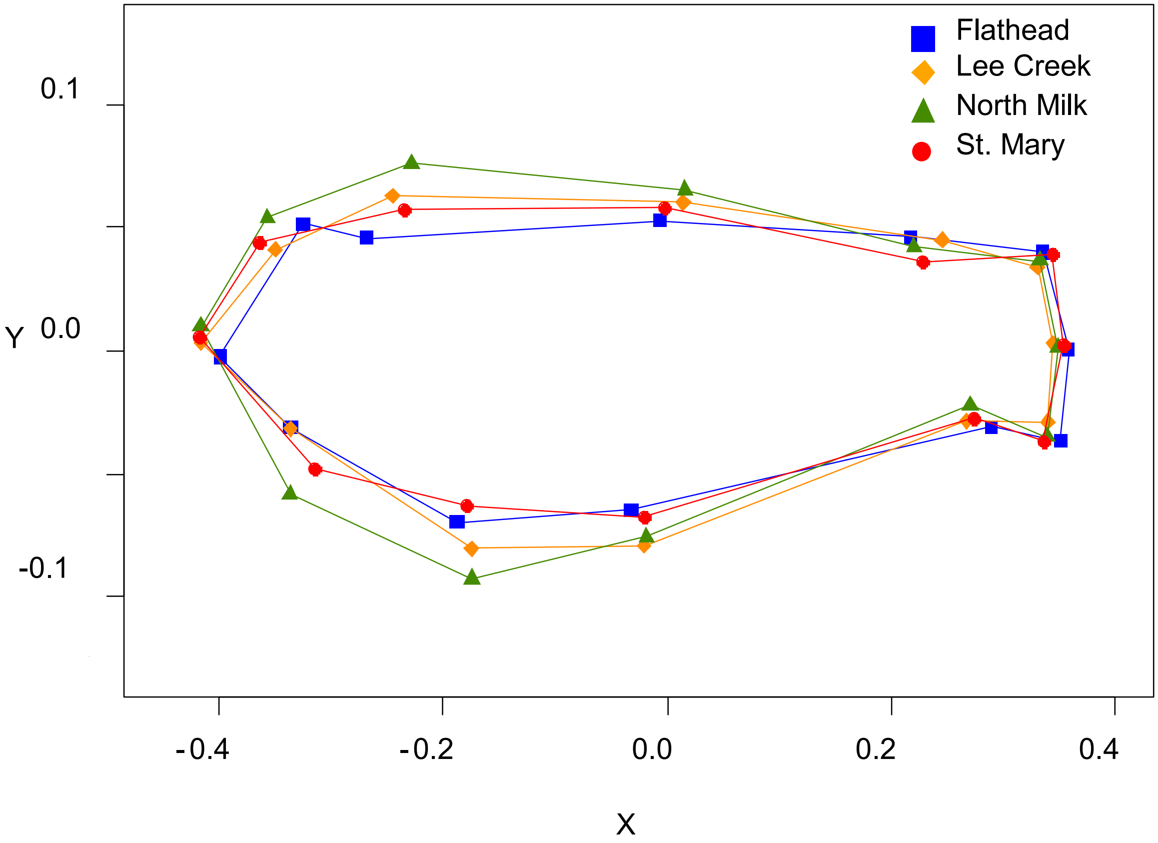
A2.1. Landmarks used in geometric morphometric comparisons across Rocky Mountain Sculpin river populations. Figure **(A)** shows landmarks used in the dorsal perspective, and **(B)** shows the landmarks used in the lateral perspective.

`



A2.2. Dorsal outline of the mean shape of each river population. Dorsal landmark placements were amplified 4.5 times to make fine-scale differences visible across populations. Landmarks were superimposed to visually show the differences across populations. The X and Y axes represent the vector scores once standardized using General Procrustes Analysis.

`



Appendix A2.3. Lateral shape outline of each river population based on mean landmarks. Lateral landmark placements were amplified 3 times to help make fine-scale differences visible. The landmarks were superimposed to visually show the differences across populations. The X and Y axes represent the vector scores once standardized using General Procrustes Analysis.

`

Chapter 3: Hybrid zones of Rocky Mountain Sculpin (*Cottus* sp.), a Threatened species, and Slimy Sculpin (*Cottus cognatus*), exhibit habitat resource partitioning in hybrid zones

3.1 Executive Summary

Freshwater biodiversity is undergoing a dramatic decline globally. Hybridization between closely related fishes is a primary concern for both conservation and fisheries management. Increasing rates of habitat degradation and climate change has led to a decline in environmental heterogeneity causing many species assemblages to become genetically homogenized. This has led to accelerated population declines in areas where closely related species co-exist on the landscape. Here, I identified hybrid zones between the threatened Rocky Mountain Sculpin (*Cottus* sp.) and Slimy Sculpin (*Cottus cognatus*) in the Flathead drainage, British Columbia, Canada. Using 731 genetic samples and 10 polymorphic microsatellite loci, two hybrid zones were identified. A logistic mixed-effects model was used with backward selection to identify environmental variables that predicted pure populations and hybrid zones. Elevation, water conductivity, turbidity, and dissolved oxygen were all significantly important factors in predicting the presence of hybrid zones. Elevation and turbidity were co-linear with temperature suggesting potential cumulative impacts of climate change. This study emphasizes the need to further understand predictors of species hybridization on species of conservation concern to ensure appropriate management of freshwater biodiversity.

`

3.2 Introduction

Freshwater biodiversity is a major conservation concern and is declining rapidly at a global scale (Jenkins 2003, Dudgeon et al. 2006). The effects of climate change and land use are of particular concern and are expected to be the main drivers of aquatic biodiversity loss (Sala et al. 2000). Water discharge reductions and increased temperatures are predicted to leave many freshwater species without suitable habitat (Eaton and Scheller 1996, Postel and Richter 2012). Meanwhile, nutrient loading, siltation, and waterway alterations can have additive effects on the persistence of fish species (Jenkins 2003).

Anthropogenic influences on freshwater environments can influence the distribution and interspecific behavior of fish species, ultimately leading to the formation of hybrid zones. For example, the warming climate is forcing species adapted to warm climates into those that were historically much cooler (Parmesan 2006). Range shifts due to climate change can result in contact with species capable of hybridization (Garroway et al. 2010). Increasing rates of habitat degradation and climate change has led to a decline in environmental heterogeneity causing many species assemblages to become genetically homogenized (Seehausen et al. 2008). Additionally, in some instances this hybridization can result in introgression, or the backcrossing of parental species with hybrid individuals (Rhymer and Simberloff 1996). Regardless of whether introgression occurs, human activities have contributed to potential increases in hybrid zone formation.

Natural hybridization, and the subsequent formation of hybrid zones, is common in freshwater systems (Hubbs 1955, Aboim et al. 2010). Freshwater hybrid zones are typically narrow portions of aquatic habitat where closely related species coexist, leading to genetically mixed individuals (Barton and Hewitt 1985, Harrison 1993a). These areas are usually ecological

`

transition zones where hybrids are in direct competition with parental species (Short 1970, May et al. 1975, Moore 1977). One of the largest reasons for the presence of hybridization zones is glacier recession at the end of the Pleistocene epoch, as the changing climate formed newly available habitats allowing species ranges to occasionally converge (Hubbs 1955, Barton and Hewitt 1985). Specifically, Hybrid zones can develop from either primary or secondary contact (Curry 2015). Primary contact occurs when a species spreads to an adjacent environment and adapts to it, leading to speciation (Endler 1977, Harrison 1993b). The two species remain in contact at the ecotone and this interaction provides opportunities for the formation of a hybrid zone. While this is similar to the events in incomplete speciation, eventual speciation can theoretically occur through parapatry when a narrow hybrid zone and introgression zones are maintained through higher adaptive fitness of parental populations. Secondary hybrid zones are formed when species populations become geographically isolated, leading to eventual speciation, and are then reunited (i.e., they converge; (Woodruff 1979, Harrison 1993a)). Two prominent theories attempt to explain how hybrid zones can persist and remain stable. The first introduces the concept of a 'tension zone,' where the parental species have a competitive advantage over hybrids, thereby preventing the radiation of hybrid genotypes throughout the parental populations (Barton and Hewitt 1985). The second theory suggests that the hybrid zone is in fact an 'ecotonal zone,' where hybrids have a competitive advantage over parental populations and thus persist (Moore 1977). Regardless of the mechanism, both scenarios threaten the genetic diversity of species and ultimately their long-term persistence

Improvements in the ability to analyze genetic hybridization has led to a renewed understanding of hybrid zones and how speciation originates (Mallet 2005, 2007). Hybrid zones are thought to represent the middle stages of speciation and are consequently studied to

`

understand the process within a reasonable time frame (Barton and Hewitt 1985, Crespin et al. 1999, Sweigart 2009). In particular, there are many examples of sculpin species forming hybridization zones when their ranges overlap (Nolte et al. 2005a, Stemshorn et al. 2011). The Rocky Mountain Sculpin (*Cottus* sp.) is a small, benthic fish that in Canada exists in only four river systems in southern Alberta and British Columbia. Its small range and sedentary nature make it particularly susceptible to anthropogenic impacts (Ruppert et al. 2017) and it has been listed as Threatened in Alberta and Special Concern in British Columbia (COSEWIC 2005, 2010). Moreover, it is known to hybridize with Slimy Sculpin (*Cottus cognatus*) in parts of the Flathead River, BC where their ranges overlap (Fig.1; COSEWIC (2010)). Previous work by Hughes and Peden (1984) determined that the hybrid zones in Rocky Mountain Sculpin were a result of elevation, occurring at around 1372 m. However, apart from surface water velocity and temperature, other habitat variables were not considered. Slimy Sculpin are more geographically widespread than Rocky Mountain Sculpin and occupy much of Canada including the upstream portions of the Flathead Drainage (Scott and Crossman 1973, COSEWIC 2010). Incidences of genetic hybridization between the two species have occurred; however, a characterization of environmental drivers that contribute to the formation of these hybrid zones have not been studied in any great depth (COSEWIC 2010). Given the imperiled Rocky Mountain Sculpin's life history coupled with the knowledge that hybridization can lead to the extinction of a species (Rhymer and Simberloff 1996), the Rocky Mountain Sculpin is an ideal species to understand what influences hybrid zones.

Here, I attempt to: 1) characterize the presence of hybrids and the extent of hybrid zones between the Rocky Mountain Sculpin and Slimy Sculpin, and 2) identify environmental factors that are contributing to the existence of those hybrid zones. Characterizing the environmental

`

factors that govern the formation and maintenance of a hybrid zone can provide valuable insight into the future trajectory of hybrid zones. Habitat features are primary determinants of hybrid zones in freshwater systems (Crespin et al. 1999), therefore, I hypothesize that broad features that contribute to environmental gradients will influence the presence and nature of hybrid zones.

3.3 Methods

3.3.1 Study Area

The Flathead River originates in southeastern British Columbia and flows southward into Montana where the majority of the watershed is found. Its mountains and riverbeds, mainly consisting of limestone and argillite, were formed in the Precambrian era and were later shaped by glaciers in the Pleistocene epoch, prior to approximately 10,000 years ago (Ross 1959). The area is known for its biodiversity and large number of rare and endangered species but also for its coal and gold deposits resulting in an ongoing dispute over whether to develop or protect it (Hughes and Peden 1984). The Flathead River is an ideal study area as the system is relatively uninfluenced by anthropogenic impacts apart from logging (Jiggins and Mallet 2000). Generally, it is thought that Slimy Sculpin are found in upstream locations of the Flathead River and its tributaries and Rocky Mountain Sculpin dominate more downstream (Hughes and Peden 1984). The Flathead River drainage is the only known area in Canada where their ranges overlap (Hughes and Peden 1984) and is therefore an ideal study area to detect habitat selection and hybrid characteristics of hybrid zones among sedentary freshwater species.

3.3.2 Genetic Data Collection

I sampled 30 1 m^2 quadrats within 300 m reaches along the Flathead River and its tributaries, including Kishinena Creek, Couldrey Creek, Middlepass Creek, and Howell Creek (Fig. 3.1). Quadrats were randomized by depth (10 to 60 cm) and distance (1 to 20 m) and were sampled for 10 seconds using a Smith-Root LR-24 backpack electrofisher using dip nets. During the 10 second sampling period, bottom substrate was gently moved and shuffled to free sculpin that were lodged between rocks, vegetation, and bottom debris.

I collected 732 sculpin and measured them for total length (mm). Pelvic fin clips were preserved in 95% ethanol for genetic analysis. Alongside genetic analysis, visual field identification using head morphology and pore counts was conducted to gauge sampling efforts along each lotic system. Despite Rocky Mountain Sculpin and Slimy Sculpin being difficult to tell apart, Hughes and Peden (1984) found that the one of the ways to reliably discern Rocky Mountain Sculpin from Slimy Sculpin in the field is to count the median occipital pores (~87% accuracy). This method was used along with other characteristics such as the presence/absence of head papillae and head ratio (see Hughes and Peden (1984)). Substrate size based on the Wentworth Scale (Wentworth 1922) and water velocity (m/s) were recorded at each quadrat. At the end of each 300m reach, additional environmental and benthic data were collected, including conductivity, elevation, turbidity, temperature, and dissolved oxygen (Table 3.1).

3.3.3 Genotyping

Genotyping protocols followed companion studies, (see Ruppert et al. (2017) for more details). DNeasy 96 Blood and Tissue Kit (Qiagen) was used following the manufacturer's protocol to isolate total genomic DNA from fin clips preserved in 95% ethanol. A total of 15 polymorphic sculpin microsatellite primer sequences were taken from peer-reviewed literature (Cba from (Fiumera et al. 2002); Cgo from (Englbrecht et al. 1999); Cott from (Nolte et al. 2005b); Cco from (Fujishin et al. 2009)). Primer sets were labelled with 6FAM, TET or HEX fluorescent dyes, this allowed for genotyping on an Applied Biosystems genetic analyzer. Loci were initially tested using a subset of individuals (*n* = 8) from across the distribution of the species to ensure

`

that all markers produced viable results. For specific details on amplification, see Ruppert et al. (2017). Ultimately, four loci had to be removed from further analysis as one locus was monomorphic and three failed to amplify. Therefore, all 732 individuals were genotyped at eleven polymorphic microsatellite loci. PCR amplifications were performed in two multiplexed 10µL reactions (Mix 1 and Mix 2), which consisted of 1x Type-it Microsatellite PCR Master Mix (Qiagen), 0.5x Q-Solution (Qiagen), 1x primer mix, and 2.5µL of extracted gDNA. Conditions and methods for all individuals when cycling (except for specific annealing temperatures), loading, and sizing were the same as those used for testing.

3.3.4 Genetic diversity analysis

All loci and individuals were not used in the completed analysis. While 11 polymorphic microsatellite loci were genotyped, only 10 were suitable to be used in subsequent analysis. Standardized screening protocols were performed (James et al. 2015, Ruppert et al. 2017) and loci were omitted if (1) there were many non-typed individuals (>5), (2) a loci was fixed (mean dominant allele frequency; GenAIEx 6.5), and (3) loci had a mean estimated null allele frequency (>0.1; ML-NULL) (Kalinowski and Taper 2006, Peakall and Smouse 2012). Using these criteria, one loci was omitted (Cco02) as there were many non-typed individuals. Further, only 731 individuals were used for analysis, as one individual had many non-typed loci (>5).

Deviations from Hardy-Weinberg Equilibrium (HWE) were tested for using the *hw.test* function in the *pegas* package in R software (Paradis 2010) and linkage disequilibrium between all loci pairs using FSTAT v2.9.3.2 (Goudet 1995). Finally, diversity measures were then calculated using the Excel GenAIEx 6.5 (Peakall and Smouse 2012).

`

3.3.5 Genetic differentiation and hybrids

Species classes were identified using STRUCTURE 2.3.4 (Pritchard et al. 2000, Falush et al. 2003), following the protocol outlined in Cullingham et al. (2012). Briefly, allele frequencies and admixture in the model were assumed to be correlated, as genetic samples came from spatially connected systems (Falush et al. 2003). It was also assumed that there were no known priors, therefore values of *K* were assessed from 1-5 and 500,000 Monte Carlo Markov Chain (MCMC) generations, after a burn-in of 50,000 generations. Each *K* was replicated with MCMC sampling 10 times. Finally, the optimal value of *K* was assessed by reviewing both the mean ln probability of *K* and Evanno method (Evanno et al. 2005).

3.3.6 Predictive Mixed-Effects Model Selection for Hybrids

Findings suggested that the best value for *K* in the STRUCTURE model was two, using the mean ln probability and Evanno method (A3.1). A combination of logistic mixed-effects models and genetic data were summarized using CLUMPP v1.1.2 (Jakobsson and Rosenberg 2007). Ancestry values (q-values) from STRUCTURE were used where values ≥ 0.90 were classified as Rocky Mountain Sculpin, ≤ 0.10 indicated Slimy Sculpin, and proportions between >0.10 and <0.90 indicated hybrids (Fig. 3.2). Waterbody, site number, and date collected were designated as random effects, as differences in space and time across samples were expected. Co-variables used in the analysis included: Elevation (m), turbidity (NTU), sample depth (cm), flow velocity (m/s), dissolved oxygen (mg/L), conductivity (µS/cm) percentage of substrate within 1m^2 (small gravel, large gravel, cobble, boulder). Finally, 538 of the 731 samples were used, as there were incomplete or inconsistent data records for one or more of the ten fixed variables with the remaining 193 samples.

Prior to analysis, correlation analysis of the variables was conducted to identify co-linearity and reduce the possibility of Type II errors (Zuur et al. 2010). Temperature was found

`

to be highly correlated with elevation (r=0.6), and was therefore not included in the mixed effects model. The environmental variable data were standardized and centered into z-scores as they were in different units and on different scales. To select the best fitting model, backward selection was conducted with the *lme4* package in R (Bates et al. 2007). Model selection was conducted based on a corrected Akaike Information Criterion (AICc) values (Akaike 1974, Akaike 1998). The corrected version of AIC is for small sample sizes; however, it gradually meets with AIC values as sample size increases were run as a precaution against over-fitting a model (Burnham and Anderson 2004). The model with the lowest AICc value was selected, as it was the most likely model having lost the least amount of information from the eliminated variables (Burnham and Anderson 2004). Furthermore, the differences of other models from the lowest AICc value (Δi) and the weightings (w_i) were calculated for each model. The Δi represented the relative amount of information lost by using a model that is not the optimal model and these models are possibly as important as the optimal model (ie. when $\Delta i \leq 2$) (Burnham and Anderson 2004). The effects of important variables were then plotted in a predictive fashion using the *effects* package in R (Fox 2015).

3.3.7 Average Stream Temperatures

Temperature loggers were used to present reliable temperature trends in study systems outside of the mixed-effects model. HOBO TidbiT v2 Water Temperature Data Loggers were deployed at one to two locations on each reach. Loggers were cemented to submerged boulders, following the protocol laid out by Isaak et al. (2013). Water temperature was recorded every 30 min from the summer of 2015 to the summer of 2016. Following the genetic analysis, three temperature loggers were selected to compare average summer stream temperatures between pure sculpin populations and hybrid zones. The upstream Flathead River temperature logger represented the average temperature for hybrid zones. The temperature logger in the downstream Flathead River near the Montana border represented the pure Rocky Mountain Sculpin populations, and a Middlepass Creek logger was located in the only area where pure Slimy Sculpin were found. A repeated measures ANOVA and *post hoc* pairwise comparison using the Tukey HSD test with Holm P-value adjustment were conducted to investigate if the average summer temperatures of each location differed significantly.

3.4 Results

3.4.1 Genetic Diversity

731 individuals were genotyped across 10 microsatellite loci. Four loci for Slimy Sculpin and two for Rocky Mountain Sculpin were not in Hardy-Weinberg equilibrium (Table 2). There was no detection of any linkage disequilibrium across loci. Allelic diversity measures were calculated for Slimy Sculpin and Rocky Mountain Sculpin separately, theseare included in Table 3.2. Diversity was higher for Rocky Mountain Sculpin in both the number of alleles and heterozygosity (Table 3.2).

3.4.2 Identifying Hybrid Zones

Of the 731 sculpin involved in the study, 367 (50%) were Rocky Mountain Sculpin (Fig. 3.2) and 101 (14%) were hybrids between Rocky Mountain Sculpin and Slimy Sculpin. Hybrid zones were found in Kishinena Creek and the Flathead River (Fig. 3.1C). The Kishinena Creek hybrid zone was approximately 6 km long, while the Flathead River hybrid zone was approximately 24 km long (Fig. 3.1C). Howell Creek had pure populations of both species but showed no indication of hybridization (Fig. 3.1C). Middlepass Creek contained only Slimy Sculpin and it is possible the heavily braided confluence of the Middlepass Creek into the Flathead River prevented genetic mixing of the pure Rocky Mountain Sculpin population in the Flathead River (Fig. 3.1C). In both the Flathead River and Kishinena Creek, there were no general areas where only Slimy Sculpin exist (Fig. 3.1C). There is, however, evidence of pure Rocky Mountain Sculpin populations in the Flathead River. Both systems also had the highest sculpin densities with, on average, 14 and 7.5 individuals per transect in Kishinena Creek and the Flathead River, respectively.

3.4.3 Predictive Habitat Variables

The logistic mixed-effects model revealed that four habitat features determined the presence of hybrid zones and pure sculpin populations: elevation, conductivity, turbidity, and dissolved oxygen (Table 3.2). Another model including water depth was also a statistically valid possibility ($\Delta i < 2$). However, the model excluding depth had the largest w_i of 0.30, indicating this is the preferred model.

Predictive effect scatterplots showed that each habitat factor in the selected mixed-effects model could predict the relative location of hybrid zones in the Flathead River and Kishinena Creek (Fig. 3.2). All covariates in the selected model had a significant effect on hybrid zones (Table 3.4). Elevation was the best determinant of hybrid zones, with two clusters of hybrids that represent the two river systems. Kishinena Creek's hybrid zone was located between 1290 and 1330 m elevation. The Flathead River's hybrid zone was located around 1430 m and exceeded 1500 m. Slimy Sculpin appeared most limited by elevation, only existing in locations above 1290 m. Turbidity closely paralleled the cluster pattern observed in the elevation plot, where Kishinena Creek hybrids were found in an area of relatively low turbidity (0.05-0.09 NTU), whereas hybrids in the Flathead were in a wider range of more turbid conditions (≥ 0.35 NTU). Slimy Sculpin were more restricted by turbid conditions than Rocky Mountain Sculpin.

Conductivity was a strong determinant of pure populations but did not appear to be a large factor in the presence/absence of hybridization. The majority of Rocky Mountain Sculpin were located in the conductivity range of 100 to 140 µS/cm while Slimy Sculpin were typically found in the 140 to 190 µS/cm range. Hybrids were found within both of these ranges, not exceeding 180 µS/cm. Rocky Mountain Sculpin tend to be restricted to lower dissolved oxygen conditions (8.3 to 9.3 mg/L), whereas Slimy Sculpin were abundant in the complete range of dissolved oxygen measurements (8.3 to 9.7 mg/L).

3.5 Discussion

The results of this study suggest that climate change can cause an imperiled species' range to expand. These findings support previous studies that identify elevation as a major factor in the presence of hybrid zones (Hughes and Peden 1984). Further, it was found that the range of Rocky Mountain Sculpin has shifted in altitude by approximately 170 m in the last thirty years. This is likely the result of the collinear relationship between elevation and temperature (Lookingbill and Urban 2003), as Slimy Sculpin are generally less common in warmer water temperatures (Otto and Rice 1977, Kowalski et al. 1978). Our temperature trends support these findings and identify that Rocky Mountain Sculpin are more common in warmer temperatures which is also a characteristic of the hybrid zones (A3.2, A3.3). The Flathead River drainage has experienced an increase in average water temperature by about 5°C since the 1980's (Muhlfeld et al. 2014). The northward encroachment of this temperature gradient closely corresponds with the Rocky Mountain Sculpin's range expansion, and is likely influencing their ability to exist at higher elevations. Finally, the results of this study suggests that Rocky Mountain Sculpin exhibits an adaptive advantage over Slimy Sculpin given current water conditions in the Flathead River as the ecotonal range shifts because of increased temperatures.

`

Genetic analysis of Rocky Mountain Sculpin and Slimy Sculpin in the Flathead drainage reveals that they are exhibiting hybridization and possibly some degree of introgression. The Rocky Mountain Sculpin's range expansion has led to increased ranges of hybridization than previously recorded (Hughes and Peden 1984), as variety of hybrid genotypes with different q-values were found (Fig. 3.2). Whether this has the potential to dilute the Rocky Mountain Sculpin gene pool or result in species loss may be a concern (Muhlfeld et al. 2014). In order to determine this, we must first further investigate if introgression is occurring.

The hybrid zones in this study are bimodal in nature, where the hybrid population is small relative to parental populations. This may suggest that these hybrid zones are the result of passive cross-fertilization (Aboim et al. 2010) as they appear to be maintained through resource partitioning due to an ecotonal boundary, which delineates parental species from hybrids. This elevates the importance of key habitat features including elevation, turbidity, conductivity, and dissolved oxygen to determine the location and extent of the hybrid zones.

As more than one key habitat feature determining hybrid zones was identified, it can be argued that additive effects such as land-use changes may augment the effects of range shifts by climate change. While climate change has previously forced shifts in species ranges causing hybrid zones, turbidity and conductivity are often representative of siltation and environmental deposits. Results suggest that Rocky Mountain Sculpin appear to be more tolerant of increased siltation, thus anthropogenic siltation event may be beneficial to species range expansion. Both increases in temperature and siltation could be additive effects that influence the direction of gene flow, provided introgression is occurring (Barton 1979, Hewitt 2001, Buggs 2007). Therefore, this suggests that Rocky Mountain Sculpin may have an adaptive advantage as this study finds that all cumulative effects do not exceed their potential physiological tolerance level.

`

While the ultimate consequences of most hybrid zones remain unknown, a cautionary approach should be employed when considering species conservation. Should climate change and land use lead to the possibility of range expansion and increased hybridization, it is possible that a reduction in overall genetic purity of imperiled species may occur (Muhlfeld et al. 2009). Rare and threatened species are especially susceptible to extinction through introgression (Rhymer and Simberloff 1996), yet these findings suggest that range changes in response to climate change and anthropogenic factors can actually be beneficial to imperiled species when it is low water temperature s that are limiting. Unfortunately, range expansion in many freshwater systems have restrictions. It is not known, but certainly this same process maybe resulting in a loss of habitat for the species in downstream, lower elevation habitats if water temperatures are increasing and approaching levels the species cannot tolerate. While climate change and urbanization may improve an imperiled species' status, it can also eventually lead to their extirpation or extinction (Parmesan 2006). Average water temperatures and siltation are the primary determinants of hybrid zone maintenance and movement and in this case these factors act as determinants of biodiversity trajectories. With this knowledge, managers might better mitigate human activities that alter these freshwater conditions.

3.6 Conclusion

Climate change and siltation from human activities appear to be driving the formation and maintenance of hybrid zones between freshwater sculpin species in the upper Flathead River. Here water temperature was the most informative determinant of species range expansions hybrid zone movement. Further, land-use acts as an additive effect, augmenting the effects of climate change on hybrid zones. This study emphasizes the temporary benefits that climate

`

change may have on imperiled freshwater fishes. Ultimately, a better understanding of hybrid interactions between hybrids and parent populations will further our understanding of the potential for extinctions by introgression in hybridizing freshwater species.

3.7 Acknowledgements

Funding for this project was provided by Fisheries and Oceans Canada, Species at Risk Program (SARCEP) (DW, MP), and NSERC Discovery Grant (MP). Thank you to Christopher Smith, Elashia Young, Kenton Neufeld, Paul Fafard, Doug Leroux, Colin Kovachik, Kyle Hamilton, and Bryan Maitland for assisting with field collection. Field safety and support was provided by Dr. Garth Mowat and Joe Caravetta. Thank you to Dr. Corey Davis for processing the genetic samples, Dr. Eric Taylor for his expertise on genotyping the sculpin genetics, and Dr. Jonathan Ruppert for genetic data analysis. Additional thanks to Dr. Jonathan Ruppert and Marie Viellard for support with mixed-effects modelling procedures, and Hedin Nelson-Chorney critiques of manuscript drafts. The procedures for this project was conducted under an approved British Columbia Fish Collection Permits CB14-149123 and CB15-171090.

3.8 Literature Cited

Aboim, M., J. Mavárez, L. Bernatchez, and M. Coelho. 2010. Introgressive hybridization between two Iberian endemic cyprinid fish: a comparison between two independent hybrid zones. Journal of Evolutionary Biology **23**:817-828.

Akaike, H. 1974. A new look at the statistical model identification. IEEE transactions on automatic control **19**:716-723.

Akaike, H. 1998. Information theory and an extension of the maximum likelihood principle. Pages 199-213 Selected Papers of Hirotugu Akaike. Springer.

Barton, N. 1979. The dynamics of hybrid zones. Heredity **43**:341-359.

`

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Annual review of Ecology and Systematics **16**:113-148.

Bates, D., D. Sarkar, M. D. Bates, and L. Matrix. 2007. The lme4 package. R package version **2**:74.

Buggs, R. 2007. Empirical study of hybrid zone movement. Heredity **99**:301-312.

Burnham, K. P., and D. R. Anderson. 2004. Multimodel inference understanding AIC and BIC in model selection. Sociological methods & research **33**:261-304.

COSEWIC. 2005. COSEWIC assessment and status report on the "eastslope" sculpin (St. Mary and Milk River Population) *Cottus* sp. in Canada. Ottawa.

COSEWIC. 2010. COSEWIC assessment and status report on the Rocky Mountain Sculpin *Cottus* sp., westslope populations, in Canada. Ottawa.

Crespin, L., P. Berrebi, and J. Lebreton. 1999. Asymmetrical introgression in a freshwater fish hybrid zone as revealed by a morphological index of hybridization. Biological Journal of the Linnean Society **67**:57-72.

Cullingham, C. I., P. James, J. E. Cooke, and D. W. Coltman. 2012. Characterizing the physical and genetic structure of the lodgepole pine× jack pine hybrid zone: mosaic structure and differential introgression. Evolutionary applications **5**:879-891.

Curry, C. M. 2015. An integrated framework for hybrid zone models. Evolutionary Biology **42**:359-365.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, and M. L. Stiassny. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological reviews **81**:163-182.

Eaton, J. G., and R. M. Scheller. 1996. Effects of climate warming on fish thermal habitat in streams of the United States. Limnology and oceanography **41**:1109-1115.

Endler, J. A. 1977. Geographic variation, speciation, and clines. Princeton University Press.

Englbrecht, C. C., C. R. Largiader, B. Hanfling, and D. Tautz. 1999. Isolation and characterization of polymorphic microsatellite loci in the European bullhead *Cottus gobio* L-(Osteichthyes) and their applicability to related taxa. Molecular Ecology **8**:1966-1969.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology **14**:2611-2620.

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics **164**:1567-1587.

`

Fiumera, A. C., B. A. Porter, G. D. Grossman, and J. C. Avise. 2002. Intensive genetic assessment of the mating system and reproductive success in a semi-closed population of the mottled sculpin, *Cottus bairdi*. Molecular Ecology **11**:2367-2377.

Fox, J., Weisberg, S., Friendly, M., Hong, J. 2015. R Package "effects": Effect.

Fujishin, L. M., F. K. Barker, D. D. Huff, and L. M. Miller. 2009. Isolation of 13 polymorphic microsatellite loci for slimy sculpin (*Cottus cognatus*). Conservation Genetics Resources **1**:429-432.

Garroway, C. J., J. Bowman, T. J. Cascaden, G. L. Holloway, C. G. Mahan, J. R. Malcolm, M. A. Steele, G. Turner, and P. J. Wilson. 2010. Climate change induced hybridization in flying squirrels. Global Change Biology **16**:113-121.

Goudet, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity **86**:485-486.

Harrison, R. G. 1993a. Hybrid zones and the evolutionary process. Oxford University Press on Demand.

Harrison, R. G. 1993b. Hybrids and hybrid zones: historical perspective. Hybrid zones and the evolutionary process:3-12.

Hewitt, G. M. 2001. Speciation, hybrid zones and phylogeography—or seeing genes in space and time. Molecular Ecology **10**:537-549.

Hubbs, C. L. 1955. Hybridization between fish species in nature. Systematic zoology **4**:1-20.

Hughes, G. W., and A. E. Peden. 1984. Life history and status of the shorthead sculpin (*Cottus confusus*: Pisces, Cottidae) in Canada and the sympatric relationship to the slimy sculpin (*Cottus cognatus*). Canadian Journal of Zoology **62**:306-311.

Isaak, D. J., D. L. Horan, and S. P. Wollrab. 2013. A simple protocol using underwater epoxy to install annual temperature monitoring sites in rivers and streams.

Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics **23**:1801-1806.

James, P. M. A., B. Cooke, B. M. T. Brunet, L. M. Lumley, F. A. H. Sperling, M. J. Fortin, V. S. Quinn, and B. R. Sturtevant. 2015. Life-stage differences in spatial genetic structure in an irruptive forest insect: implications for dispersal and spatial synchrony. Molecular Ecology **24**:296-309.

Jenkins, M. 2003. Prospects for biodiversity. Science **302**:1175-1177.

Jiggins, C. D., and J. Mallet. 2000. Bimodal hybrid zones and speciation. Trends in ecology & evolution **15**:250-255.

Kalinowski, S. T., and M. L. Taper. 2006. Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. Conservation Genetics **7**:991-995.

Kowalski, K. T., J. P. Schubauer, C. L. Scott, and J. R. Spotila. 1978. Interspecific and seasonal differences in the temperature tolerance of stream fish. Journal of Thermal Biology **3**:105-108.

Lookingbill, T. R., and D. L. Urban. 2003. Spatial estimation of air temperature differences for landscape-scale studies in montane environments. Agricultural and Forest Meteorology **114**:141-151.

Mallet, J. 2005. Hybridization as an invasion of the genome. Trends in ecology & evolution **20**:229-237.

Mallet, J. 2007. Hybrid speciation. Nature **446**:279.

May, R. M., J. A. Endler, and R. E. McMurtrie. 1975. Gene frequency clines in the presence of selection opposed by gene flow. The American Naturalist **109**:659-676.

Moore, W. S. 1977. An evaluation of narrow hybrid zones in vertebrates. The Quarterly Review of Biology **52**:263-277.

Muhlfeld, C. C., S. T. Kalinowski, T. E. McMahon, M. L. Taper, S. Painter, R. F. Leary, and F. W. Allendorf. 2009. Hybridization rapidly reduces fitness of a native trout in the wild. Biology Letters:rsbl. 2009.0033.

Muhlfeld, C. C., R. P. Kovach, L. A. Jones, R. Al-Chokhachy, M. C. Boyer, R. F. Leary, W. H. Lowe, G. Luikart, and F. W. Allendorf. 2014. Invasive hybridization in a threatened species is accelerated by climate change. Nature Climate Change **4**:620-624.

Nolte, A. W., J. Freyhof, K. C. Stemshorn, and D. Tautz. 2005a. An invasive lineage of sculpins, *Cottus* sp.(Pisces, Teleostei) in the Rhine with new habitat adaptations has originated from hybridization between old phylogeographic groups. Proceedings of the Royal Society of London B: Biological Sciences **272**:2379-2387.

Nolte, A. W., K. C. Stemshorn, and D. Tautz. 2005b. Direct cloning of microsatellite loci from *Cottus gobio* through a simplified enrichment procedure. Molecular Ecology Notes **5**:628-636.

Otto, R. G., and J. O. H. Rice. 1977. Responses of a freshwater sculpin (*Cottus cognatus gracilis*) to temperature. Transactions of the American Fisheries Society **106**:89-94.

Paradis, E. 2010. pegas: an R package for population genetics with an integrated-modular approach. Bioinformatics **26**:419-420.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annu. Rev. Ecol. Evol. Syst. **37**:637-669.

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics **28**:2537-2539.

Postel, S., and B. Richter. 2012. Rivers for life: managing water for people and nature. Island Press.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics **155**:945-959.

Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. Annual review of Ecology and Systematics **27**:83-109.

Ross, C. P. 1959. Geology of Glacier National Park and the Flathead region, northwestern Montana. 2330-7102, US Geological Survey.

Ruppert, J. L. W., P. M. A. James, E. B. Taylor, T. Rudolfsen, M. Veillard, C. S. Davis, D. Watkinson, and M. S. Poesch. 2017. Riverscape genetic structure of a threatened and dispersal limited freshwater species, the Rocky Mountain Sculpin (*Cottus* sp.). Conservation Genetics:1-13.

Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, and A. Kinzig. 2000. Global biodiversity scenarios for the year 2100. Science **287**:1770-1774.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin **184**.

Seehausen, O., G. Takimoto, D. Roy, and J. Jokela. 2008. Speciation reversal and biodiversity dynamics with hybridization in changing environments. Molecular Ecology **17**:30-44.

Short, L. L. 1970. A reply to Uzzell and Ashmole. Systematic zoology **19**:199-202.

Stemshorn, K. C., F. A. Reed, A. W. Nolte, and D. Tautz. 2011. Rapid formation of distinct hybrid lineages after secondary contact of two fish species (*Cottus* sp.). Molecular Ecology **20**:1475-1491.

Sweigart, A. 2009. Sculpin hybrid zones: natural laboratories for the early stages of speciation. Molecular Ecology **18**:2547-2548.

Water Survey of Canada. 2016. Wateroffice: Historical Hydrometric Data. Environment Canada, Government of Canada.

Wentworth, C. K. 1922. A scale of grade and class terms for clastic sediments. The Journal of Geology **30**:377-392.

Woodruff, D. S. 1979. Postmating reproductive isolation in Pseudophryne and the evolutionary significance of hybrid zones. Science **203**:561-563.

`

Zuur, A. F., E. N. Ieno, and C. S. Elphick. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution **1**:3-14.

`

3.9 Tables

Table 3.1. Summary of fixed effects statistics used in mixed effects selection process identifying the factors that determine the hybrid zones between Rocky Mountain Sculpin (*Cottus* sp.) and Slimy Sculpin (*Cottus cognatus*).

Variables	**Units Measured**	**Mean**	**±SD**	**Maximum**	**Minimum**
Water Quality					
Elevation	m	1371	94.2	1540	1196
Turbidity	NTU	0.46	0.50	2.96	0.02
Dissolved Oxygen	mg/L	9.07	0.67	10.72	7.43
Conductivity	µS/cm	132.5	30.9	208.6	79.8
Sample Depth	cm	29.9	12.9	60	0
Velocity	m/s	0.49	0.32	2.51	0
Benthic Substrate					
Boulder	%	13.7	21.0	100	0
Cobble	%	53.1	31.0	100	0
Large Gravel	%	21.1	24.8	100	0
Small Gravel	%	7.0	11.6	80	0

Table 3.2. Genetic diversity measures for the 10 microsatellite loci that were typed for all individuals, Slimy Sculpin and Rocky Mountain Sculpin from the Flathead River. Given is the number of individuals typed at each loci (N), number of alleles (N_a), observed heterozygosity (H_o), unbiased expected heterozygosity (UH_e) and fixation rate (F). All values were calculated using GenAlEx 6.5. Bold values indicate loci out of Hardy-Weinberg equilibrium.

		All				Slimy Sculpin				Rocky Mountain Sculpin			
Locus	N	N_a	H_o	UH_e	F	N_a	H_o	UH_e	F	N_a	H_o	UH_e	F
Cba42	731	6	0.316	0.630	0.498	5	0.167	0.495	**0.661**	6	0.411	0.606	0.320
Cgo114	731	4	0.276	0.345	0.200	4	0.179	0.258	**0.307**	4	0.322	0.383	0.160
Cco13	731	3	0.093	0.496	0.812	3	0.034	0.440	0.922	3	0.123	0.406	0.698
Cco15	731	8	0.356	0.661	0.462	7	0.186	0.479	0.610	7	0.463	0.685	0.323
Cco17	730	14	0.518	0.753	0.312	10	0.384	0.605	**0.364**	11	0.591	0.778	**0.239**
CottES10	729	6	0.115	0.503	0.771	6	0.069	0.461	**0.850**	5	0.142	0.414	0.657
Cott130	730	5	0.158	0.196	0.196	4	0.236	0.290	0.185	3	0.115	0.137	0.161
Cott687	728	14	0.242	0.575	0.579	9	0.160	0.502	0.681	11	0.298	0.506	0.411
CottES19	731	4	0.008	0.038	0.782	2	0.004	0.004	-0.002	3	0.011	0.069	**0.841**
Cgo310	727	8	0.314	0.619	0.493	6	0.245	0.565	0.565	8	0.349	0.550	0.365
Average				0.482	0.510			0.410	0.514			0.453	0.417

`

Table 3.3. Mixed-effects model using backward selection depicts the habitat factors important in determining hybridization zones between Rocky Mountain Sculpin and Slimy Sculpin in the Flathead Drainage. Standardized z-scores of the variables were used in to allow comparison across measurement units. The k column represents the number of variables including the response variable (Q-score) used in the habitat model. The optimal model (in bold font) was selected based corrected Akaike Information Criteria (AICc*i*), the difference between AICc$_i$ values and the smallest AICc$_i$ value (AICc Δ_i), and the weightings of each model showing the probability of a given model being the most optimal (AICc w_i).

Mod #	Fixed Effects	Random Effects	*k*	AICc$_i$	AICc Δ_i	AICc w_i
1	Elevation	1|Waterbody, 1|Date, 1|Site	2	492.04	30.32	0.00
2	Elevation, Conductivity	1|Waterbody, 1|Date, 1|Site	3	474.86	13.14	0.00
3	Elevation, Conductivity, Turbidity	1|Waterbody, 1|Date, 1|Site	4	466.67	4.95	0.02
4	**Elevation, Conductivity, Turbidity, Dissolved Oxygen**	**1|Waterbody, 1|Date, 1|Site**	**5**	**461.72**	**0.00**	**0.30**
5	Elevation, Conductivity, Turbidity, Dissolved Oxygen, Depth	1|Waterbody, 1|Date, 1|Site	6	461.85	0.13	0.28
6	Elevation, Conductivity, Turbidity, Dissolved Oxygen, Depth, Cobble	1|Waterbody, 1|Date, 1|Site	7	462.81	1.09	0.17
7	Elevation, Conductivity, Turbidity, Dissolved Oxygen, Depth, Cobble, Boulder	1|Waterbody, 1|Date, 1|Site	8	463.25	1.53	0.14
8	Elevation, Conductivity, Turbidity, Dissolved Oxygen, Depth, Cobble, Boulder, Velocity	1|Waterbody, 1|Date, 1|Site	9	464.86	3.14	0.06
9	Elevation, Conductivity, Turbidity, Dissolved Oxygen, Depth, Cobble, Boulder, Velocity, Lg. Gravel	1|Waterbody, 1|Date, 1|Site	10	466.74	5.02	0.02
10	Elevation, Conductivity, Turbidity, Dissolved Oxygen, Depth, Cobble, Boulder, Velocity, Lg. Gravel, Sm. Gravel	1|Waterbody, 1|Date, 1|Site	11	468.78	7.06	0.01
11	Elevation, Conductivity, Turbidity, Dissolved Oxygen, Depth, Cobble, Boulder, Velocity, Lg. Gravel, Sm. Gravel	-	11	645.93	184.2	0.00

`

Table 3.4. Estimates and significance levels describing the key covariates that determine q-values of sculpin in the Flathead River drainage, BC. Values are based on the selected logistic mixed-effects model (model #4). A * next to P-values indicate covariate significance (≤0.05).

Covariates	**Estimate**	***t* value**	***P***
Elevation	-0.003	-3.26	<0.001*
Conductivity	-0.006	-3.28	<0.001*
Turbidity	0.353	3.30	0.006*
Dissolved Oxygen	0.237	4.07	0.002*
Response: *Q-value*			

`

3.10 Figure Legends

Figure 3.1. (A) The Canadian distribution of Rocky Mountain Sculpin (top). The Slimy Sculpin distribution overlaps with Rocky Mountain Sculpin in the Flathead Drainage in southeastern BC, outlined in red (ranges modified from Scott and Crossman (1973) and COSEWIC (2010)). **(B)** Sample sites (n=95) in the Flathead River Drainage (bottom) were chosen based on areas that would possibly have hybrid zones based on findings by Hughes and Peden (1984). **(C)** Map of the Flathead Drainage showing the areas where hybrids and pure populations are found. Pies represent the proportion of parental and hybrid populations present in a given area of each lotic system.

Figure 3.2. Frequency distribution of q-values based on microsatellite analyses. Q-values represent the proportion of Rocky Mountain Sculpin microsatellites present in the genetic fin clips. Q-values of 0-10% (black) were Slimy Sculpin, 11-90% (gray) were hybrids, and 91-100% were Rocky Mountain Sculpin.

Figure 3.3. Effects plots showing the influence of each significant variable on q-value based on the environmental conditions each sculpin (points) was collected in. A fitted regression line and 95% confidence intervals (dashed lines) show the general trends of each environmental variable

`

3.11 Figures

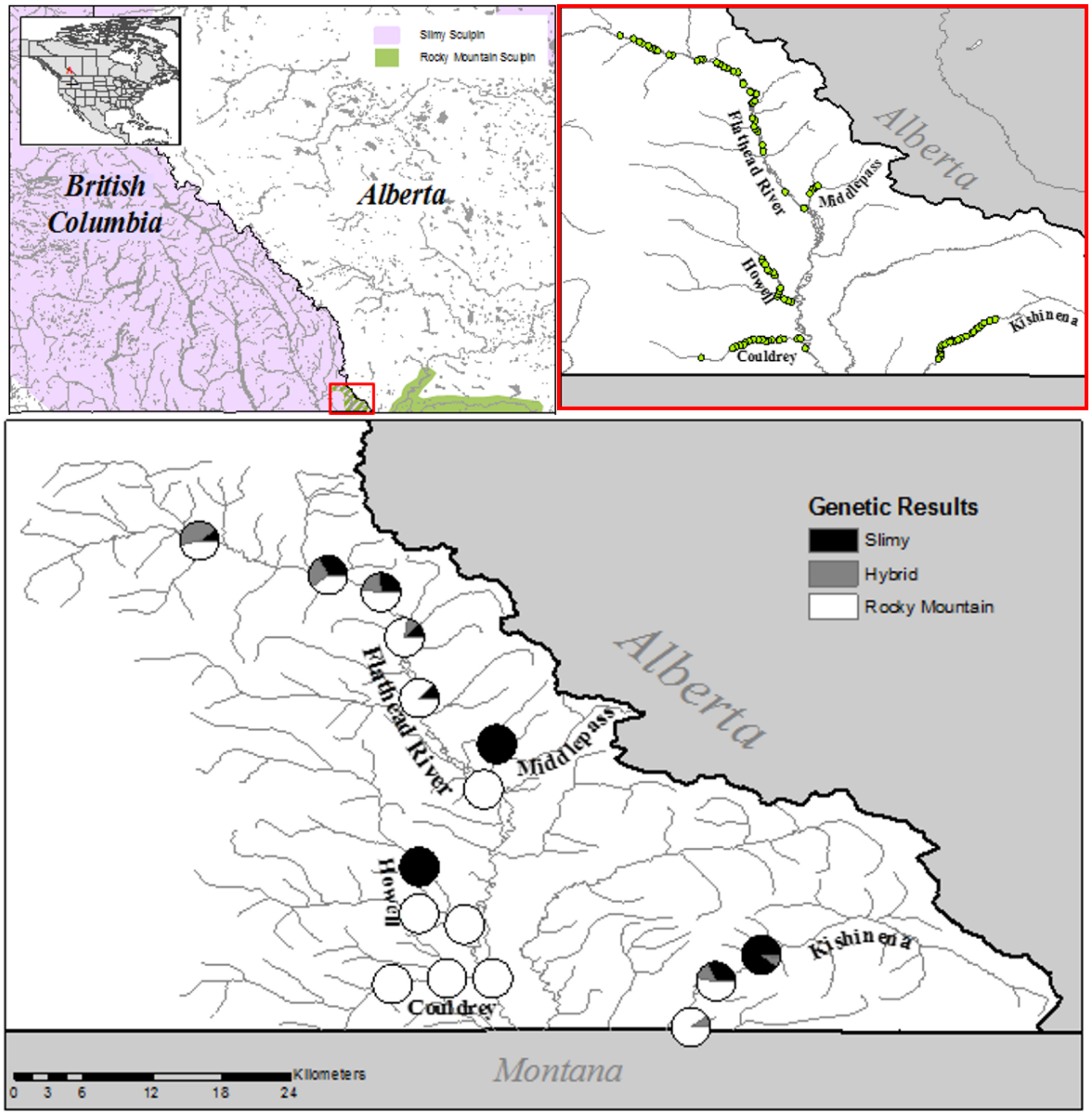
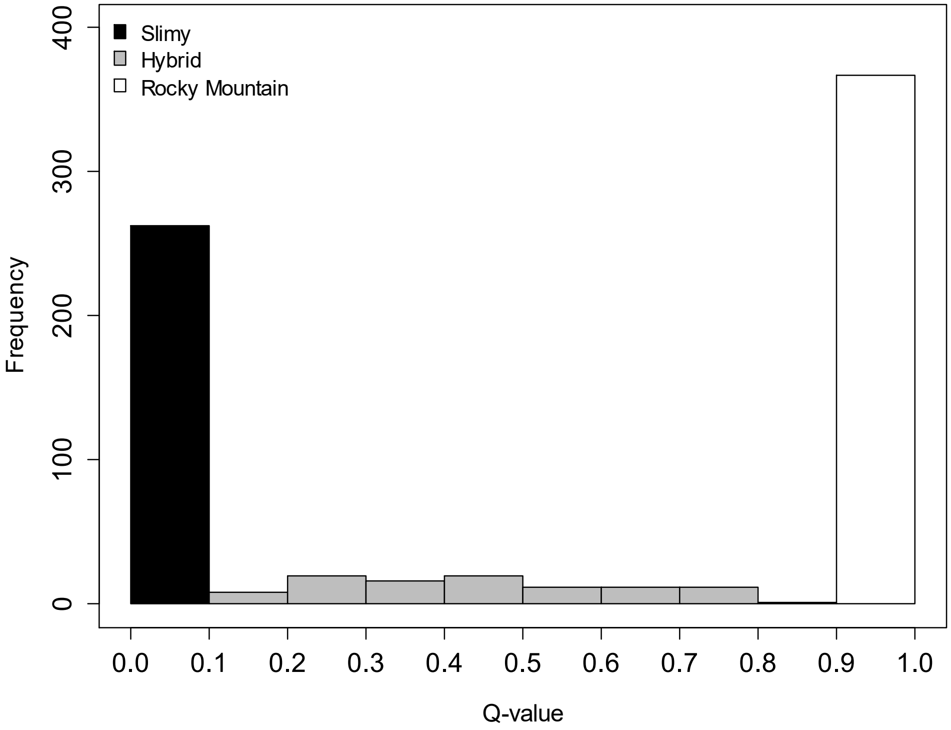


Figure 3.1

`



■ Slimy
▨ Hybrid
☐ Rocky Mountain
Frequency
0
100
200
300
400
Q-value
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

Figure 3.2

`

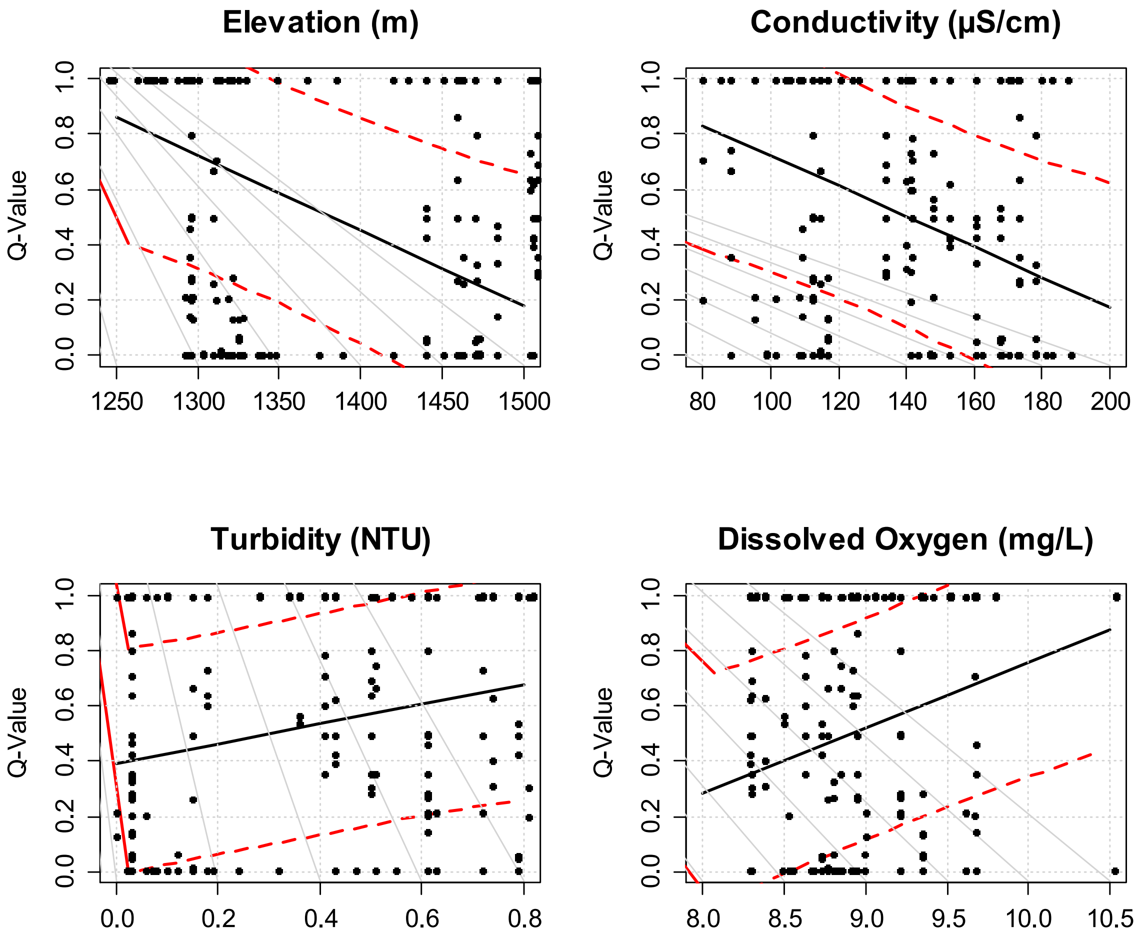
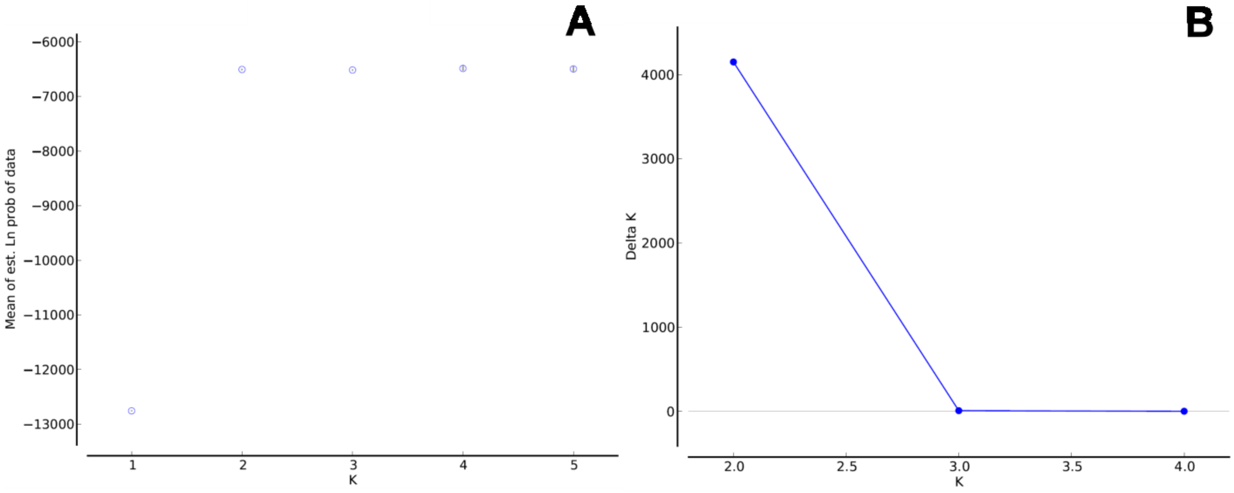

Elevation (m)
Q-Value
0.0
0.2
0.4
0.6
0.8
1.0
1250
1300
1350
1400
1450
1500
Conductivity (μS/cm)
Q-Value
0.0
0.2
0.4
0.6
0.8
1.0
80
100
120
140
160
180
200
Turbidity (NTU)
Q-Value
0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.2
0.4
0.6
0.8
Dissolved Oxygen (mg/L)
Q-Value
0.0
0.2
0.4
0.6
0.8
1.0
8.0
8.5
9.0
9.5
10.0
10.5

Figure 3.3

`

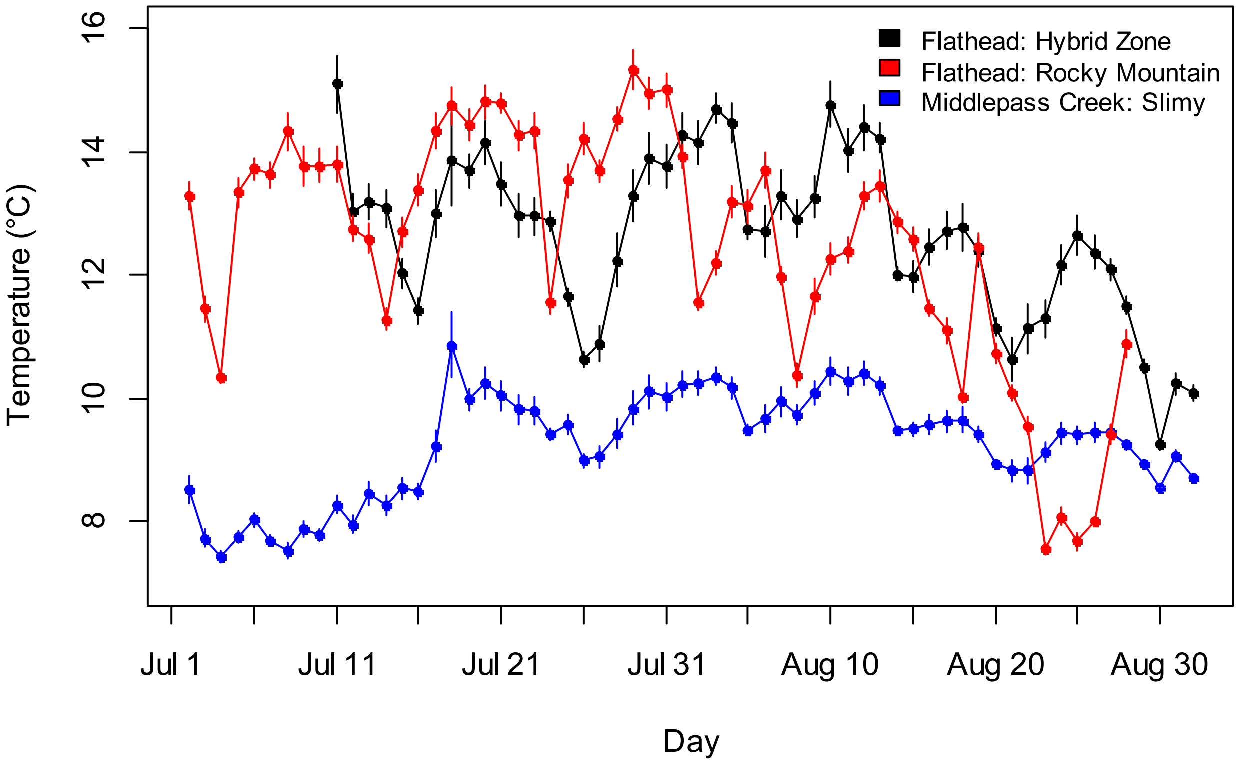
3.12 Appendix

A3.1. Results from the STRUCTURE model including the (**A**) mean ln probability and (**B**) delta K value. Shown are values for K between 1 and 5.



`

A3.2. Average water temperature trends in the Flathead drainage where Rocky Mountain Sculpin, Slimy Sculpin, and hybrid zones were found. Plot includes means ± standard errors across July and August of 2015 and 2016.



`

A3.3. Results from submerged temperature loggers relative to the presence or absence of Rocky Mountain Sculpin (RMS), Slimy Sculpin (SSC), and hybrid zones (HYB). Table **(A)** represents the results from the repeated measures Analysis of Variance (ANOVA). Table **(B)** shows the pairwise comparison of thermal regime between groups using the Tukey HSD test with Holm *P*-value adjustment. An asterix represents significant P-values.

A)

	df	**Sum of Squares**	**Mean Square**	***F***	***P*-value**
Sculpin Group	2	464.0	232.0	113.1	<0.001*
Residuals	170	348.6	2.05		

B)

Comparison	**Mean Difference**	***P*-value**	**Confidence Interval 95%**	
			Upper	Lower
RMS-HYB	-0.30	0.52	0.35	-0.94
SSC-HYB	-3.56	0.00*	-2.93	-4.19
SSC-RMS	-3.27	0.00*	-2.65	-3.88

`

Chapter 4: General Conclusion

Current biodiversity loss in freshwater systems is surpassing that of terrestrial or marine systems. Anthropogenic impacts such as water withdrawal and diversion are unavoidable as human populations and the demand for freshwater increase. Meanwhile, climate change is not an issue that can be reversed in the short term. There is a need to compromise the human demand for water resources with maintaining aquatic biodiversity. In order to find this balance, key environmental factors must be identified to direct conservation efforts on how to best mitigate impacts.

The Rocky Mountain Sculpin (*Cottus* sp.) is a species that can provide insight on how to prevent the degradation of freshwater systems and promote biodiversity. As a threatened, sedentary indicator species, it is ideal to identify its susceptibility to habitat change, and key environmental factors that cause acute perturbations in freshwater ecosystems. The objectives of this thesis were to: 1) identify the Rocky Mountain Sculpin's susceptibility to varying flow regime, and whether or not it displays morphological adaptation to flow, and 2) Characterize hybrid zones and the driving environmental factors that lead to their formation. In achieving these objectives, this thesis should bring clarity on how to minimize human impacts on freshwater biodiversity.

Rocky Mountain Sculpin appear able to cope with a certain range of different flow regimes. In faster moving water, they tend to be more dorso-ventrally flattened to reduce drag in moving water, have more pectoral fin rays to optimize their position among cobble substrate, and exhibit an increase in head pores to better detect prey items drifting by. There are some discrepancies however, that are more answerable by biogeographical isolation between populations. Some populations have likely been separated for upwards of 10,000 years, allowing for natural evolutionary divergence. The degree to which biogeography is influencing morphological differences is unknown; therefore, it cannot be concluded with certainty that Rocky Mountain Sculpin are capable of adopting quickly to flow regimes. As a result, human-induced flow alterations within the Rocky Mountain Sculpin range should be mitigated or implemented with caution.

Habitat features can be major drivers of species hybridization. The findings of this study support previous research that suggests Rocky Mountain Sculpin are hybridizing with Slimy Sculpin. However, it was also discovered that the Rocky Mountain Sculpin range was expanding upstream, likely due to climate change and changing water quality conditions such as increased turbidity and dissolved oxygen, and decreased salinity levels. This study is an example of how some human activity is not always detrimental to an imperiled species; however, there are limits to the benefits of these changes, especially as a sedentary species in a restricted river range. Further increases in temperatures and siltation can have the equal potential of eventually extirpating the species in those habitats that exceed the limits for the species.

While increasing stream temperatures from climate change is causing an upstream range expansion of Rocky Mountain Sculpin the scope of this study was unable to determine whether there was a concurrent contraction in the southern portion of their range in Montana. There, stream temperatures would also be increasing, likely to the point where the species could no longer inhabit those areas. This problem extends beyond the Flathead River. Natural water temperatures of the Milk River are too warm for the Rocky Mountain Sculpin to survive in. It is only with the influx of cool St. Mary water from the augmentation canal that the species is able to survive. The St. Mary canal also provides the added benefit of increasing flow to the Milk

`

River, which has a natural tendency to be shallow and slow moving. While this is better for the Rocky Mountain Sculpin however, it may not make favorable conditions to other fish species like the Western Silvery Minnow (*Hybognathus argyritis*), another species at risk. The Western Silvery Minnow prefers the natural regime of the Milk River, therefore conservation measures should account for both its requirements and those of the Rocky Mountain Sculpin.

Rocky Mountain Sculpin appear to be relatively tolerant of varying habitat features. This may provide them with an adaptive advantage over similar co-inhabitants. Their level of tolerance has limits however, and there can be detrimental consequences to populations if that limit is breached. Conservation managers should view these findings as an indicator that sculpin habitats are changing due to direct and indirect human activities. Efforts should be made to minimize further direct influences, as climate change is likely to augment any impacts to an unpredictable degree.

The findings of this thesis suggest that benthic sedentary fish species are likely to exhibit varying phenotypes and corresponding adaptations. With this, management efforts should be directed at the population level. In presence of hybrid zones, this management scope should be amended to a more local scale, as there are likely more fine-scale habitat features influencing the success of a given species at different parts along the reach. If these habitat features are dynamic through time they may lead to increased interspecific competition within the population’s range.

`

Literature Cited

Aboim, M., J. Mavárez, L. Bernatchez, and M. Coelho. 2010. Introgressive hybridization between two Iberian endemic cyprinid fish: a comparison between two independent hybrid zones. Journal of Evolutionary Biology **23**:817-828.

Adams, D., M. Collyer, and E. Sherratt. 2014. geomorph: Software for geometric morphometric analyses. R package version **2**.

Aickin, M., and H. Gensler. 1996. Adjusting for multiple testing when reporting research results: the Bonferroni vs Holm methods. American journal of public health **86**:726-728.

Akaike, H. 1974. A new look at the statistical model identification. IEEE transactions on automatic control **19**:716-723.

Akaike, H. 1998. Information theory and an extension of the maximum likelihood principle. Pages 199-213 Selected Papers of Hirotugu Akaike. Springer.

Allan, J. D., R. Abell, Z. Hogan, C. Revenga, B. W. Taylor, R. L. Welcomme, and K. Winemiller. 2005. Overfishing of Inland Waters. BioScience **55**:1041-1051.

Anderson, M. J. 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian Journal of Fisheries and Aquatic Sciences **58**:626-639.

Barton, N. 1979. The dynamics of hybrid zones. Heredity **43**:341-359.

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Annual review of Ecology and Systematics **16**:113-148.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2013. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-5.

Beauchamp, D. A. 1990. Seasonal and diel food habits of rainbow trout stocked as juveniles in Lake Washington. Transactions of the American Fisheries Society **119**:475-482.

Brinsmead, J., and M. Fox. 2002. Morphological variation between lake-and stream-dwelling ock bass and pumpkinseed populations. Journal of Fish Biology **61**:1619-1638.

Buggs, R. 2007. Empirical study of hybrid zone movement. Heredity **99**:301-312.

Burnham, K. P., and D. R. Anderson. 2004. Multimodel inference understanding AIC and BIC in model selection. Sociological methods & research **33**:261-304.

Cheever, B., and K. Simon. 2009. Seasonal influence of brook trout and mottled sculpin on lower trophic levels in an Appalachian stream. Freshwater Biology **54**:524-535.

Cohen, J. E. 2003. Human population: the next half century. Science **302**:1172-1175.

Collin, H., and L. Fumagalli. 2011. Evidence for morphological and adaptive genetic divergence between lake and stream habitats in European minnows (*Phoxinus phoxinus*, Cyprinidae). Molecular Ecology **20**:4490-4502.

Collyer, M., D. Sekora, and D. Adams. 2015. A method for analysis of phenotypic change for phenotypes described by high-dimensional data. Heredity **115**:357-365.

COSEWIC. 2005. COSEWIC assessment and status report on the "eastslope" sculpin (St. Mary and Milk River Population) *Cottus* sp. in Canada. Ottawa.

COSEWIC. 2010. COSEWIC assessment and status report on the Rocky Mountain Sculpin *Cottus* sp., westslope populations in Canada. Ottawa.

Crespin, L., P. Berrebi, and J. Lebreton. 1999. Asymmetrical introgression in a freshwater fish hybrid zone as revealed by a morphological index of hybridization. Biological Journal of the Linnean Society **67**:57-72.

Cullingham, C. I., P. James, J. E. Cooke, and D. W. Coltman. 2012. Characterizing the physical and genetic structure of the lodgepole pine× jack pine hybrid zone: mosaic structure and differential introgression. Evolutionary applications **5**:879-891.

Curry, C. M. 2015. An integrated framework for hybrid zone models. Evolutionary Biology **42**:359-365.

Dineen, C. F. 1951. A comparative study of the food habits of Cottus bairdii and associated species of Salmonidae. The American Midland Naturalist **46**:640-645.

Dryden, I. L., and K. V. Mardia. 2016. Statistical Shape Analysis: With Applications in R. John Wiley & Sons.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, and M. L. Stiassny. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological reviews **81**:163-182.

Eaton, J. G., and R. M. Scheller. 1996. Effects of climate warming on fish thermal habitat in streams of the United States. Limnology and oceanography **41**:1109-1115.

Endler, J. A. 1986. Natural selection in the wild. Princeton University Press.

Englbrecht, C., C. Largiader, B. Hänfling, and D. Tautz. 1999. Isolation and characterization of polymorphic microsatellite loci in the European bullhead *Cottus gobio L.*(Osteichthyes) and their applicability to related taxa. Molecular Ecology **8**:1966-1969.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology **14**:2611-2620.

Facey, D. E., and G. D. Grossman. 1990. The metabolic cost of maintaining position for four North American stream fishes: effects of season and velocity. Physiological Zoology **63**:757-776.

Fagan, W. F., P. J. Unmack, C. Burgess, and W. Minckley. 2002. Rarity, fragmentation, and extinction risk in desert fishes. Ecology **83**:3250-3256.

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics **164**:1567-1587.

Fisheries and Oceans Canada. 2012a. Recovery Strategy for the Rocky Montain Sculpin (*Cottus* sp.), Eastslope populations, in Canada. Fisheries and Oceans Canada, Ottawa.

Fisheries and Oceans Canada. 2012b. Recovery Strategy for the Rocky Mountain Sculpin (*Cottus* sp.), eastslope populations, in Canada. . Ottawa.

Fiumera, A. C., B. A. Porter, G. D. Grossman, and J. C. Avise. 2002. Intensive genetic assessment of the mating system and reproductive success in a semi-closed population of the mottled sculpin, *Cottus bairdi*. Molecular Ecology **11**:2367-2377.

Fox, J., Weisberg, S., Friendly, M., Hong, J. 2015. R Package "effects": Effect.

Fraser, D. J., and L. Bernatchez. 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecular Ecology **10**:2741-2752.

Friendly, M., and J. Fox. 2010. Candisc: R package for canonical discriminant analysis. Accessed at: http://cran. r-project. org/web/packages/candisc.

Fujishin, L. M., F. K. Barker, D. D. Huff, and L. M. Miller. 2009. Isolation of 13 polymorphic microsatellite loci for slimy sculpin (*Cottus cognatus*). Conservation Genetics Resources **1**:429.

Garroway, C. J., J. Bowman, T. J. Cascaden, G. L. Holloway, C. G. Mahan, J. R. Malcolm, M. A. Steele, G. Turner, and P. J. Wilson. 2010. Climate change induced hybridization in flying squirrels. Global Change Biology **16**:113-121.

Goodall, C. 1991. Procrustes methods in the statistical analysis of shape. Journal of the Royal Statistical Society. Series B (Methodological):285-339.

Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. Journal of Heredity **86**:485-486.

Gray, M. A., R. A. Curry, and K. R. Munkittrick. 2005. Impacts of nonpoint inputs from potato farming on populations of slimy sculpin (*Cottus cognatus*). Environmental Toxicology and Chemistry **24**:2291-2298.

Harrison, R. G. 1993a. Hybrid zones and the evolutionary process. Oxford University Press on Demand.

Harrison, R. G. 1993b. Hybrids and hybrid zones: historical perspective. Hybrid zones and the evolutionary process:3-12.

Hewitt, G. M. 2001. Speciation, hybrid zones and phylogeography—or seeing genes in space and time. Molecular Ecology **10**:537-549.

Hoekstra, D., and J. Janssen. 1985. Non-visual feeding behavior of the mottled sculpin, *Cottus bairdi*, in Lake Michigan. Environmental Biology of Fishes **12**:111-117.

Hubbs, C. L. 1955. Hybridization between fish species in nature. Systematic zoology **4**:1-20.

Hughes, G. W., and A. E. Peden. 1984. Life history and status of the shorthead sculpin (*Cottus confusus*: Pisces, Cottidae) in Canada and the sympatric relationship to the slimy sculpin (*Cottus cognatus*). Canadian Journal of Zoology **62**:306-311.

Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics **23**:1801-1806.

James, P., B. Cooke, B. M. Brunet, L. M. Lumley, F. A. Sperling, M. J. Fortin, V. S. Quinn, and B. R. Sturtevant. 2015. Life-stage differences in spatial genetic structure in an irruptive forest insect: implications for dispersal and spatial synchrony. Molecular Ecology **24**:296-309.

Jenkins, M. 2003. Prospects for biodiversity. Science **302**:1175-1177.

Jiggins, C. D., and J. Mallet. 2000. Bimodal hybrid zones and speciation. Trends in ecology & evolution **15**:250-255.

Kalinowski, S. T., and M. L. Taper. 2006. Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. Conservation Genetics **7**:991-995.

Kane, E. A., and T. E. Higham. 2012. Life in the flow lane: differences in pectoral fin morphology suggest transitions in station-holding demand across species of marine sculpin. Zoology **115**:223-232.

Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. Ecology letters **7**:1225-1241.

Koehl, M. 1984. How do benthic organisms withstand moving water? American Zoologist **24**:57-70.

Koumoundouros, G., C. Ashton, D. Sfakianakis, P. Divanach, M. Kentouri, N. Anthwal, and N. Stickland. 2009. Thermally induced phenotypic plasticity of swimming performance in

European sea bass *Dicentrarchus labrax* juveniles. Journal of Fish Biology **74**:1309-1322.

Kowalski, K. T., J. P. Schubauer, C. L. Scott, and J. R. Spotila. 1978. Interspecific and seasonal differences in the temperature tolerance of stream fish. Journal of Thermal Biology **3**:105-108.

Langerhans, R. B. 2008. Predictability of phenotypic differentiation across flow regimes in fishes. Integrative and Comparative Biology **48**:750-768.

Langerhans, R. B., C. A. Layman, A. K. Langerhans, and T. J. Dewitt. 2003. Habitat-associated morphological divergence in two Neotropical fish species. Biological Journal of the Linnean Society **80**:689-698.

Lele, S. 1993. Euclidean distance matrix analysis (EDMA): estimation of mean form and mean form difference. Mathematical Geology **25**:573-602.

Levêque, C., T. Oberdorff, D. Paugy, M. Stiassny, and P. Tedesco. 2008. Global diversity of fish (Pisces) in freshwater. Hydrobiologia **595**:545-567.

Levin, D. A., J. Francisco-Ortega, and R. K. Jansen. 1996. Hybridization and the extinction of rare plant species. Conservation Biology **10**:10-16.

Lookingbill, T. R., and D. L. Urban. 2003. Spatial estimation of air temperature differences for landscape-scale studies in montane environments. Agricultural and Forest Meteorology **114**:141-151.

Lytle, D. A., and N. L. Poff. 2004. Adaptation to natural flow regimes. Trends in ecology & evolution **19**:94-100.

Mallet, J. 2005. Hybridization as an invasion of the genome. Trends in ecology & evolution **20**:229-237.

Mallet, J. 2007. Hybrid speciation. Nature **446**:279.

May, R. M., J. A. Endler, and R. E. McMurtrie. 1975. Gene frequency clines in the presence of selection opposed by gene flow. The American Naturalist **109**:659-676.

McGuigan, K., C. E. Franklin, C. Moritz, M. W. Blows, and P. Wainwright. 2003. Adaptation of rainbow fish to lake and stream habitats. Evolution **57**:104-118.

McKee, J. K., P. W. Sciulli, C. D. Fooce, and T. A. Waite. 2004. Forecasting global biodiversity threats associated with human population growth. Biological Conservation **115**:161-164.

McKinney, M. L. 2002. Urbanization, biodiversity, and conservation: the impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. BioScience **52**:883-890.

McLaughlin, R. L., and J. W. Grant. 1994. Morphological and behavioural differences among recently-emerged brook charr, *Salvelinus fontinalis*, foraging in slow-vs. fast-running water. Environmental Biology of Fishes **39**:289-300.

Moore, W. S. 1977. An evaluation of narrow hybrid zones in vertebrates. The Quarterly Review of Biology **52**:263-277.

Muhlfeld, C. C., S. T. Kalinowski, T. E. McMahon, M. L. Taper, S. Painter, R. F. Leary, and F. W. Allendorf. 2009. Hybridization rapidly reduces fitness of a native trout in the wild. Biology Letters:rsbl. 2009.0033.

Muhlfeld, C. C., R. P. Kovach, L. A. Jones, R. Al-Chokhachy, M. C. Boyer, R. F. Leary, W. H. Lowe, G. Luikart, and F. W. Allendorf. 2014. Invasive hybridization in a threatened species is accelerated by climate change. Nature Climate Change **4**:620-624.

Natsumeda, T., T. Tsuruta, H. Takeshima, S. Awata, and K. i. Iguchi. 2014. Variation in morphological characteristics of Japanese fluvial sculpin related to different environmental conditions in a single river system in eastern Japan. Ecology of Freshwater Fish **23**:114-120.

Nelson, J. S., and M. J. Paetz. 1992. The fishes of Alberta. University of Alberta.

Nolte, A. W., J. Freyhof, K. C. Stemshorn, and D. Tautz. 2005. An invasive lineage of sculpins, *Cottus* sp.(Pisces, Teleostei) in the Rhine with new habitat adaptations has originated from hybridization between old phylogeographic groups. Proceedings of the Royal Society of London B: Biological Sciences **272**:2379-2387.

Nolte, A. W., and H. D. Sheets. 2005. Shape based assignment tests suggest transgressive phenotypes in natural sculpin hybrids (Teleostei, Scorpaeniformes, Cottidae). Frontiers in zoology **2**:11.

Oksanen, J., F. Blanchet, R. Kindt, P. Legendre, P. Minchin, R. O'Hara, G. Simpson, P. Solymos, M. Henry, and H. Stevens. 2013. Vegan: Community Ecology Package. 2013. R-package version 2.0-10. URL http//:CRAN.R-project. org/package= vegan.

Otto, R. G., and J. O. H. Rice. 1977. Responses of a freshwater sculpin (*Cottus cognatus gracilis*) to temperature. Transactions of the American Fisheries Society **106**:89-94.

Pakkasmaa, S., and J. Piironen. 2000. Water velocity shapes juvenile salmonids. Evolutionary Ecology **14**:721-730.

Paradis, E. 2010. pegas: an R package for population genetics with an integrated–modular approach. Bioinformatics **26**:419-420.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annu. Rev. Ecol. Evol. Syst. **37**:637-669.

`

Parmesan, C., N. Ryrholm, C. Stefanescu, and J. K. Hill. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature **399**:579.

PE, P. R. S. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics **28**:2537-2539.

Poff, N. L., J. D. Allan, M. B. Bain, J. R. Karr, K. L. Prestegaard, B. D. Richter, R. E. Sparks, and J. C. Stromberg. 1997. The natural flow regime. BioScience **47**:769-784.

Poff, N. L., and J. K. Zimmerman. 2010. Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. Freshwater Biology **55**:194-205.

Postel, S., and B. Richter. 2012. Rivers for life: managing water for people and nature. Island Press.

Pringle, C. M., M. C. Freeman, and B. J. Freeman. 2000. Regional Effects of Hydrologic Alterations on Riverine Macrobiota in the New World: Tropical–Temperate Comparisons. BioScience **50**:807-823.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics **155**:945-959.

Quinn, J. W., and N. Mundahl. 1994. Effects of trout stream habitat rehabilitation projects on Nongame fish communities in five southeastern Minnesota streams. Report to the Conservation Biology Research Grants Program, Division of Ecological Services, Minnesota Department of Natural Resources, St. Paul.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2015. .

Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. Annual review of Ecology and Systematics **27**:83-109.

Ricciardi, A., and J. B. Rasmussen. 1999. Extinction rates of North American freshwater fauna. Conservation Biology **13**:1220-1222.

Richter, B. D., D. P. Braun, M. A. Mendelson, and L. L. Master. 1997. Threats to imperiled freshwater fauna. Conservation Biology **11**:1081-1093.

Riffel, M., and A. Schreiber. 1998. Morphometric differentiation in populations of the Central European sculpin *Cottus gobio L.*, a fish with deeply divergent genetic lineages. Canadian Journal of Zoology **76**:876-885.

RL&L Environmental Services Ltd. 2002. Fish species at risk in the Milk and St. Mary drainages. Edmonton, AB.

`

Rohlf, F. J. 1999. Shape statistics: Procrustes superimpositions and tangent spaces. Journal of Classification **16**:197-223.

Rohlf, F. J. 2005. tpsDig, digitize landmarks and outlines, version 2.05. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F. J., and L. F. Marcus. 1993. A revolution in morphometrics. Trends in ecology & evolution **8**:129-132.

Ross, C. P. 1959. Geology of Glacier National Park and the Flathead region, northwestern Montana. 2330-7102, US Geological Survey.

Ruppert, J. L. W., P. M. A. James, E. B. Taylor, T. Rudolfsen, M. Veillard, C. S. Davis, D. Watkinson, and M. S. Poesch. 2017. Riverscape genetic structure of a threatened and dispersal limited freshwater species, the Rocky Mountain Sculpin (*Cottus* sp.). Conservation Genetics:1-13.

Sagnes, P., and B. Statzner. 2009. Hydrodynamic abilities of riverine fish: a functional link between morphology and velocity use. Aquatic Living Resources **22**:79-91.

Sala, O. E., F. S. Chapin, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, and A. Kinzig. 2000. Global biodiversity scenarios for the year 2100. Science **287**:1770-1774.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin **184**.

Sherratt, E. 2014. Quick guide to Geomorph v. 2.0.

Short, L. L. 1969. Taxonomic aspects of avian hybridization. The Auk **86**:84-105.

Short, L. L. 1970. A reply to Uzzell and Ashmole. Systematic zoology **19**:199-202.

Slice, D. E. 2001. Landmark coordinates aligned by Procrustes analysis do not lie in Kendall's shape space. Systematic biology **50**:141-149.

Stearns, S. C., and T. J. Kawecki. 1994. Fitness sensitivity and the canalization of life-history traits. Evolution:1438-1450.

Stemshorn, K. C., F. A. Reed, A. W. Nolte, and D. Tautz. 2011. Rapid formation of distinct hybrid lineages after secondary contact of two fish species (*Cottus* sp.). Molecular Ecology **20**:1475-1491.

Sweigart, A. 2009. Sculpin hybrid zones: natural laboratories for the early stages of speciation. Molecular Ecology **18**:2547-2548.

Tabor, R. A., E. J. Warner, K. L. Fresh, B. A. Footen, and J. R. Chan. 2007. Ontogenetic diet shifts of prickly sculpin in the Lake Washington basin, Washington. Transactions of the American Fisheries Society **136**:1801-1813.

Taft, N., G. Lauder, and P. Madden. 2008. Functional regionalization of the pectoral fin of the benthic longhorn sculpin during station holding and swimming. Journal of Zoology **276**:159-167.

Taylor, E. B., and J. McPhail. 1985. Variation in body morphology among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. Canadian Journal of Fisheries and Aquatic Sciences **42**:2020-2028.

Water Survey of Canada. 2016. Wateroffice: Historical Hydrometric Data. Environment Canada, Government of Canada.

Webb, P. W. 1984. Form and function in fish swimming. Scientific American.

Webster, M., and H. D. Sheets. 2010. A practical introduction to landmark-based geometric morphometrics. Quantitative Methods in Paleobiology **16**:168-188.

Wentworth, C. K. 1922. A scale of grade and class terms for clastic sediments. The Journal of Geology **30**:377-392.

Whiteley, A. R., S. M. Gende, A. J. Gharrett, and D. A. Tallmon. 2009. Background matching and color-change plasticity in colonizing freshwater sculpin populations following rapid deglaciation. Evolution **63**:1519-1529.

Woodruff, D. S. 1979. Postmating reproductive isolation in Pseudophryne and the evolutionary significance of hybrid zones. Science **203**:561-563.

Zelditch, M. L., D. L. Swiderski, and H. D. Sheets. 2012. Geometric morphometrics for biologists: a primer. Academic Press.

Zuur, A. F., E. N. Ieno, and C. S. Elphick. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution **1**:3-14.