AN ABSTRACT OF THE DISSERTATION OF

Chanté D. Davis for the degree of Doctor of Philosophy in Fisheries Science presented on August 17 2017.

Title: Riverscape genetics of *Oncorhynchus tshawytscha* (Chinook salmon) in Siletz River, OR

Abstract approved: _____

Michael A. Banks

Traditional analysis in population genetics evaluates differences among groups of individuals and, in some cases, considers the effects of distance or potential barriers to gene flow. However, many forces may shape genetic variation of organisms in riverine systems. Similarly complex research linking habitat heterogeneity and configuration to genetic structure has integrated methods from landscape ecology, population genetics, and spatial statistics in approaches known as landscape or seascape genetics. However, challenges exist when translating these approaches into freshwater river networks due to functional differences in riverscape topography that create constrained pathways for movement. The overall goal of my dissertation was to combine the approaches applied in population genetics to identify genetic diversity within and among populations, with concepts derived from network theory to better understand how the riverscape influenced spatial genetic structure of Chinook salmon (*Oncorhynchus tshawytscha*) populations in Siletz River. I provide a perspective on how riverscape genetics could be used to provide a more comprehensive conceptual and applied understanding of connectivity and dispersal in freshwater systems. I describe four thematic areas of study representing current and future research opportunities and propose a basic

methodology for conducting riverscape genetics analysis. I applied the proposed riverscape genetics method to attempt a novel analysis of spatial genetic structure of Chinook salmon within Siletz River, Oregon and compared results with interpretation of spatial genetic structure using traditional population genetics methods.

Chinook salmon are a culturally important and economically valuable fish that express diverse life histories characterized by the season of their return migration to spawning habitat (called a "run") and duration of freshwater or estuarine residence. Population structure among Chinook salmon of alternate run times observed in the Siletz River was investigated using 11 microsatellite markers, 96 Single Nucleotide Polymorphisms (SNPs), and three candidate gene markers that are linked to spawn time and body size. Results from all marker types identified two genetically distinct populations in the watershed (microsatellites; $F_{ST} = 0.02$, $p < 0.05$) that included a previously unrecognized spring run. This finding is an important consideration for management of the species, as spring run populations have not been recognized in smaller watersheds. Using riverscape genetics methods I characterized the spatial relationships among fall run Chinook salmon. Analysis assessed the effect of indicators of hydrology on dispersal and identified patterns of genetic variation were associated with site-specific differences in elevation of spawning habitat (MRDM; $R^2 = 0.11$ $p < 0.05$). Further investigation using path-based methodology identified that the cumulative changes in gradient among stream reaches also significantly affected spatial genetic structure (MRDM; $R^2 = 0.14$ $p < 0.01$). The combination of approaches that were used to investigate spatial genetic variation highlighted the utility of riverscape genetics to enhance our understanding of the relationships that contributed to observed population structure. Although fall run Chinook salmon within Siletz River exhibited high gene flow and were considered a single spawning population using traditional population genetic methods, there was evidence of differential habitat use within the group that was driven by the location of spawning habitat and the resistance to dispersal caused by habitat between these locations. Chinook

salmon that traveled over steeper gradients to reach spawning habitat at higher elevations *were different than* individuals that traveled over shallower gradients to reach spawning habitat at lower elevations. The riverscape genetics approach applied in this chapter enhanced our understanding of habitat heterogeneity in shaping gene flow and spatial genetic structure at a fine spatial scale. Expanding quantitative genetic research, in river systems to explicitly consider riverscape scale network configurations would help develop a clear understanding of the importance of these factors in terms of population persistence.

Riverscape genetics of *Oncorhynchus tshawytscha* (Chinook salmon) in Siletz River, OR

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Chanté D. Davis, Author

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CONTRIBUTION OF AUTHORS

Dr. Michael A. Banks contributed to this project throughout its course including: acquiring funding and interpretation of results for chapter three. He also provided editing in various capacities for all chapters.

Dr. John Carlos Garza provided the SNP panel and sample processing that was used in the analysis of data for chapter three. His technical expertise and editorial comments assisted in the completion of the manuscript resulting from this chapter.

Dr. Clinton Epps provided technical expertise of landscape and population genetics during lengthy theoretical discussions that helped conceptualize components of chapter two. Additionally, Dr. Epps provided guidance and mentoring for the design and analysis of chapter four as well as editing subsequent manuscripts that resulted from both chapters.

Dr. Rebecca Flitcroft contributed technical expertise in Salmonid ecology and life history, and knowledge of freshwater river ecosystems during lengthy theoretical discussions that greatly improved the direction of chapter two. She also assisted with project design of chapter four and provided editorial comments for manuscripts of all chapters.

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Chapter 1:

General Introduction

Habitat connectivity has been a central theory in ecology. Within a river, hydrology is the force that provides connectivity among habitat patches (Allan and Castillo, 2007). Connectedness between patches shape structure and location of biological communities throughout the system and is essential to long-term viability of riverine fish populations (Flitcroft et al., 2012; Kocick and Ferreri, 1998; Le Pichon et al., 2006). Resource quality within a habitat patch and connectivity among patch types are constraints for the abundance and proliferation of biotic communities within the river (Flitcroft et al., 2012; Kocick and Ferreri, 1998; Le Pichon et al., 2006). Ecological disturbance, both natural (e.g., landslides) and anthropogenic (e.g., water impediment) that alter hydraulic processes can impart profound and lasting alterations on the natural flow of water (Allan and Castillo, 2007). Depending on magnitude of the disturbance and length of its effect permanent shifts to topography (i.e., bedrock scour, channel widening, branching, or waterfalls) can develop, fragmenting habitat, isolating communities, or reconnecting previously isolated communities (Allan and Castillo, 2007). As the patterns of dispersal within the ecological community respond to changes in connectivity, there is a potential to alter neutral and functional genetic diversity (Banks et al., 2013). Genetic diversity influences the fitness of individuals, the adaptability of a species to changing environments and the structure of communities. Therefore, any alteration in connectivity that disrupts or enhances dispersal ability and subsequent gene flow may result in changes in genetic diversity that will have important ecological ramifications on long-term population viability (Banks et al., 2013).

Riverscape genetics is a field with methodologies that are an amalgamation of ecology, population genetics and spatial statistics Davis et al., (*in review*), and is related to land and seascape genetics (Manel et al., 2003; Selkoe et al., 2016). The aim is to understand how changes in dispersal affect spatial genetic structure and the role of connectivity in mediating these effects. Unlike terrestrial systems, the temporal and spatial distribution of habitat patches within riverscapes are driven by natural hydraulic forces within the river (Allan and Castillo, 2007; Anlauf et al., 2011; Durance et al., 2006; lose et al., 2006), in ways that are shared with marine systems (Selkoe et al., 2008). Hydrology is a major distinction that has important

consequences on sample collection, methodology and analysis, necessitating careful thought when selecting appropriate tools of land-and seascape genetics for application in rivers.

The overall goal of my dissertation was to combine the approaches applied in population genetics to identify genetic diversity within and among populations, with concepts derived from freshwater ecology to better understand how the riverscape influenced spatial genetic structure of Chinook salmon (*Oncorhynchus tshawytscha*) populations in Siletz River. First in chapter two I discussed the applicability of transferring land- and seascape genetic analysis into freshwater systems. I defined riverscape genetics and proposed a riverscape-specific model that integrated genetic, habitat, and hydraulic variables to test for the effect of dispersal on genetic structure and gene flow of riverine fishes. Next, in chapter three I identified spatial genetic structure among spawning groups and described the spatial and temporal genetic structure using standard population genetics analysis. Then in Chapter four, I investigated the utility of riverscape genetics (RG) to identify further substructure within fall run Chinook salmon.

Unlike terrestrial counterparts, research in lotic systems has only recently begun to consider genetics and riverscape variables simultaneously (Bowlby et al., 2016; Cowen and Sponaugle, 2009; Selkoe et al., 2016). Given the dynamics of river systems, there is need for thinking of genetic isolation in a context that accounts for the dynamic riverine environment in a network context and across multiple spatial scales. The most common pattern for modeling genetic distance that has been applied in a riverine environment was measures of geographic distance: waterway distance and Euclidian distance (Merimans and Hedrick, 2011). These approaches were translated from landscape genetics, and seascape genetics, but new approaches have considered the complex spatial network of rivers (Johnson and Host, 2010; Le Pichon et al., 2006). There are recognized difficulties with the use and application of terminology, limited analytical methodology, and lack of cohesive theoretical frameworks (Balkenhol et al., 2009; Dyer, 2015; Johnson and Host, 2010; Richardson et al., 2016; Selkoe et al., 2016). In chapter two I addressed the challenge of translating land- and seascape genetic methodology and analysis into freshwater

systems. I designed relevant research themes that would be enhanced by a RG approach, I reviewed current methodologies in both fields, and evaluated the efficacy of selected methods for application in rivers. The unique value of RG is its flexibility to accommodate multiple scales, thereby enhancing our ability to address questions of ecosystem structure and function.

Ecologists have demonstrated correlations between physical and geomorphological river processes, and fluctuations of Chinook salmon populations (Brenkman et al., 2011; Geist and Dauble, 1998). The availability of suitable spawning ground and nursery areas have been correlated with maintenance of genetic diversity (Ozerov et al., 2012). Habitat variables, drainage area, gradient, temperature, and water flow have been correlated with suitable habitat necessary for long-term salmon survival ((PFMFC), 2016; Geist and Dauble, 1998; Moir and Pasternack, 2010). Concurrently, geneticists are providing evidence of coarse-grain and fine-grain genetic structuring across the range of the species (Banks et al., 2000; Seeb et al., 2007; Teel et al., 2000). In Chapter Three I described genetic variation of distinct groups within the Siletz River. A notable finding that resulted from my research was the identification of a genetically distinct spring run life history. This is noteworthy because spring run Chinook salmon life history is associated with larger rivers that are composed of multiple watersheds like the San Joaquin – Sacramento river in California (Banks et al., 2000; Yoshiyama et al., 1998), or the Columbia river in the Pacific Northwest that has tributaries that crosses into seven state boundaries (Narum et al., 2011; Waples et al., 2004; Yoshiyama et al., 1998).

Identifying features of the landscape that promote or impede dispersal provides an ecological context for understanding how populations are structured and help to prevent further decline in population abundances or extinctions (Manel, Schwartz, Luikart, & Taberlet, 2003). When functional connectivity of the riverscape is impeded, the ability of an individual to disperse becomes limited (Brenkman et al., 2012; Schick and Lindley, 2007). Therefore, gene flow is decreased and populations are able to proceed down independent evolutionary trajectories that are affected by adaptation to their local environments. Connectivity is essential for individuals within populations to maintain genetic exchange and has implications for long-term

viability. In Chapter Four I investigated the relationship between fine-scale genetic structure and dispersal of fall run. I achieved this goal by reanalyzing the genetic dataset that I generated in chapter three, using a finer grain (*i.e.,* stream reach). I assessed whether indicators of hydrology partially explained observed genetic structure, after accounting for the influence of geographic distance. I approached this objective using two methods. The first followed a more traditional framework and compared the difference in hydrologic variables measured among sites to pairwise genetic distance. However, there are costs of migration that accumulate during dispersal among habitat patches and also effect gene flow (Cushman et al., 2010). Therefore, in a second approach, I measured effective distance as the cumulative cost of hydrologic variables along the path of travel between sites. This approach incorporated network position into analysis, adding a level of inquiry that is not possible to achieve when only considering site-based comparisons.

My analysis and continued development of riverine specific models have begun to assess the affects of dispersal on long-term population viability that are associated with changes in connectivity. In the final chapter I speculated on a few of the possibilities and encourage continued development of methodology in riverscape genetics.

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Chapter 2:

Refining and defining riverscape genetics: how rivers influence population genetic structure

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Abstract

Traditional analysis in population genetics evaluates differences among groups of individuals and, in some cases, considers the effects of distance or potential barriers to gene flow. Genetic variation of organisms in complex landscapes, seascapes, or riverine systems, however, may be shaped by many forces. Recent research has linked habitat heterogeneity and landscape or seascape configuration to genetic structure by integrating methods from landscape ecology, population genetics, and spatial statistics in approaches known as landscape or seascape genetics. However, challenges when translating these approaches into freshwater river networks result from functional differences in riverscape topography that create constrained pathways for movement pose and directional water flow pose necessitates development of new methodology. Studies that may be described as riverscape genetics have linked temperature, stream gradient, and confluences to genetic variability. Lack of consistency in methodology has made comparisons across species and scales difficult. We provide a perspective on how riverscape genetics could be used to provide a more comprehensive conceptual and applied understanding of connectivity and dispersal in freshwater systems. We describe four thematic areas of study representing current and future research opportunities and describe a basic workflow for conducting riverscape genetics analysis. Although numerous methodological challenges remain, a riverscape genetics approach can enhance our understanding of habitat heterogeneity in shaping gene flow and spatial genetic structure. These characteristics of populations are critical components for interpreting demographic and evolutionary consequences of habitat loss and fragmentation.

Introduction

In most natural systems, gene flow and species dispersal are key processes with fundamental influences on demography and evolution of spatially-structured populations. Gene flow and dispersal are influenced by the interaction of locally-adapted life history traits and habitat heterogeneity. Genetic structure is influenced most strongly by genetic drift, gene flow, and in some cases, natural selection (Banks

et al., 2013). Maintaining genetic diversity and gene flow may enable populations to respond to environmental change through the spread of locally adapted genes (Kawecki and Ebert, 2004). Yet, the rate and timing of changes to landscapes may be more rapid than the potential adaptive response by organisms, thereby increasing the threat of local and regional extirpations (Kawecki and Ebert, 2004). This may be particularly critical for freshwater species whose movement is restricted to connectivity within river networks that are highly vulnerable to fragmentation.

Understanding the role of habitat heterogeneity in shaping spatial genetic structure is necessary to interpret the evolutionary consequences of habitat loss, fragmentation, and environmental change. Yet, only recently have researchers begun to address this systematically. A fundamental component of traditional population genetic analysis is the comparison among groups of samples, relating genetic differences to geographic distance (also referred to as Euclidean distance) and considering "panmixia" (no spatial pattern) as a null hypothesis. The expected correlation between genetic structure and geographic distance is known as isolation by distance, or IBD (Rousset, 1997; Wright, 1943). In rivers, distance along waterways ("waterway distance") has been recognized as a more biologically meaningful measure to relate to genetic structure, but even that measure may not capture other key characteristics of the river that may influence movement of a particular species or life stage (Selkoe et al., 2016).

In terrestrial and oceanic systems, increasing numbers of studies have used systematic characterizations of land- or seascapes to assess how habitat heterogeneity and configuration influence genetic structure. These approaches are known respectively as "landscape genetics"(Manel et al., 2003) and, more recently, "seascape genetics" (Selkoe et al., 2008). These approaches move beyond explaining genetic structure solely as a function of IBD by generating models that describe how specific habitat enhance or provide resistance to the movement of individuals, and testing whether those models explain genetic differences better than IBD or panmixia. This is accomplished by integrating theory and methods from landscape ecology, population genetics, and spatial statistics (Manel et al., 2003; Selkoe et al., 2016).

Most landscape genetic (LG) analyses seek to use genetic data to test hypotheses about the influence of landscape on gene flow. Early LG studies often tested for barrier effects ("isolation by barrier", hereafter IBB), where a specific landscape barrier (e.g., road, rivers) is identified and levels of genetic variation among populations separated by the barrier are compared (Holderegger and Wagner, 2008; Storfer et al., 2007). Methods were developed to explore the complex effects of multiple landscape variables (e.g., precipitation, temperature, elevation) on individual movements leading to gene flow; genetic structure resulting from such habitat heterogeneity is often referred to as "isolation by environment", hereafter, IBE (Wang and Bradburd, 2014). A common approach to test IBE hypotheses, as pioneered in LG, assigns "resistance" values to different habitats based on their presumed effect on individual movements, which are then mapped across the study area (Cushman et al., 2006; Epps et al., 2007). The estimated cumulative "cost" of movement between sampling locations (hereafter, "effective distance") is then estimated (Shirk et al., 2010). Each set of resistance values can be regarded as a hypothesis to explain genetic structure among sampling locations. Typically, many such hypotheses are generated, and researchers determine which set of effective distance estimates is most strongly correlated with genetic differences (Cushman et al., 2006; Epps et al., 2007; Spear et al., 2010).

Studies using seascape genetics (SG) face challenges different from LG. Life histories of many marine organisms are characterized by large population sizes, high fecundity, external fertilization, and planktonic larvae. Ocean currents transport organisms during their planktonic larval phase leading to populations that are spatially distributed across considerable distances. The time and cost associated with adequate environmental and genetic sampling of such broadly distributed populations is a difficulty when conducting SG analysis (Lal et al., 2017; Riginos and Liggins, 2013). As a result, empirical data describing dispersal for the majority of marine organisms are limited (Cowen and Sponaugle, 2009; Liggins et al., 2013). Due to the variable and complex nature of ocean currents, a single oceanographic value cannot be attributed to a specific location, therefore LG-type approaches based on resistance surfaces have rarely if ever been employed (Hansen and

Hemmer-Hansen, 2007; Riginos and Liggins, 2013). Instead, SG studies have revealed the influence of hydrogeomorphic properties on spatial genetic structure by combining genetic data with a variety of non-genetic information derived from complex models (e.g., sea surface temperature), current speed and direction, chlorophyll, or coupled biological-physical models) summarized over an appropriate time scale (Banks et al., 2007; Hansen and Hemmer-Hansen, 2007; Liggins et al., 2013; Riginos and Liggins, 2013; Selkoe et al., 2010). With increased computing power, simulations have provided a way to circumvent the lack of empirical data. For instance, larval dispersal models can be created by compiling data from physical oceanographic conditions and particle tracking models. Those simulated data can be used in a graph-theoretic approach, discussed in a later section, where migration probabilities derived from biophysical models define "edges" that connect patches of genetically distinct populations, or "nodes" to create a network (Johansson et al., 2015; Urban et al., 2009). Node clusters and individual nodes or edges defined within the network reflect complex processes that contribute to spatial genetic structure. Each alteration to modelled migration probabilities reflects a hypothesized explanation of how functional connectivity affects genetic structure. Multiple hypotheses can be tested allowing researchers to determine which model generated patterns are most similar to observed genetic structure.

Recently, land- and seascape genetic approaches have been extended to studies of riverine organisms in research that can be called "riverscape genetics" (RG). Defined as an aquatic counterpart to landscape genetics by Kanno et al. (2011), RG analysis is becoming more common, but the term is not universally used or consistently applied in practice. In the context of RG, use of the term "riverscape" bears resemblance to the use of the terms landscape and seascape in LG and SG. Therefore, we define the riverscape as a hierarchically structured mosaic of differentially distributed habitat within freshwater environments. Fully extending land- and seascape approaches into riverine networks will require consideration of the dynamic features of rivers, notably, the directional movement of water flow and physical network structure that is highly constrained and hierarchically organized

(Isaak et al., 2014). Studies that can be described as RG have shown that elements of riverscapes related to temperature, stream gradient, culverts, and confluences are often linked to genetic differences (Bowlby et al., 2016; Leclerc et al., 2008; Ozerov et al., 2012; Torterotot et al., 2014), but many other variables (e.g., seasonal water flow, high flow events, precipitation) act inconsistently across species and scales. Such differences may reflect inherent characteristics of different species and systems, but may also result from variation in the strength of study designs. These inconsistencies suggest that the riverscape presents analytical challenges not presented by terrestrial landscapes or seascapes, and may require the development of new analytical approaches (Isaak et al., 2014).

Implications of water flow and rivers as networks in the development of methodologies for riverscape genetics

Riverscapes include features that are rarely encountered in landscapes, and hydrologic processes that influence dispersal in aquatic environments differ from those in marine environments. For example, freshwater hydrologic connectivity is driven by downstream water flow; (Poff et al., 1997) while in marine systems, ocean currents are largely responsible for this interaction (Hughes et al., 2009). Seascapes lack strong ties to terrestrial landscapes, while many river systems exhibit complex branching patterns formed by iterative tributary junctions that are embedded in terrestrial habitat. This branching pattern is often called the “river network”, (Benda et al., 2004; Thorp et al., 2006) and is much more physically constrained with respect to potential connections among locations as compared to seascapes (Rodriguez-Iturbe et al., 2009). Changes in hydrology add variability that is rarely observed in terrestrial landscapes. Directional water flow and river network pattern underlie movement pathways for aquatic species, directly affecting patterns of gene flow, dispersal, and increasing the potential for isolation. Existing analytical methods developed for LG and SG may not adequately account for the affect of these physical differences on expectations of gene flow, dispersal, and connectivity.

The spatial configuration of the network is another important characteristic of riverscapes, but fitting spatial statistical models to stream networks is a

particularly challenging task because of the flow-connected nature of freshwater habitats (Ver Hoef and Peterson, 2010). The pressing need to understand how connectivity, flow, and stream hierarchy affect dispersal or movement has led to development and adaptation of spatial statistical models in freshwater ecology (e.g., variograms and graph-theory; for reviews see Isaak et al. (2014), Dale and Fortin (2010); Isaak et al. (2014)). Development of more refined tools that incorporate geophysical properties and better reflect the spatial configuration of the river is an important step toward understanding connectivity (Ganio et al., 2005; Peterson et al., 2013; Ver Hoef et al., 2006). These methods have not been fully incorporated into analysis of spatial genetic variation of freshwater organisms. Therefore, developments of spatial statistical tools and models that expressly use the physical stream network in ecological studies have potential to be foundational for novel genetic analysis in freshwater systems (Brauer et al., 2016).

Understanding how changes to rivers influence dispersal and genetic structure is essential for appropriate management and attempts to restore connectivity. Therefore, continued development of the RG approach could provide important tools for conservation, management, ecology, and evolutionary biology of species in these ecosystems. In this paper, we provide a perspective on the utility of such an approach for providing a more comprehensive and holistic understanding of dispersal and connectivity in river networks. We evaluate studies that self-identify, or could be described, as using a RG approach. Based on those studies as well as directions for future work, we describe four broad themes: 1) detecting the impacts of anthropogenic and natural barriers on dispersal, connectivity, and genetic structure, 2) identifying riverscape factors that affect the scale and pattern of spatial genetic structure in a stream, 3) separating effects of historical, and contemporary riverscapes on genetic structure, and 4) linking spatial adaptive genetic variation to the heterogeneous riverscape. Within each theme we summarize what current research has contributed to understanding correlations between gene flow and environmental variation while also identifying avenues for continued exploration. Then, we describe three methods commonly used to quantify the effect of environmental variables on genetic structure. We provide a perspective

on how to expand analysis so that each method may be more appropriate for RG and discuss the implications of such methodologies for study design.

Linking genetic structure with the geophysical template of the river system

Our review of the literature, although not exhaustive, has demonstrated that studies have linked dispersal barriers (Theme 1) and environmental variables (Theme 2) to contemporary gene flow and genetic structure within and among populations of riverine species. While the gains in understanding linkages between the geomorphological riverscape and genetic structure have been illuminating, substantial opportunities for growth remain. For example, incorporation of ancient riverscapes (Theme 3) would provide a control for effects of historic events on observed gene flow, thereby allowing for the correct correlation of contemporary genetic structure with effects from modern riverscape features. We found that the majority of studies have used markers with no effect on fitness (neutral genetic variation) but markers that are experiencing selection may bear more direct link to the environment. Therefore, a more visionary application of gene-environment associations may co-develop with RG and other leading edges of inquiry (e.g., evolutionary and molecular ecology, phylogeography, or epigenetics) into the genetic basis of local adaptation using markers that are associated with direct effects on fitness (adaptive genetic variation; Theme 4). The following themes provide a glimpse of the breadth of knowledge that a RG perspective can bring to our collective understanding of dispersal and spatial genetic variation.

Theme 1: Detecting the impacts of anthropogenic and natural barriers on dispersal, connectivity, and genetic structure.

The structure of the river network is inherently vulnerable to fragmentation that alters hydrologic connectivity (Fagan, 2002). Long-term population persistence is specifically related to connectivity. The physical distribution of habitat patches (structural connectivity) and the ways organisms navigate the river network to access specific habitat (functional connectivity) are components of connectivity that influence genetic structure in different ways. As organisms migrate among habitat

patches at different places in the network, they experience environmental variation (e.g., temperature, stream gradient, or waterfalls) that produce variable resistances to movement. Naturally-occurring disturbances (e.g., debris flows or fires) fragment habitat at seasonal and intermittent time scales, presenting barriers to dispersal that may erode over time, changing patterns of structural and functional connectivity throughout the river network. Anthropogenic barriers (e.g., dams, culverts, dikes) fragment the riverscape over longer timescales and exacerbate the effects of ongoing natural disturbance events (Benda et al., 2004; Reeves, G.H. et al., 1995). Additionally, river network structure rarely allows alternative dispersal pathways; therefore, changes in connectivity caused by either type of fragmentation have potential to greatly influence dispersal and genetic structure (Hughes et al., 2009; Yamamoto et al., 2004).

Barrier effects are especially evident for upstream passage of migratory species that encounter dams and fish ladder operations. In some cases, permanent extirpation of fish has occurred above such barriers, while in other river networks fish are able to persist (Lindley et al., 2004). For example, Torterotot et al. (2014) evaluated effects of fragmentation by natural and non-natural barriers on *S. fontinalis* and found that the cumulative number of barriers correlated significantly with patterns of genetic diversity. Genetic structure is often identified among populations that are up- or downstream of these types of physical barriers for species including Chinook, brook trout, and chum salmon (Neville, Helen M. et al., 2006; Torterotot et al., 2014). Natural barriers also affect connectivity of habitat within the river network by altering genetic structure for migratory and non-migratory fishes. Castric et al. (2001) associated waterfalls with decreased heterozygosity among populations of brook charr but the authors were unable to find significant genetic structure that was related to IBD or IBB. Leclerc et al. (2008) evaluated spatial genetic structure of yellow perch (*Perca flavescens)*, finding distinct populations that were separated by a dam or zones of high velocity water flow that prevented migration.

Although the effect of barriers on genetic diversity was commonly investigated among studies we reviewed, distance (IBD) and barriers (IBB) alone

rarely explained the majority of genetic variation in freshwater systems (Cook et al., 2011; Dionne et al., 2008; Earnest et al., 2014; Sprehn et al., 2015; Torterotot et al., 2014). Kanno et al. (2011) used eight microsatellite loci (Table 2.2) to calculate genetic diversity measures of anadromous fish (*Salvelinus fontinalis*) and associated weak structure with barriers that partitioned individuals among three tributaries. However, one tributary within the study lacked a physical barrier but still supported genetically structured populations. To better understand how genetic structure is shaped within the river we should look beyond barrier and distance hypotheses to include IBE.

Theme 2: Quantifying the effect of the riverscape on scale and pattern of spatial genetic structure

The effects of hydrologic connectivity on fish ecology have been studied in freshwater systems because hydrology is one of the primary contributors to the spatial configuration of habitat within the river network (Fagan, 2002; Fullerton et al., 2010). For example, Flitcroft et al. (2014) determined that the spatial distance between habitats and the availability of habitats for specific life histories are determinants in the pattern of juvenile coho salmon distribution throughout river networks. The authors hypothesized that stream flow contributed to observed spatial patterns but were unable to test this hypothesis with their dataset. Studies using multiple genetic and riverscape datasets to test hypothesis of IBE have demonstrated correlations between spatial genetic structure and temperature, stream gradient, number of confluences, drainage basin, seasonal precipitation, seasonal water flow, and high flow events (Cook et al., 2011; Kanno et al., 2011; Olsen, Jeffrey B. et al., 2010). For example, Castric et al. (2001) quantified spatial genetic patterns in *Mogurnda mogurnda* sampled at 17 sites within multiple watersheds. They tested if genetic variation was correlated with linear distance, maximum stream gradient, elevation, or discharge. IBE models have successfully quantified genetic structure for a wide variety of fish species including Salmonids, (Hand et al., 2016; Olsen, J. B. et al., 2010; Ozerov et al., 2012) topminnows, (Earnest et al., 2014) tropical freshwater fish, (Brauer et al., 2016; Cook et al., 2011)

headwater chub, (Pilger et al., 2015) and electric fish, (Cooke et al., 2014) and in a more limited capacity, freshwater mussels (Galbraith et al., 2015) and parasites (Sprehn et al., 2015). Collectively, these studies provide evidence that complex interaction between climate variation and habitat heterogeneity have shaped elements of spatial genetic structure in freshwater species, and further emphasize limitations of IBD used alone to explain genetic variation in streams.

Theme 3: Separating effects of ancient and contemporary riverscapes on genetic structure

Genetic diversity is the result of cumulative environmental and geologic processes that have occurred at varying temporal and spatial scales, each leaving interpretable marks in the genome. As addressed above, dispersal barriers resulting from riverscape fragmentation may strongly influence spatial genetic structure of populations. Traditionally, species or populations that occupy high-elevation, dendritic tributaries are expected to be more physically isolated because of human alteration to downstream reaches. The increased probability of barriers increases resistance to gene flow with upstream populations. However, ancient historical events (e.g., the last glacial maximum) have shaped genetic structure and species diversification (Avise, 2000; Hickerson et al., 2010). Geological processes that restructured the range and distribution of biota across entire river networks included ancient mega-flood events and Pleistocene glacial cycles that dramatically changed connections among populations (Hickerson et al., 2010). Without accounting for the effects of ancient riverscapes on contemporary spatial genetic structure, genetic structure from such ancient legacies could be incorrectly attributed to a modern riverscape feature, complicating efforts to understand and predict changes in dispersal or genetic structure.

Molecular techniques and computer simulation have aided in the ability to detect legacies of ancient geographic, geologic, and climatic events on extant populations (Hickerson et al., 2010). Identifying evidence of bottlenecks, decreased genetic variation associated with long periods of isolation, and other genetic signatures have helped illuminate ancient colonization events (Swatdipong et al.,

2009). For instance, Waples (2001) describes the contemporary patterns of diversity among Pacific salmon lineages as resulting from interspecific diversification following the last glacial maximum and evolution during the Holocene. High-magnitude but low-frequency disturbance regimes around the last glacial maximum caused massive extinction events and reshaped entire river networks enabling species diversification in Pacific Northwest salmonids. Similar historic relationships exist between present day diversification and historical geologic events within the lineages of marine organisms. For instance, genetic discontinuities among the greenshell mussel (*Perna canaliculus*) have been attributed to sea level fluctuations that occurred throughout the Pleistocene when dynamic geological and hydrological processes established Cook Strait. Without accounting for this legacy it would not be possible to distinguish between current and past processes that are influencing genetic variation.

LG and SG studies offer several examples of such approaches (Epps and Keyghobadi, 2015). For instance, in a LG study of a desert-dwelling plant, Dyer et al. (2010) analysed the correlation of genetic distances among sampling locations to a distance matrix summarizing phylogeographic variation. Then, from the residuals of that relationship, they evaluated effects of IBE using effective distance matrices estimated from the current landscape. Similarly, based on recognition that greenshell mussel divergence among the North and South islands of New Zealand had been attributed to historical events, Wei et al. (2013) used a SG approach to quantify contemporary genetic diversity within the two historically diverged populations correlated to regional environmental data. They explored the relationship between pairwise genetic differentiation and distance matrices of environmental and geological data by using regression analysis for each island separately. In a freshwater example, Osborne et al. (2014) investigated landscape-scale spatial genetic structure among three Great Plains fish species using a simple linear regression of allelic richness (A_r) and latitude. The authors found that increasing genetic variation correlated with increasing latitude, reflecting a postglacial colonization history. Then, after accounting for the effect of latitude, the authors investigated whether species-specific genetic diversity (A_r) and structure

(F_{ST}) was influenced by modern fragmentation. They found that genetic variation among the sites did not reflect the influence of contemporary barriers on gene flow, highlighting the importance of first controlling for influences of historical processes (Gouin et al., 2011; Hanfling et al., 2002).

Theme 4: Linking spatial adaptive genetic variation to the heterogeneous riverscape

Individuals that occupy the same local riverscape experience selective pressures that act to maximize individual fitness within a specific habitat. However, local adaptation is not a guaranteed outcome for individuals in all populations because it is mediated by the life-history of the organism, evolutionary processes, and environmental interaction. For instance, gene flow will counter effects of selection by maintaining frequencies of alleles, genetic drift balances selection by buffering increased frequency of adaptive loci (especially in small populations), and the underlying genetics of traits (e.g., plasticity and epigenetic effects) can constrain adaptation (Kawecki and Ebert, 2004). Furthermore, selective pressure from the heterogeneous riverscape will vary spatially and temporally, favoring genotypes differentially over time and space. Nevertheless, many studies find convincing evidence of local adaptation. For instance, Xu et al. (2013) identified loci that were related to stress response of a cyprinid fish, and demonstrated adaptation to alkalinity by sequencing RNA. Torres-Dowdall et al. (2012) found significant correlations between genetic differentiation among multiple traits among Trinidadian guppies reared in high- and low-predation conditions. Whitehead et al. (2011) studied plasticity in killifish (*Fundulus beteroclitus*) and found genes related to osmotic shock correlated with local adaptation to pollution tolerance.

Evidence of local adaptation in natural populations is challenging to acquire because demonstrating these types of effects typically requires that a gene x environment association leads to higher fitness by an organism in one environment relative to another environment (Hereford, 2009). This can be accomplished by translocation or common garden experiments, which provide estimates of heritability and genetic effects from replicated experimentation. These types of studies are difficult to conduct on wild populations because of difficulties associated

with rearing in laboratory conditions or limitations associated with listed species status, among other complications. In absence of experimentation, computer simulations have provided the means to identify molecular markers that may be experiencing selection (Antao et al., 2008; Gunther and Coop, 2013; Narum and Hess, 2011). For example, the program LOSITAN produces an estimate of F_{ST} for each locus from an empirical dataset and then simulates the expected distributions of F_{ST} and heterozygosity (H_e) under neutral processes. Loci that fall outside of the distribution are "outliers" and may be experiencing selection (Antao et al., 2008). This method was used by Chang et al. (2013) to test if genetic differentiation among cyprinid fish (*Leuciscus waleckii*) sampled from alkaline and freshwater environments was caused by selection pressure from the increase in alkalinity. Using microsatellite loci and mtDNA sequences the authors detected a single outlier locus that may play a role in local adaptation to alkalinity for this species. Continued discovery of markers that are potentially under selection will improve the quantification of patterns of adaptive genetic differentiation, advancing research in evolutionary ecology and epigenetics that seeks to understand adaptive evolution in natural populations.

The adaptability of spatially structured populations is affected by dispersal of organisms across their spatially heterogeneous environment. Therefore, understanding how dispersal is affected by functional and structural connectivity would provide useful information applicable for the study of adaptive evolution. The movement or redistribution of local adaptive genetic variation among sub-populations has fitness consequences. Evaluating adaptive genetic variation as well as neutral genetic variation in a RG context may clarify processes influencing genetic structure and provide predictive capabilities for interpreting the influence of new challenges to native species (e.g., biological invasions, hybridization resulting in decreased fitness, disease vectors).

Applying the mechanics of landscape genetics to riverscapes

The themes that were identified above illuminate the importance of incorporating a RG perspective in analysis of freshwater organisms. To achieve this

goal, a robust study design and novel analytical methods that account for directionality of flowing water and spatial layout of the river network river system are needed. Although there is not a unifying or "one-size-fits-all" approach in LG or SG, shared similarities can be used as the basis for a unifying set of approaches. Therefore, we recommend RG practitioners carefully consider reviews of LG (e.g., Balkenhol et al. (2009), Segelbacher et al. (2010)) and SG (e.g., Selkoe et al. (2016), Liggins et al. (2013)). In this section, we discuss the similarities in approaches with regard to application of dissimilarity matrices, resistance surfaces, and network and graph theories (Dyer et al., 2010; Murphy et al., 2008; Proulx et al., 2005).

Riverscape genetics analysis requires a dataset representing genetic variation (Table 2.3) among samples at an individual or a population level, and a suite of measured environmental variables (Table 2.4) that are hypothesized to affect genetic variation. The more complex task, once datasets are available, is to select an appropriate suite of analytical tools with the power to detect effects while minimizing Type I and Type II error. Common analytical methods include the Mantel test and its derivatives, which assess the correlation between matrices of pairwise measures,(Bowlby et al., 2016; Cooke et al., 2014; Dionne et al., 2008; Earnest et al., 2014; Galbraith et al., 2015; Kanno et al., 2011; Pilger et al., 2015) multiple-regression on distance matrices (MRDM), which tests for effects of multiple variables on genetic variation, (Bowlby et al., 2016; Diniz-Filho et al., 2009; Kanno et al., 2011) and regression modeling (Hand et al., 2016). Of these, many are widely criticized (e.g., Mantel tests and partial Mantel test) for propensity to underestimate Type I error. For additional review of the applicability and utility of Mantel test in LG and SG analysis we refer to Raufaste and Rousset (2001), Legendre and Fortin (2010), and Legendre et al. (2015).

Dissimilarity matrix: comparisons among sites across large spatial distances

The simplest comparison that can be made is to test if genetic differences among individuals or populations are related to differences in factors such as elevation, temperature, or stream depth that has been measured at each sampling site (Figure 2.1). Pairwise differences are calculated for each factor, forming a dissimilarity matrix. Genetic variation is measured at each site and pairwise

differences between sites are calculated. The datasets are compared to identify if changes in any variables correlate with identified genetic differences. In an example of this approach, Castric et al. (2001) hypothesized that a greater occurrence of permanent waterfalls existed in areas with high altitudinal differences, and therefore hypothesized that genetic structure would exist across the permanent barriers. To test this, the authors formed a dissimilarity matrix of altitude variation following the shortest waterway distance between 30 Brook Charr (*Salvelinus fontinalis*) populations and compared this data to a pairwise genetic distance matrix generated from a suite of six microsatellite markers. Similarly, Kanno et al. (2011) evaluated if mean stream temperature, mean stream gradient, waterway distance, number of seasonal barriers, and number of confluences were related to pairwise genetic variation among populations of *S. fontinalis*. To test for IBE, the authors created a dissimilarity matrix of each variable by reach using regression analysis to determine which predictor explained genetic structure.

While the flexibility provided by the dissimilarity matrix makes it a versatile tool for addressing a variety of questions, the method does not allow for accurate interpretation of processes between sampling sites. For instance, the above example where Castric et al. (2001) used the difference in elevation among sites as a measure of potential waterfall barriers could have been strengthened by a detailed estimation of such barriers along each section of waterway using a fine-scaled Digital Elevation Model (DEM). Thus, dissimilarity matrices provide some context of the differences among patches that contribute to overall genetic variation, but cannot easily capture the continuous exposure to environmental selection and resistance experienced by organisms moving through the riverscape. The next methods we discuss were developed as a way to quantify the contribution of these "en-route" effects on genetic variation and gain greater insight of structural and functional connectivity.

Resistance surface and path-based analysis to calculate effective distance

The resistance surface transforms the landscape into numerical values that depict different habitats, substrate, vegetation, or other features of interest so that

hypotheses about the cost or presumed influence of those features on movement (i.e., effective distance) can be tested (Zeller et al., 2012). Analytical GIS tools have enabled widespread use of resistance surfaces to calculate effective distances for species in heterogeneous environments. A raster image is a grid of cells (pixels) representing individual or multiple variables (Figure 2.2). Each cell in the raster is assigned a numerical value. In the simplest raster map, values can reflect the presence or absence of a variable (e.g., road or barrier). In more complex models, weights reflect the presumed influence of each variable on species dispersal, movement, or gene flow (Epps et al., 2007; Shirk et al., 2010). Cells in the final raster represent cumulative weights that are derived by independently summing each cell across all variables. The values or weights on the resistance surface allow researchers to quantify the influence of covariates (e.g., elevation, gradient, temperature) on some response variable of interest (e.g., movement, genetic differentiation, gene flow). For example, Epps et al. (2007) employed resistance surfaces to understand the role of slope and distance on genetic structure of 26 bighorn sheep populations using 14 microsatellite loci. To test for IBE, Epps et al. (2007) created raster maps from DEMs and established 18 topographic resistance models that represented a range of weighting schemes based on slope. Then, using least cost path analysis, they estimated the cumulative cost along the least costly path between each pair of populations for each resistance model. The resulting matrices of effective distance were tested to see which was most strongly correlated with matrices of pairwise estimates of gene flow.

Rasterized maps work well for depicting difference in terrain across a terrestrial landscape. The hypothetical landscape shown in figure 2.1 includes roads, river, forested, and urban areas. For a terrestrial organism the weighted rasterized grid may represent features such as roads, non-forested habitat, or wide high-flow river sections as somewhat or highly resistant to passage (e.g., weight = 3). However, there are multiple routes of travel that would avoid these impediments. Identification of the most likely route that will be travelled is based on knowledge of species-specific movement and biological needs of the organism, and the assumption that organisms will tend to move through the habitat in a pattern

that represents the least costly path of resistance (e.g., least-cost path analysis (Wang et al., 2009)). The increase in use of resistance surfaces and path-based analysis for LG reflects, in part, the potential for such analyses to inform conservation efforts by clarifying how complex landscapes influence movement.

Although resistance surfaces have worked well in a terrestrial landscape, this technique may not be appropriate for all river networks, such as the dendritic river depicted in Figure 2.2. In a freshwater river context, weights for the grid cells could reflect areas with a dam, culvert, or waterfall as somewhat or highly resistant to passage, while different velocities of free-flowing water could also vary in resistance. In contrast to Figure 2.3, the dendritic river network depicted lacks alternative pathways. Therefore, path-based analysis in this situation may not provide additional knowledge about the costs of travel within the network, although larger river systems or non-dendritic river systems could have a greater diversity of pathways that vary in resistance (e.g., a river in flood stage that expands laterally into a mangrove forested system). Even where the variation in riverscape resistance can be modelled, existing methods for acquiring accurate measurements of the riverscape at fine spatial scales within these dynamic systems are limited. Thus, path-based analyses based on different resistance models will tend to produce highly correlated estimates of IBE in these systems, restricting inference to detecting only variables with very large impacts on resistance.

Spatial graphs and network theory model connectivity

Theoretical models available in graph-theoretic analysis are well suited to model a dendritic environment such as freshwater rivers. In a graph-theoretic approach, the user is able to interpret structural or functional connectivity of a population by assigning habitat patches or populations as “nodes” (e.g., spawning or breeding sites, reef structure or discrete habitat) that are connected by “edges” representing any measure of connectivity (e.g., pairwise genetic distance, dispersal rates, or migration patterns) (Hines and Borrett, 2014; Urban et al., 2009). The resulting graph or network aids visual depiction of levels of clustering or metapopulation structure among the nodes, revealing relationships that are not easily identifiable in other ways (Proulx et al., 2005; Urban et al., 2009). More

importantly, this method can provide an efficient characterization of connectivity at multiple spatial scales. Graph topology analysis provides additional interpretation of network structure (Urban et al., 2009). In Figure 2.4, the sampling sites from the hypothesized riverscape shown in Figure 2.1 were transformed into two hypothetical networks representing different life histories. In each, nodes represent spawning habitats that are connected by dispersal. Examining node "degree", the number of edges connected to a node, may identify populations that have possible genetic isolation (i.e., fewer or weak edges). Sequences of node-edge pairs that are oriented in closed loop "cycles" provide information about potential subpopulations that may experience gene flow. For example, site 3 (Figure 2.4b) is in a reservoir created by a dam. Few edges connect it to the network, suggesting the potential for isolation that may be reflected by increased genetic structure and lower heterozygosity than other sites. In this example, a mobile aquatic organism may experience limited connectivity with the rest of the network if migration into and out of the reservoir was not completely restricted. In Figure 2.4b, a directed network was used to reflect life history of a sessile organism that experiences dispersal during the larval phase. The direction of water flow drives patterns of dispersal. In a directed network, sites upstream connect to all other flow-connected sites that are downstream. Site 2 may therefore reflect similarity with both upstream sites and have greater heterozygosity, while upstream sites are increasingly dissimilar from each other. Thus, in a distinctly different approach than the distance-based statistics commonly employed in LG, network statistics can be used to summarize testable hypotheses about the influence of riverscape on the characteristics of nodes, such as genetic diversity.

In an example of a weighted network analysis applied in rivers, Schick and Lindley (2007) depicted source-sink dynamics of spring-run Chinook salmon (*Oncorhynchus tshawytscha)* with a historical perspective. Nodes reflected the size of spawning populations; edges that linked nodes were constructed from a migration matrix calculated as a function of distance, dispersal probability, and population size. The authors tested if changes in topology reflected cumulative effects of historical migration barriers by creating multiple networks from which

they identified extirpations, loss of source nodes, and changes in structure of node cycles. If applying a RG approach to this analysis, the Schick and Lindley network could be used to establish predictions that could be tested using empirical genetic data. For example, a prediction that cycles reflect potential sub-populations within the network could be evaluated from genetic samples collected at all sites (nodes). Significant pairwise measures of genetic structure or identification of genetically-distinct clusters could provide support for presence of sub-populations that may be meaningful for conservation and management of the species.

SG demonstrates the utility and flexibility of networks and graph-theoretic analysis to incorporate genetic, biophysical and hydrogeological model into analysis. Johansson et al. (2015) investigated connectivity (IBD) of giant kelp (*Macrocystis pyrifera*) throughout their northeast Pacific distribution to evaluate if biogeographic population structure was explained by ocean transport. A network built from empirical data consisted of nodes, genetically distinct populations that were identified by individual assignment tests (Table 2.3), and edges that were weighted by pairwise values of genetic differentiation (F_{ST}). To test if clustering observed within the network resulted from propagule dispersal by ocean transport, the authors constructed a network where edges reflected transport time between nodes based on seasonal oceanographic transport distance as modelled by Lagrangian particle simulations. Network theory as applied by SG has great potential for development of RG where similar relationships between geographic space, organism life history, and hydrology also exist.

Using computer simulations in riverscape genetics

Computer simulations are used in a variety of scientific fields to validate findings, make predictions, build scientific theories, and test hypotheses. Computer simulations offer tools to explore natural systems and provide insights about system functions when it may be impractical to do so with empirical data (Epperson et al., 2010; Hoban et al., 2011). For example, larval dispersal in marine systems results from the physical movement of water; therefore, larval settlement and resulting gene flow is connected to oceanographic conditions. Empirical data describing larval

dispersal for marine organisms are sparse but computer simulations have provided a way to estimate these processes. The utility of simulations is vast and varied; they can be used to evaluate current conditions, predict future conditions, and even recreate past conditions (Epperson et al., 2010). A spatially-explicit individual modelling program (Landguth and Cushman, 2010) was used by Castillo et al. (2014) to simulate mating and dispersal of American Pika at Crater Lake National Park in Oregon as a function of environmental resistance. The authors also collected empirical data, and using resistance surfaces to analyze effective distance they determined that gene flow was affected by topographic complexity, water, and aspect. The modelled predictions of genetic variation were compared against empirical data to evaluate if resistance values could generate the observed genetic variation, and more importantly, whether the analytical methods used to pick the "best" model were likely to select the correct explanatory model. Simulations provide a useful method to test multiple hypotheses about the evolutionary mechanisms that underlie the observed spatial patterns. User-friendly programs have made simulation more accessible to researchers who conduct such analysis in aquatic environments (e.g., CDFISH, (Landguth and Cushman, 2010; Landguth et al., 2014) AQUASPLATCHE, (Neuenschwander, 2006) SPLATCHE2 (Ray et al., 2010)).

The predictive capability of computer simulation is another useful function with direct application to SG and RG. Computer simulations are used to build hydrologic models that predict flow and runoff in freshwater rivers or current fluctuations and ocean circulation in marine environments. Typically, models predict processes that are not easily acquired through physical sampling of the environment. For instance, Galindo et al. (2010) modelled larval dispersal of the intertidal acorn barnacle *Balanus glandula* to predict larval settlement. The authors coupled a Regional Ocean Modelling System (ROMS) with an ecosystem model (Carbon, Silicate, and Nitrogen Ecosystem – CoSINE) as well as models of larval development and particle-tracking to simulate larval dispersal. Genetic structure was calculated for the settlement sites that were predicted by the simulated data. Predictions of genetic structure were compared against empirical data to determine which model best described genetic structure. Computer simulations can be

powerful and cost-effective tools that enable researchers to understand the effects of complex processes on evolution and demography but should be used with care. Simulated models are essentially detailed hypotheses about processes; therefore, without empirical data to confirm or deny findings and without quality inputs (model parameters), modeled predictions may differ quite considerably from reality.

Considerations for building an appropriate sample design

Sample collection

Sampling design is a fundamental component of robust research but it is often unclear how different sampling strategies affect interpretation of analysis and conclusions. Of the many decisions associated with design and implementation of a sampling plan, sampling locations, sample number, marker type, and the ideal number of loci to characterize genetic differences at a particular scale are often the most difficult. Efforts in LG to understand the effects of different sampling schemes on landscape genetic inference have provided valuable insight. These types of simulation studies are particularly needed in RG, but are lacking (Landguth et al., 2012a; Murphy et al., 2008). Sampling is often associated with time, space, and cost limitations that ultimately may dictate overall feasibility of a project, but other factors are sometimes overlooked. In the context of RG, collecting an appropriate number of samples relates to whether sampling will occur at the level of individuals or populations. LG has embraced individual level sampling and analysis, while both SG and RG studies are primarily reliant on population level sampling. LG researchers have compared spatially-explicit individual-based models with population based sampling designs typical of classic population genetics to understand the difference between sampling individuals and populations in interpreting population structure (Landguth and Cushman, 2010). Simulation tools are now available that allow researchers to evaluate interactions between gene flow and selection in terrestrial systems but are limited in flexibility for modeling diverse reproductive and dispersal strategies of marine and freshwater organisms (Landguth et al., 2012b).

Nevertheless, evaluating the difference among inferences of each sampling level is an important question to answer in RG.

Genetic Data

Selection of an appropriate molecular marker requires understanding the capabilities and limitations of each marker and what type of genetic information is necessary to test the hypothesis (Table 1.2). For example, SNPs are easy to generate and many sequencing platforms are available for SNP discovery, but single SNPs have limited information content because there are few polymorphisms per locus. Therefore, hundreds may be needed for appropriate power to detect genetic structure (Schlötterer, 2004). In comparison, microsatellites are more time consuming to isolate but tend to have more polymorphisms per locus; therefore, fewer are needed to garner the necessary power to detect genetic differentiation (Table 1.2). Mitochondrial DNA (mtDNA) would be useful marker choice when tissue samples are degraded because its structure resists degradation, but because inheritance of mtDNA originates from a single parent, the applicability of this marker is limited (Table 1.2). Next generation sequencing has improved the ability to acquire genetic data of all marker types at increasingly affordable costs. Increasing interest in the development of novel genetic tools will continue. Deciding on the appropriate marker will require trade-offs between costs of acquiring the number of loci that have statistical power to identify differences, and the number of individual samples to collect. Ultimately, the number of polymorphisms per marker and the number of loci needed to amplify are two of the main decisions for selecting a marker. As demonstrated by Landguth et al. (2012a), a greater number of polymorphic loci, rather than increased sample number, resulted in greater power to detect LG relationships. To identify this correlation, the authors employed a spatially explicit individual assignment program, CDPOP, to simulate genetic differentiation and modelled scenarios that varied in number of loci, number of alleles per locus, and the number of sampled individuals. In subsequent analysis, Oyler-McCance et al. (2012) replicated the Landguth et al. (2012a) study and assessed how variations in sampling design would affect the conclusion of spatial genetic variation. This topic remains a pressing issue and continued effort in this

area will be necessary as statistical methodology and molecular markers develop within LG and the related fields of SG and RG.

Environmental data

Selecting a sampling design that is best suited to address the hypothesis and targeted species requires consideration of organism life history and population demography in order to avoid flawed inferences. Many life histories of marine organisms are characterized by large population sizes, high fecundity, external fertilization, and planktonic larvae, which often necessitates modelling to predict possible routes of connectivity. Likewise, in freshwater organisms, similar considerations of life history are required. Life histories of freshwater organisms are diverse and each is influenced by the riverscape at different spatial scales. For example, freshwater mussels release gametes into the water column, where broad scale influences of water temperature and discharge affect distribution of larvae downstream (Wei et al., 2013). Therefore, it is expected that individuals at settlement sites distributed along a flow-connected path are expected to be more closely related than individuals at settlement sites along flow-unconnected paths. This relationship describes spatial autocorrelation among sites that are along the hydrologically-driven dispersal path and violates the assumptions of independence needed for parametric statistics. Yet, the consequence of a sample design at a sampling distance that would avoid autocorrelation may result in loss of biologically relevant interpretations of spatial genetic variation. A similar association exists within SG, where methods developed using simulation modelling (e.g., biophysical models) can predict dispersal. As these models lack inclusion of environmental variables, however, they are more useful for discerning relationships associated with movement (IBD) rather than genetic-environment associations (IBE).

Discussion

Understanding the relationship between connectivity and spatial genetic variation remains a growing area for novel research in freshwater systems. Attempts to quantify the factors that shape spatial genetic variation are ongoing in landscapes and seascapes but available analytical tools for riverscapes are limited. A

primary challenge in RG is development of models that adequately describe the contribution of physical and geomorphological processes in shaping genetic variation in rivers. These challenges separate RG from terrestrial studies of LG that often calculate effective distance from resistance surfaces. Thus, LG methods are not easily transferrable into RG because the branched physical structure of riverscapes form constrained pathways for dispersal and migration. Not surprisingly, studies of seascapes offer opportunities that seem more applicable for river systems. The use of biophysical models in SG to generate estimates of potential larval dispersal and settlement site is a novel interpretation of connectivity. Genetic connectivity for sessile organisms and any life history that includes a water-dispersed larval phase depends on larvae reaching their settlement site and surviving to reproduce. This mechanism of dispersal sets seascape genetics apart from many terrestrial systems, although wind-dispersed plants may provide interesting analogs, but larval dispersal adaptations are shared with organisms in riverscapes. RG would benefit from development of models to describe the genetic structure of larval dispersing organisms similar to the biophysical modeling used in SG. Although larval phases of freshwater organisms share similarities in their use of water as a dispersal mechanism, biophysical models are not widely used in freshwater; however, this framework may provide a fertile ground for development of new ways to consider connectivity.

The advantages of a graph theoretic approach warrant continued development in freshwater systems. SG has incorporated simulated data of oceanographic processes to connect nodes in a network using edges derived from models of connectivity relevant for aquatic life histories. But not all freshwater species “go with the flow”, some are able to resist movement. The additional flexibility of directed networks allows researchers to weight edges and assign direction, thereby accommodating a greater variety of connectivity scenarios that exist in riverscapes (Campbell Grant et al., 2007). The progress and growth of LG and SG indicates the utility of both approaches to better understand dispersal and connectivity in their respective environments, encouraging the continued development of methods in these systems.

Our overview has illustrated the potential of a RG approach to better understand structural and functional connectivity in freshwater rivers. An increasing body of evidence shows barriers (IBE) and environmental factors (IBE) contribute to spatial genetic structure of populations at large (across multiple basins) and small (within local watersheds) spatial extents. As research examining the effects of barriers and environment on spatial genetic structure continues, it would be useful to consider local adaptation and history. Habitat fragmentation, sedimentation, and human water uses are likely to change in the future given predictions of climate change and alterations to natural processes within river systems. Correct interpretation of spatial genetic structure and how ancient and contemporary riverscapes contribute to observed diversity would improve predictions of population response in future scenarios, and better inform studies in evolutionary biology, conservation genetics, phylogenetics, and other disciplines that seek to examine such linkages.

Conclusion

This review brings together a growing field that has yet to be formally defined or recognized. The literature supports a variety of hypothesis that have been tested using methodology that can be considered RG, but in doing so several limitations have surfaced. Although advances in LG and SG are relevant and should be applicable for riverine research, dynamic processes that drive and constrain biological and physical processes in rivers necessitate novel approaches and methods of analysis. As these methodological challenges are resolved, there is potential to significantly advance scientific understanding of processes that influence spatial genetic variation in riverscapes. Identifying and working with factors specific to RG will continue to challenge and bring together transdisciplinary teams with expertise in genetics, ecology and fluvial morphology. We trust that this brief synthesis will inspire new innovation in this field.

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Table 2.1: Glossary of terms. Synthesis of terms from references Smouse and Peakall (1999), Selkoe et al. (2016), Manel and Holderegger (2013), Riginos and Liggins (2013), Hartl and Clark (2007).

Term	Definition
Adaptive genetic variation	Genetic differences with an effect on fitness.
Allelic richness (A_r)	A measure of genetic diversity based on the average number of alleles per locus, sometimes considered indicative of a population's long-term potential for adaptability and persistence.
Allele	A variant at a locus.
Biophysical model	Spatially explicit modelling that uses mathematical formulations to simulate the interaction of biological and physical factors of a system.
Closeness	The mean shortest path between a focal node and all other nodes in the network.
Collinearity	The non-independence of predictor variables.
Digital elevation model (DEM)	Terrain elevation data provided in digital form.
Dispersal	Movement of individuals to different localities that has the potential to lead to gene flow.
Edge	Connections between nodes in a graph theoretic network.
Fixation index (F_{ST})	Measure of population genetic differentiation that reflects the proportion of allelic variation contained in subpopulations relative to the total genetic variation; may be calculated "pairwise" as a measure of differentiation between any two groups.
Gene flow	The transfer of genes from one population to another via movement followed by reproduction.
Genetic differentiation/ structure	A measure of allele frequencies among subpopulations .
Graph theory	A branch of mathematics that deals with statistical descriptions of static networks.
Heterozygosity expected; H_e observed; H_o	A measure of genetic variation. The proportion of loci expected to be heterozygous (H_e) and the observed proportion of heterozygotes, averaged over loci (H_o).
Hydrodynamic connectivity	Connectivity that is mediated by the flow of water.
Least-cost path	Length of a path minimizing the cumulative resistance (distance weighted by the cost of traversing a particular habitat type) between two localities.
Locus	A specific location on a chromosome; to be informative in a population genetic study, should show variation among individuals.
Neutral genetic variation	Genetic differences with no direct effect on fitness.
Node	An individual element within a network that represents a discrete unit (*e.g.*., a population, spawning site, or sampling location).
Raster image	A grid image created in geographic information systems (GIS).

Riverscape	A mosaic of freshwater river habitat that is spatially structured and hierarchically organized across multiple scales.
Riverscape genetics	An area of study that evaluates the effects of riverscape features on spatial genetic variation. Shares methodological similarities with seascape and landscape genetics.
Scale	The ratio or relationship between distance on a map and the corresponding distance on the ground.

Table 2.2: Defining molecular markers for genetic analysis, modified from Selkoe and Toonen (2006), Morin et al. (2004), Schlötterer (2000), Seeb et al. (2011)

Mitochondrial DNA (mtDNA): A maternally inherited, small, circular strand of DNA that is found in the mitochondria of cells. The molecule consists of a coding region, the majority of the molecule, and a control region (D-loop), responsible for regulating the production of gene products from the coding region. mtDNA is stable over time because it is present in multiple copies within the cell and the circular form resists degradation. However, it is maternally inherited and therefore haploid which provides less information than bi-parentally inherited nuclear DNA. Amplification is accomplished through polymerase chain reaction (PCR) and visualized by gel electrophoresis. Newer automated platforms have improved the ability to sequence mtDNA, resulting in the rapid sequencing of hypervariable regions and decreasing the time it takes to sequence the whole genome. Microsatellites: Short repeats of nucleotides (i.e., guanine (G), thymine (T), cytosine (C), adenine (A)) that are found throughout the nuclear genome. A microsatellite locus repeat consists of two (dinucleotide repeat), three (trinucleotide repeat), or four (tetranucleotide repeat) nucleotides, although more are possible. A locus has variable repeat lengths (alleles) that will vary by locus, species, and population. For instance, a heterozygous individual may show one allele with four GT repeats (GTGTGTGT), while the second allele has 7 repeats (GTGTGTGTGTGTGT). This difference in length can be assessed by gel electrophoresis or by using automated sequencing platforms after PCR amplification. Marker isolation and optimization are time consuming but once identified they are easy to amplify at relatively low costs. Microsatellites that are associated with neutral genetic variation have been used to identify bottlenecks, parentage analysis, gene flow, population structure and many other evolutionary effects. Although the advent and growth of next generation sequencing, contributed to the decline in use of microsatellites, they are still a useful marker. Single Nucleotide Polymorphism (SNP): Individuals of the same species share many DNA sequences that are almost identical and differ only at a few nucleotide positions within the sequence. At these sites, the two copies of a gene in a heterozygous individual show different nucleotides, whereas a homozygous individual shows only a single nucleotide. Finding SNPs first requires the sequencing of many genes or regions of a genome, a process that has decreased in cost and time as more automated platforms have become available. A single SNP offers little power to distinguish genetic structure among populations, but automated sequencing platforms now enable discovery of 10K's – 100K's SNPs with relative ease. SNPs are also used in similar contexts as microsatellite markers. Many journals require that molecular markers (mtDNA, microsatellites, and SNPs) are published and offer specific journals and databases for the purpose (i.e., Molecular Ecology Notes, National Center fro Biotechnology Information NCBI provides a searchable link for GenBank (www.ncbi.nlm.nih.gov/genbank/)).

Table 2.3: Quantifying genetic variation in population genetic analysis

DNA is extracted from tissue samples following an extraction protocol. Polymerase chain reaction amplifies specific portions of the genome, based on the molecular marker selected for use (Table 2.2). Amplified DNA fragments are visualized using gel-electrophoresis and genotypes (e.g., microsatellites or SNPs), or whole sequences are identified. Data are evaluated for quality and evidence of genotyping or sampling errors (Morin et al., 2010). The resulting dataset is used to calculate genetic diversity values that describe the diversity of gene variants among samples (allelic richness; A_r), differences in variation of each population compared to the total population (F_{ST} or other measures;) and heterozygosity (H_e). Individual assignment analysis and clustering algorithms (e.g., STRUCTURE (Pritchard et al., 2000), BAPS (Corander et al., 2008)) can be used identify patterns among individuals that may reflect spatial structure.

Table 2. 4Quantifying the riverscape in ecological analysis

The selection of environmental features for analysis that characterize the riverscape is predicated on the expectation that they have potential influence on target species. Therefore *a priori* use of expert opinion or the scientific literature can identify potential factors that are expected to impede movement. Data are then collected following an appropriate sampling design (discussed in text) targeting a relevant spatial and temporal scale for the phenomena investigated. As remote sensing has increased in accuracy and quality, providing data that is widely available at multiple spatial scales (e.g., LIDAR (Light Detection and Ranging) or drone imagery), geoprocessing needs also increase. In response, technical toolboxes have been and continues to be developed for use in geographic information system (GIS) applications. For example, to investigate physical and chemical parameters, STARS (Peterson and Ver Hoef, 2014), SSN (Ver Hoef et al., 2014) and FLoWS (Theobald et al., 2006) provide tools that will predict catchment-scale information. Once extracted, spatial data should be evaluated for presence of heteroskedasticity or bimodal distributions and transformed if necessary to avoid violating assumptions when applying regression analysis. The relationships among data are also evaluated for collinearity to identify evidence of spatial autocorrelation. In rivers, autocorrelation among some variables is likely when sites are flow-connected (i.e., temperature, pH, or dissolved O_2). Additional correlation between variables occurs regardless of flow-connectedness. For example, elevation typically increases with increasing distance from the river mouth, while stream width often decreases. In general, only one of the correlated variables is retained, although this approach is debated given that the correlation may be more realistic of natural processes. Finally, the variable list may be further transformed into a smaller number of uncorrelated variables by reducing the dimensions of the dataset and identifying which variables account for the majority of the variability in the data (e.g., Principal Components Analysis (PCA) or Principal Coordinates Analysis (PCoA)), although such approaches may complicate biological interpretation.

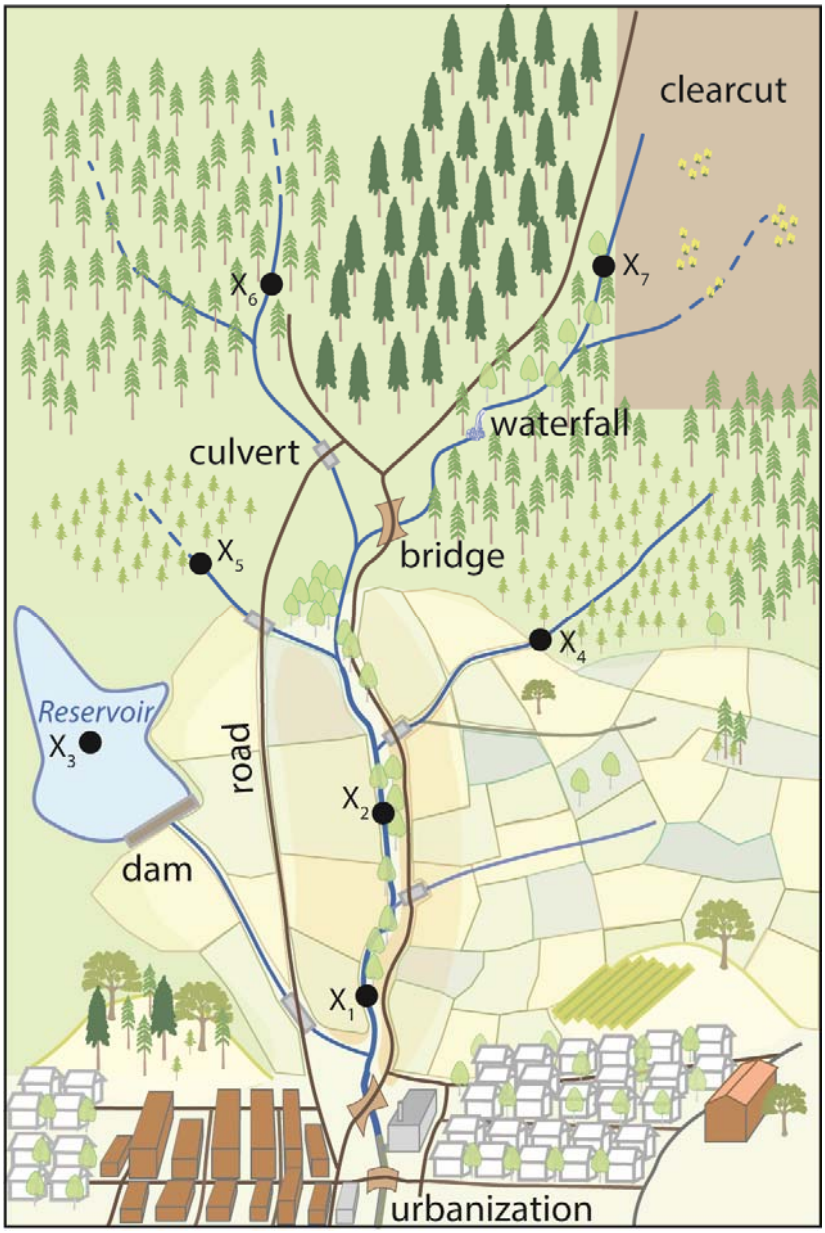

Figure 2.1: A hypothetical watershed that includes a forest, dendritic river network, and land that has been developed for industry, agriculture, and urban use. Sampling locations are represented by black circles.

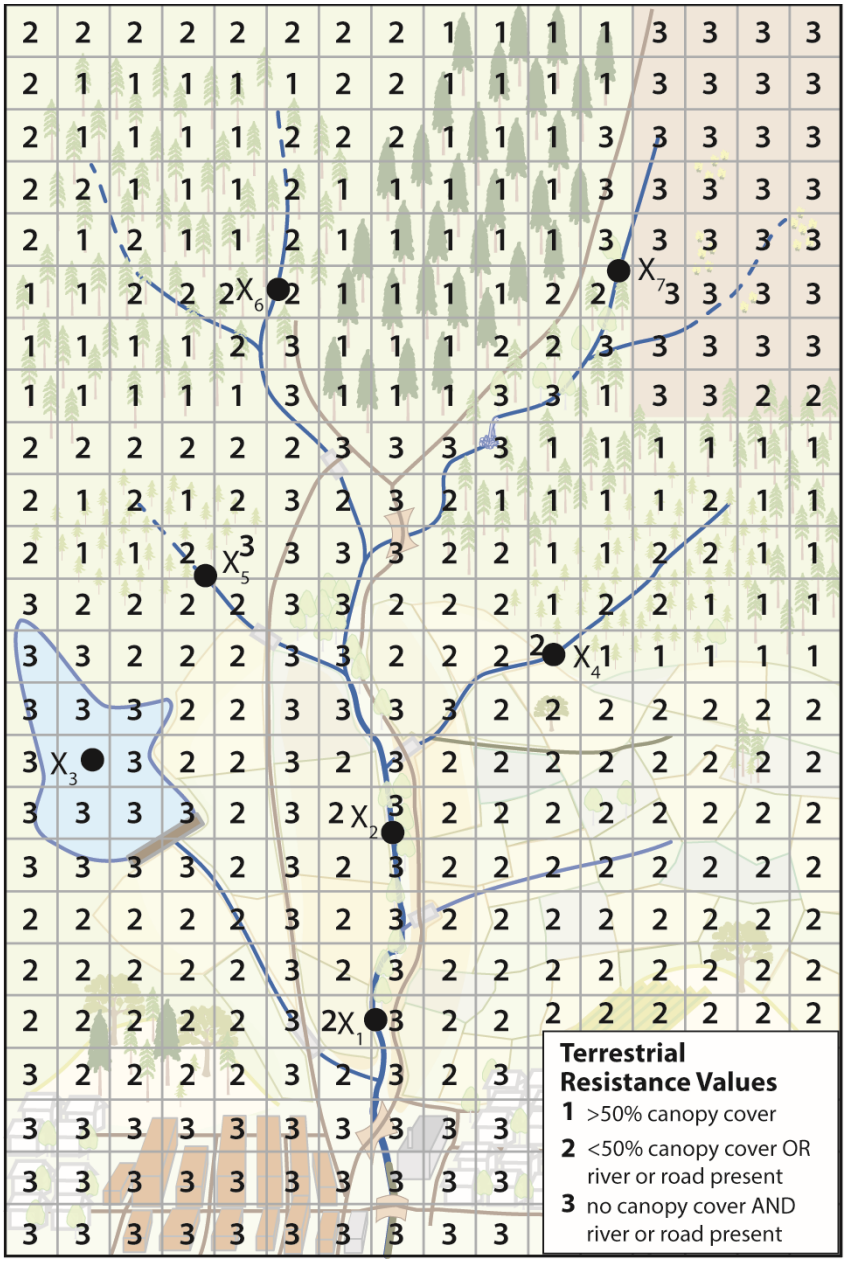

Figure 2.2: The hypothetical watershed presented in Figure 2.1 has been transformed into a raster image. The grid cells are weighted to reflect costs of travel for a terrestrial organism (A).

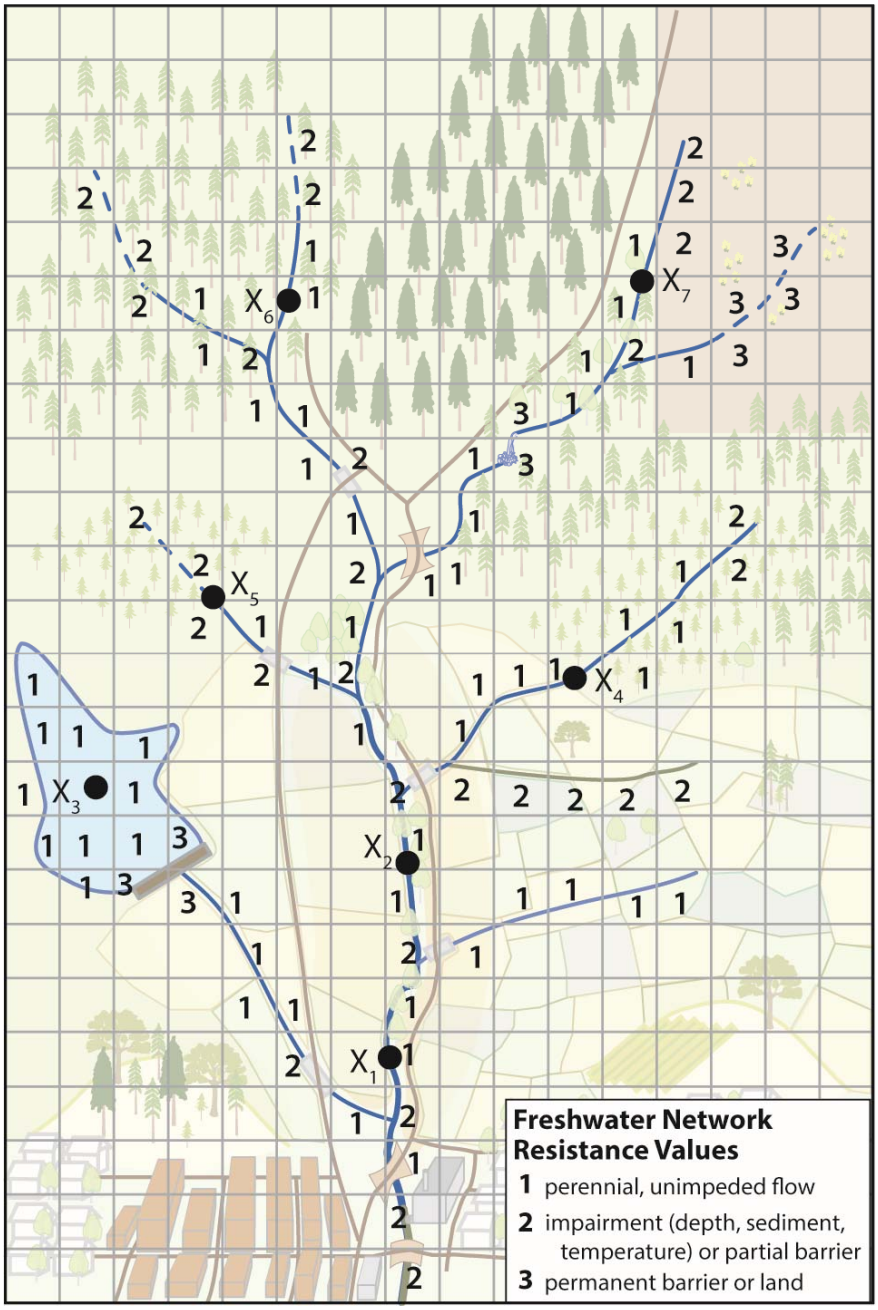

Figure 2.3: The hypothetical watershed presented in Figure 2.1 has been transformed into a raster image. The grid cells are weighted to reflect costs of travel for a freshwater organism (B).

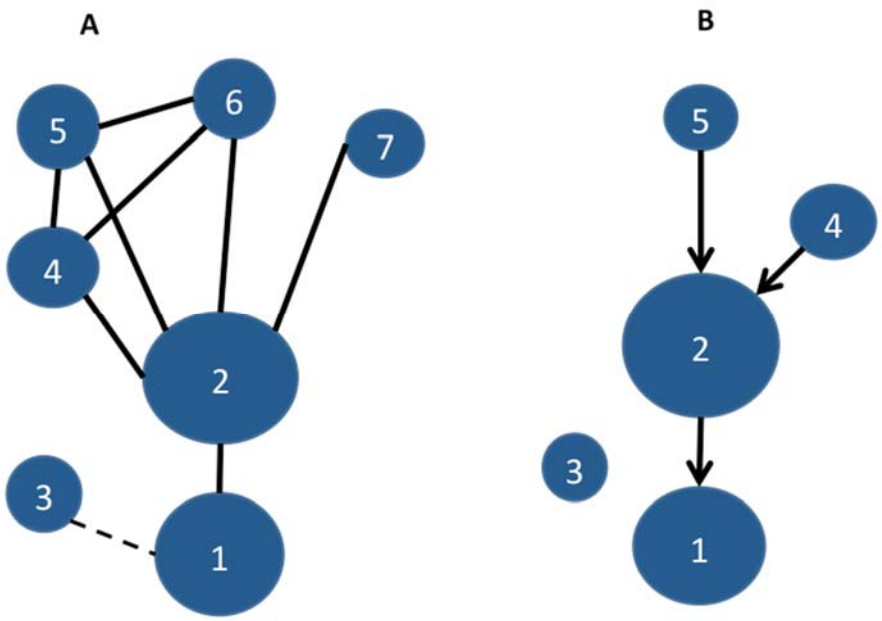

Figure 2.4: A diagram of genetic relationships among populations. Differences in node size reflect genetic variation within a population, while edges connecting nodes reflect between population genetic variation. A) sampling locations across a river network, B) sampling locations within the network reflecting migration (edges) and A_r (nodes).

Chapter 3:

Identification of multiple genetically distinct populations of Chinook salmon (*Oncorhynchus tshawytscha*) in a small coastal watershed

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Abstract

Management and restoration planning for Pacific salmon is often characterized by efforts at broad multi-basin scales. However, finer-scale genetic and phenotypic variability may be present within individual basins and can be overlooked in such efforts, even though it may be a critical component for long-term viability. Here, we investigate Chinook salmon (*Oncorhynchus tshawytscha)* within the Siletz River, a small coastal watershed in Oregon, USA. Adult Chinook salmon were genotyped using neutral microsatellite markers, single nucleotide polymorphisms and "adaptive" loci, associated with temporal variation in migratory behavior to investigate genetic diversity. Results from all three marker types identified two genetically distinct populations in the basin, corresponding to early returning fish that spawn above a waterfall, a spring-run population, and later returning fish spawning below the waterfall, a fall-run population. This finding is an important consideration for management of the species, as spring-run populations generally only have been recognized in large watersheds, and highlights the need to evaluate population structure of salmon within smaller watersheds, and thereby increase the probability of successful conservation of salmon species.

Introduction

Genetic and life-history diversity contribute to the resilience of native species in dynamic environments. Chinook salmon (*Oncorhynchus tshawytscha*) in western North America historically exhibited at least four behavioral life histories that are associated with the season of adult upstream migration (fall, spring, winter, and summer runs) and maturation status (Yoshiyama et al., 1998). Contributing to evolution of these migratory patterns is their homing fidelity to natal spawning rivers that allow for reproductive isolation and development of unique evolutionary trajectories (Quinn, 2004; Waples, 2001). Anthropogenic activities including harvest, waterway development, hatchery production, and land use practices have altered salmon populations and their associated freshwater ecosystems; the result is often reduced life-history and genetic variability (Brenkman et al., 2012; Yoshiyama et al., 1998).

To counter the effects of declining populations, substantial focus has been directed toward increasing abundance (supplementation programs) while also maintaining genetic diversity within evolutionarily significant units (ESUs) or other management units (Eldridge and Killebrew, 2007; Lindley et al., 2004; Olsen et al., 2000; Waples, 1998). As genetic techniques have improved, they have been used increasingly to enable better management (Clemento et al., 2014; Shafer et al., 2015). Salmonid fishes were among the first groups of organisms to be studied with molecular techniques, when the available methods were limited to the evaluation of genetically determined variation in a handful of proteins that could be reliably stained for detection (Utter and Hodgins, 1972). One important application of molecular techniques has been genetic stock identification (GSI), which uses a "baseline" reference dataset of genotypes from individuals of known origin to identify the most likely provenance of individuals of unknown origin on the basis of their genotype (Milner et al., 1985). A North America-wide baseline dataset has been developed for Chinook salmon that includes 42 major reporting units (Seeb et al., 2007) and, more recently, baseline datasets using single nucleotide polymorphisms (SNPs) have been developed for regional applications, including in the California Current ecosystem (Clemento et al., 2014).

Pacific salmon management and restoration planning is characterized by efforts at broad multi-basin scales; however, fine-scale genetic structure that may be present within small watersheds (< 120 river km) is often overlooked in recovery planning. Homing to natal stream of origin is an influential driver of isolation among spawning groups and contributes to the genetic structure that has been observed at the landscape scale within salmon species, as do other ecological factors (i.e., habitat fragmentation, water development, episodic landslides or fires etc.). However, proper management of this fine-scale life history variability is a critical component in the long-term population persistence in a dynamic landscape.

In addition to geographically structured genetic variation, salmon species demonstrate substantial life-history variation (for detailed descriptions of life history variation see (Groot and Margolis, 1991; Waples, 2001). In Chinook salmon, much of this variation manifests as run-timing "ecotypes" which are characterized

by mean date of freshwater entry and reproductive status (Moran et al., 2013; Waples et al., 2004). The 'fall-run', which enter freshwater as reproductively mature adults in late summer and fall, is regionally dominant in the southern extreme of the species range in North America. The 'spring-run' ecotype, which enter freshwater as reproductively immature adults in spring and early summer and hold in deep pools before maturing in the fall, are much less abundant and are derived from proximate fall-run populations in coastal basins (Kinziger et al., 2013).

Here, we investigated if fine-scale population structure of Chinook salmon exists in the Siletz River (523 km^2), Oregon. Previous studies that examined salmon populations from the Siletz River using data from neutral genetic variation included only samples from the fall-run, and were part of large-scale efforts to characterize genetic structure in the species and provide methods for identification of fish of unknown origin (Clemento et al., 2014; Moran et al., 2013; Seeb et al., 2007). We studied salmon of different life-history types and used data from three types of molecular marker to: i) identify patterns of neutral genetic variation within the watershed, ii) evaluate whether temporal patterns of life-history variation are correlated with variation at genetic markers known to be associated with run-timing, and iii) determine phylogeographic patterns within the river and compare them to other coastal basins in the North Oregon Coast region. We then address the implications of our results for current conservation and management activities.

Methods

Study area

The Siletz River system (~109 river km) has headwaters in the Central Oregon Coast Range and meets the ocean at Siletz Bay. The geology of the watershed is principally volcanic. A waterfall that may be a barrier to upstream migration by aquatic organisms is located at river kilometer 103.8 (Figure 1). A fish ladder was constructed in 1953 to allow fish passage during the winter for migratory fishes and Oregon Department of Fish and Wildlife (ODFW) operates it year round to control fish passage. In 1994, management practices began limiting access of anadromous fish that compete for spawning habitat with wild summer steelhead (Siletz basin

fish management plan; ODFW, 1997). Substantial changes to water flow and suitable salmon spawning habitat also occurred as a result of historical logging and splash-damming and alteration to the floodplains.

As many as three Chinook salmon ecotypes may exist in the basin: spring-, summer-, and fall-run (*Stan van de Wetering, Siletz Tribe, Pers. Comm*). The largest of these is the fall-run, which enters the river in September. The spring-run enters the river in May and moves the farthest upstream; while the summer-run ecotype returns in July and spawns between mid-September and mid-October (*Stan van de Wetering, Siletz Tribe, Pers. Comm.)*.

Sample collection

Tissue samples from Chinook salmon were collected by ODFW during their yearly carcass surveys and through monitoring at the trap on the fish ladder (Figure 1). Samples that were collected during the fall carcass survey consisted of a small section of the least degraded flesh from each carcass. Body condition and geographic (reach ID) location was recorded for all samples. Collections occurred September through December in 2011 and 2012 and were considered to be from the fall-run ecotype (*SIFA*). These samples were collected downstream of the fish trap (Reach IDs 2 – 23; Figure 1) and a total of 565 samples of sufficient quality for successful genotyping were collected.

Samples from carcasses of fish that appeared in the main stem Siletz River prior to October 15th in 2012 and 2013 were hypothesized to be a unique group, based on adult return and spawn time and were designated lower river early (*SILE*) fish. These samples were collected downstream at sampling locations Reach IDs 17 – 21 (Figure 1). There were 55 such samples of sufficient quality for successful genotyping

The fish ladder and trap are operated year round, and enable the upstream passage of all early returning Chinook salmon. Fish that entered the trap were considered to be the spring-run ecotype (*SISP*) and were passed over the waterfall and allowed access to spawning grounds located upstream (Figure 1). Scales were removed for age analysis and genotyping from all passed fish for which it was possible (i.e. body condition). During the sampling years, 700 fish were passed

upstream at the fish trap and, of these, 258 individuals were sampled and genotyped.

Genetic analysis

Total genomic DNA was isolated from each tissue sample following the extraction protocol of Ivanova *et al.,* (2006). Samples were genotyped with up to three types of molecular marker: presumably neutral microsatellites, putatively adaptive microsatellites, associated with run-timing variation, and single nucleotide polymorphisms (SNPs). *Neutral microsatellite markers* are not known to be associated with phenotypic expression and are considered to be selectively neutral; therefore, we were able to infer demographic processes that shaped population structure. Polymerase chain reaction (PCR) was used to amplify 21 neutral microsatellites following published thermocycling protocols. These included 10 microsatellites that are standardized range-wide (Moran et al. 2013) and an additional 11 microsatellites (Nelson and Beacham 1998; (Naish and Park, 2002; Williamson et al., 2002). The standardized microsatellite panel was developed for range-wide genetic stock identification (GSI) of Chinook salmon and included genotypes from populations throughout North America (Moran et al., 2013; Seeb et al., 2007). Fluorescent PCR products were electrophoresed on a 96-capillary DNA sequencer (3730XL; Applied Biosystems Inc.) and genotypes called using GENEMAPPER v3.7 software (Applied Biosystems).

Putatively adaptive microsatellite markers are associated with phenotypic expression and may be useful for interpreting adaptation of individuals to their local environments. The circadian clock gene network has been identified in salmon as contributing to the genetic control of adult migration timing in salmon(O'Malley et al., 2007; O'Malley et al., 2010). Three circadian clock gene markers*, Ots515NWFSC (Ots515), Cryptochrome3 (Cry3)*, and *OtsClock1b (clock1b)*, were amplified via published PCR and thermal cycling protocols. *Ots515* is a QTL-linked marker that is associated with spawn time and body weight in rainbow trout (O'Malley et al., 2007). *Cry3* is linked to flavoproteins that mediate circadian rhythms in plants

(O'Malley et al., 2010). *Clock1b* contains a polyglutamine repeat tract that has been shown to vary in Chinook salmon (O'Malley et al., 2010).

Single nucleotide polymorphisms (SNPs) are sites in the genome that have two nucleotides segregating and are ubiquitous in vertebrates. A panel of 96 SNPs specific for Chinook salmon (Clemento *et al.* 2014) were genotyped on 96.96 Dynamic Arrays with an EP1 System (Fluidigm Corp., South San Francisco, CA), and genotypes were called with Fluidigm Genotyping Analysis software v2.1.1. These SNPs have been used previously to construct a GSI "baseline" database that is comprised of genotypes from more than 69 populations, including fall-run from the Siletz River. A total of 188 samples, 94 fall-run and 94 spring-run, chosen representatively from the larger sets of samples was genotyped with these markers to determine phylogenetic relationships among proximate coastal basins.

Statistical analysis of genetic variation

Loci were assessed for genotyping problems including null alleles or allelic dropout using MICROCHECKER (Van Oosterhout et al., 2004). Observed allele frequencies were tested for evidence of deviations from Hardy-Weinberg expectations (HWE) and for significant linkage disequilibrium (LD) between loci with GENETIX (BELKHIR ET AL., 1996-2004). Summary statistics of genetic diversity were calculated for each hypothesized population. Characterization of the genetic diversity among loci was evaluated using allelic richness (A_r) a measure of allelic number that corrects for unequal sample sizes using a rarefaction method with HP_RARE (Kalinowski, 2005). The number of alleles (A), and observed (H_o) and expected (H_e) heterozygosity were calculated in GENALEX (Peakall and Smouse, 2012).

Spatial structure was evaluated in three ways: pairwise estimates of the fixation index (F_{ST}), exact tests for genic and genotypic frequencies, and individual assignment tests. Pairwise F_{ST} values (θ; (Weir and Cockerham, 1984)) were calculated in the program GENALEX and the data set was permuted 1000 times to determine if the values differed significantly from zero, an indication that populations may be genetically distinct. Exact tests for differences in genic and genotypic frequencies were performed with the program GENEPOP (RAYMOND AND

ROUSSET, 1995), which applies a Markov Chain Monte Carlo algorithm to account for small sample sizes or low-frequency alleles. Significant values of genic and genotypic frequencies may occur even though sufficient power to detect genetic differentiation through other methods is not possible, as is the case with populations where high gene flow exists.

A model-based Bayesian clustering method was used as an additional method to identify the degree of differentiation between the hypothesized populations. The software package STRUCTURE v2.2 (PRITCHARD ET AL., 2000) estimates the likelihood for hypothesized values of *k*, the number of genetically distinct clusters or populations from which the sampled individuals were drawn. This method allows the data to define the clusters and assigns individuals to the *k* clusters without *a priori* information about their sampling locations. Five independent runs were performed for each value of *k* (2 - 6), using 50,000 burn-in and 150,000 retained iterations. An additional STRUCTURE run to assess the association of SISP and SIFA to the Central Oregon Coast reporting unit was conducted using published data provided in (Clemento et al., 2014). Five independent runs were also performed for values of *k* = 4 - 10, using the same burn-in and iterations as above.

Phylogeography

Phylogeographic patterns of fish within the Siletz River were inferred with a dendrogram based on SNP genotypes, constructed using chord distances (Cavalli-Sforza and Edwards, 1967) and with the topology determined using the neighbor-joining algorithm, in the PHYLIP package (Felsenstein, 1993). Majority-rule consensus values were calculated from 1000 bootstrap replicates of the data by the PHYLIP components SEQBOOT and CONSENSE. Only bootstrap values above 80% were reported.

Results

Neutral genetic variation

Null alleles or other problems were identified in three microsatellite loci (*Ots209, Ots211,* and *Omm1080*), due to the presence of more homozygous individuals than would be expected. Significant departures from HWE existed for

seven markers *(Ots9, Ots104, OtsG409, Ogo4, Ots208, Ots249,* and *OtsG83*) after adjusting for multiple comparisons, the remaining 11 loci were used for analysis. Allelic richness and heterozygosity of SISP was greater than that of either SIFA or SILE (Table 1). Linkage disequilibrium was found in all populations; the largest fraction of locus pairs in LD was within SISP (Table 1). Overall accuracy of individual assignment to population of origin using the microsatellite data was greater than 83% (Table 2). Pairwise F_{ST} values across years (2011 and 2012) were low (SIFA, $F_{ST} = 0.001$, $p = 0.006$; SISP, $F_{ST} = 0.003$, $p = 0.001$) and marginally significantly different from zero, but did not likely represent biologically meaningful differentiation (Hedrick 1999). Data were therefore pooled across years within groups for subsequent analyses.

Of the 96 SNP loci, four loci *(Ots_108735-302, Ots_118175-479, Ots_128302-57,* and *Ots_Pr12*) did not yield genotypes within the SISP population, and *OkiOts_120255* functions to discriminate Chinook salmon from closely related coho salmon (*O. kisutch*); the remaining 91 loci were used for subsequent analyses (suppl. 1). There were no departures from HWE among loci following correction for multiple comparisons. Allelic richness and heterozygosity of SISP was again greater than that of either SIFA or SILE (Table 1). There was no evidence of linkage disequilibrium within SIFA; however, a small amount of LD was present in the SISP group (Table 1). The SNP dataset had similar ability to accurately assign individuals to population of origin as the microsatellite dataset (accuracy > 85%; Table 2).

Pairwise F_{ST} differed significantly from zero between SILE and SISP, but not between SIFA and SILE (Table 3). Model-based clustering analysis with STRUCTURE provided evidence of two major genetic groups in the Siletz River that corresponded to the fall-run and spring-run Chinook salmon ecotypes (Figure 2a and 2b) and was consistent with the pattern identified by other analyses with both datasets.

Temporal adaptive genetic variation

Variation at the three markers associated with circadian clock genes provided further evidence for differentiation between migratory ecotypes of Chinook salmon in the Siletz River. All pairwise F_{ST} values and exact tests of genic

and genotypic divergence between SISP and both SIFA and SILE, except those for locus *Clock1b*, were significant, indicating differentiation of SISP and both SIFA and SILE (Table 4). In contrast, these loci provided minimal evidence for differentiation between the earlier returning (SILE) and later returning lower river fish (SIFA), with non-significant F_{ST} values and significant tests of genic and genotypic differentiation at loci *OTS515* only (Table 4).

Phylogeography

A neighbor-joining dendrogram was created with the SNP dataset and was compared to a larger published study of Chinook salmon by Clemento *et al.,* (2014), that used the same loci and in which "reporting groups" of populations from the same geographical regions were identified. Siletz River fall-run Chinook salmon were grouped with the North Oregon Coast reporting unit in that study, as they were in several other genetic studies (Narum et al., 2008; Moran et al., 2013). Our analysis also placed SIFA in the North Oregon Coast reporting unit (bootstrap 83%; Figure 3), which is consistent with the fact that the SIFA samples were collected from the same general location as the fall-run Siletz River fish that were analyzed in these previous studies. The Siletz River spring-run ecotype (SISP) population also branched with the North Oregon Coast reporting unit. Model-based clustering analysis with STRUCTURE also supports this finding (Figure 2c).

Discussion

Here, we used genetic data to identify a previously unrecognized population of early returning (i.e., spring-run) Chinook salmon within the Siletz River, a basin that is < 120km from source to ocean exit. The use of three different types of molecular genetic data to investigate population genetic structure of Chinook salmon in this watershed allowed us to resolve fine-scale structure not previously recognized. Patterns of genetic variation within the watershed indicated that individuals spawning downstream of the waterfall (i.e., SILE and SIFA) are a fall-run population, and that a genetically distinct population of the spring-run ecotype (i.e., SISP) spawns upstream of the waterfall. Concordance among results from analyses

of the multiple marker types lent strength to the resulting conclusion of significant structure in the Siletz River that corresponds to fish with different freshwater entry timing and to fish spawning above and below a waterfall. Pairwise F_{ST} values for populations spawning upstream and downstream of the waterfall were significant for all three molecular markers. The presumably neutral microsatellite loci that were used in this study were highly polymorphic and provided substantial power for resolution of genetic structure. However, persistent LD was found between some of these markers. The SNP markers were biallelic, and therefore less polymorphic per locus, but more numerous and did not have significant LD. The adaptive genetic markers correlate with behavioral variation in some salmon populations that have a temporal component and could therefore potentially discriminate such populations in the absence of other genetic differentiation. Previous work has shown that the combination of data from multiple marker types improved resolution of population structure in salmon, especially among populations with potentially high gene flow, as can be expected in smaller watersheds (Narum et al., 2008; Hess et al*.,* 2011; DeFaveri et al., 2013; Garvin et al., 2013). Below, we discuss the patterns of genetic differentiation as inferred from the three sets of molecular markers and summarize the implications of our findings for continued conservation efforts.

Temporal variation

In contrast to the finding of significant differentiation between fall- and spring-run ecotypes returning above and below the waterfall with all three types of molecular marker, none of the datasets found differentiation between the lower river, early fall returning fish (SILE) and the lower river, fall-run ecotype (SIFA). There is no physical barrier between these two groups, but the timing of their return to freshwater and spawning dates suggested that heritable behavioral differences might exist. None of the pairwise F_{ST} values between SIFA and SILE were significant, indicating that these two groups of fish are likely experiencing high levels of gene flow. However, significant exact tests for genic and genotypic frequencies may indicate slight differentiation associated with this early spawning phenotype at a locus (*Ots 515*) that has been found to be associated with spawn time

(O'Malley et al., 2007). We did not identify fine-scale genic and genotypic diversity using *Clock1b* and *Cry 3*, but these markers may only be informative for adaptive variation across basins, as suggested by O'Malley and Banks (2008). Although the existence of an early fall-run is informally acknowledged in some coastal rivers, only two non-fall-run populations of Chinook salmon (two spring-run populations in the upper Umpqua River) are formally delineated in the Oregon Coastal Multi-Species Conservation and Management Plan for the purposes of management and recovery (ODFW 2014). The finding of a genetically unique, wild population of spring-run Chinook salmon in the Siletz River indicates that greater life history diversity exists within this species in these smaller coastal rivers.

The adaptive genetic markers provided signals of structure that were similar to the other types of genetic markers, although variation in them has been found in other studies to be strongly associated with temporal variation in migration by salmon (O'Malley et al., 2007). Clock genes are part of the molecular mechanism of long-term timekeeping for tracking season-specific activities in response to photoperiod in many animals (Bradshaw and Holzapfel, 2007; Leder et al., 2006; Liedvogel et al., 2009). Timing of freshwater entry and reproductive maturity in salmonid fishes is a complex array of interrelated behavioral and physiological traits that has heritable components and exhibits phenotypic plasticity in response to environmental variability (Abadia-Cardoso et al., 2013; Carlson and Seamons, 2008). Chinook salmon populations have been able to exploit a wide range of habitats because of evolution at this trait, often in the face of ongoing gene flow (Waples 2001; O'Malley et al. 2013). Using markers associated with the circadian clock gene network might have provided additional insight in the differentiation among these closely related groups and they did discriminate the spring-run population above the waterfall from all downstream fish, although with genetic differentiation similar to the other markers.

Conservation implications

It is important for species to maintain genetic variability in order to respond to dynamic environments. The extent and scale of intraspecific genetic diversity is

therefore a crucial consideration from both conservation and management perspectives (Funk et al., 2012; Manel et al., 2010). Chinook salmon populations are characterized by hierarchical genetic structure (Seeb et al., 2007; Moran et al., 2013) with evidence of isolation across their range in the north Pacific (> 10,000km), within larger river systems (> 1,000km), and regionally among watersheds (< 1,000 km). Finer-scale structure in Chinook salmon within smaller coastal watersheds (e.g., the Siletz River < 120km) has been relatively unstudied.

Matala et al. (2012) identified spatial structuring of Chinook salmon in the South Fork Salmon River (~ 90km), which is part of the Columbia River system. Chinook salmon that spawned within the main stem South Fork Salmon River were significantly different from individuals returning to two other tributaries within the subbasin. To reach these isolated tributaries, salmon must travel hundreds of kilometers up the main stem Columbia River. The F_{ST} values that we report between fish above and below the waterfall in the Siletz River are of similar magnitude to those reported among these Columbia River tributaries, but on a much smaller geographic scale. This demonstrates that such life history variation and genetic differentiation is not limited to large river systems and can be found in smaller watersheds.

Population structure described solely on the basis of divergence at one type of molecular marker, particularly presumably neutral ones, may fail to identify distinct populations that warrant separate management. Life history diversity of salmon is most often associated with spatial diversity and larger river systems (e.g., Columbia River or Sacramento-San Joaquin river systems) that typically have both numerous genetically distinct salmon populations and a greater number of life history strategies associated with them (Groot and Margolis, 1991; Taylor, 1991; Waples, 2001).

Identifying and preserving genetic and phenotypic diversity is an important component of formulating strategies to maintain resiliency and fitness of salmon, particularly in smaller watersheds (McElhany et al., 2000). It has become evident that genetic data can inform many decisions relating to management strategies, especially those that are aimed at maintaining abundance and genetic diversity in

natural salmon populations (Brenkman et al., 2012; Eldridge and Killebrew, 2007; Grandjean et al., 2009; Matala et al., 2012; Olsen et al., 2000)). Without such information there is great risk of losing important life history variation that enables resilience of anadromous species to changing environments.

Conclusions

We have demonstrated how the application of molecular genetic data from multiple types of markers provides strong support for existence of two genetically and phenotypically distinct salmon populations in a small coastal watershed where only one is currently recognized. Much of fishery management and conservation is based upon status of larger, regional management units (e.g., the North Oregon Coast Chinook Salmon ESU). These management units are combinations of unique spawning groups from multiple, smaller river basins (e.g., the Siletz, Alsea, Coquille, and Siuslaw Rivers). Management solely at such larger scales may not take into account fine-scale genetic and phenotypic variability that is present within smaller watersheds, such as has been demonstrated here. This fine-scale variability is a necessary component of long-term resilience and its maintenance should be explicitly considered to ensure successful conservation and management.

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Table 3. 1: Summary of genetic markers for 11 microsatellites, 3 adaptive loci and 91 SNPs by population (SIFA= fall-run, SILE= lower river early, SISP = spring-run) across all marker types. A_r = allelic richness, H_o and H_e are observed and expected heterozygosity, respectively, LD = percentage of locus pairs with significant genotypic linkage disequilibrium within populations.

	Microsatellites					**Pooled Adaptive Loci**					**SNPs**				
	A_r	**A**	**H_o**	**H_e**	**LD**	**A_r**	**A**	**H_o**	**H_e**	**LD**	**A_r**	**A**	**H_o**	**H_e**	**LD**
SIFA	18.5	28.3	0.87	0.87	7.0%	19.5	36.00	0.70	0.70	-	1.9	1.9	0.33	0.32	5.8%
SILE	18.0	18.8	0.86	0.87	10%	20.4	21.25	0.70	0.70	-	-		-	-	-
SISP	19.6	25.6	0.90	0.90	25%	18.2	28.00	0.71	0.69	-	2	1.9	0.35	0.35	10.0%

Table 3. 2: Individual assignment to population of origin for each molecular marker. Accuracy reflects the percent of correct assignment to population of origin. The percent correct assignment of populations below the waterfall to either of the populations sampled below the waterfall is in parentheses.

	Microsatellites				Pooled Adaptive Loci				SNPs		
	SIFA	**SILE**	**SISP**	**Accuracy**	**SIFA**	**SILE**	**SISP**	**Accuracy**	**SIFA**	**SISP**	**Accuracy**
SIFA	490	53	22	87(96)	381	121	63	67 (89)	76	6	92
SILE	27	19	9	35(83)	24	19	12	34(78)			
SISP	20	11	227	88	31	28	199	77	13	76	85

Table 3.3: Matrix of pairwise F_{ST} values calculated from the microsatellite dataset (below diagonal) and the SNP dataset (above diagonal). F_{ST} values significantly different from zero are denoted by an asterisk.

	SIFA	**SILE**	**SISP**
SIFA	-	-	0.009*****
SILE	0.00253	-	-
SISP	0.02075*	0.01709*	-

Table 3.4: Genic and genotypic exact test results and pairwise F_{ST} values for all populations (SIFA= fall-run, SILE= lower river early, SISP = spring-run).

	SIFA vs. SILE			SIFA vs. SISP			SILE vs. SISP		
	Genotypic	Genic	F_{ST}	Genotypic	Genic	F_{ST}	Genotypic	Genic	F_{ST}
Clock1b	0.719	0.339	0.000	0.042*	0.040*	0.003*	0.104	0.073	0.004
Ots515	0.003*	0.010*	0.003	0.000*	0.000*	0.009*	0.001*	0.000*	0.008*
Cry3	0.094	0.068	0.001	0.000*	0.000*	0.008*	0.001*	0.001*	0.008*

* Significant values

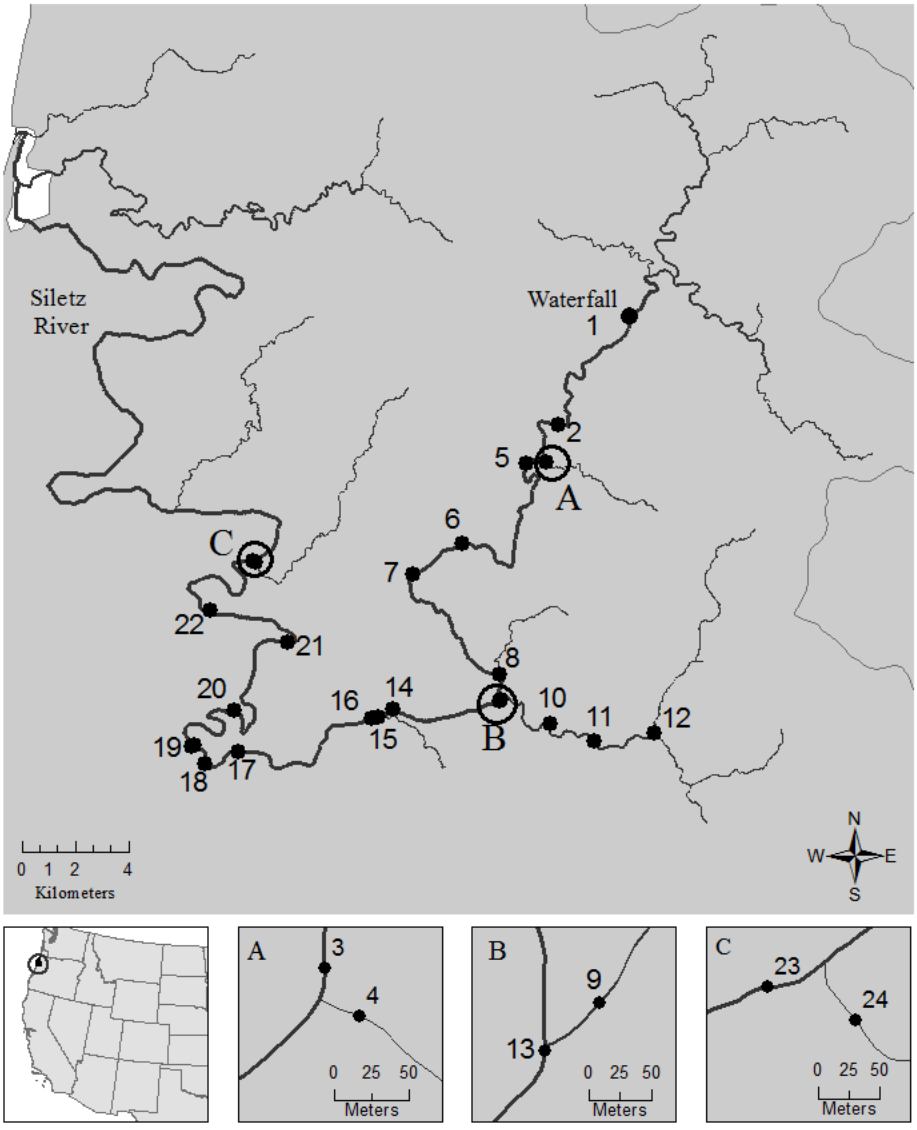

Figure 3.1: Map of sample sites for Chinook salmon from the Siletz River basin. Sampling locations are identified numerically

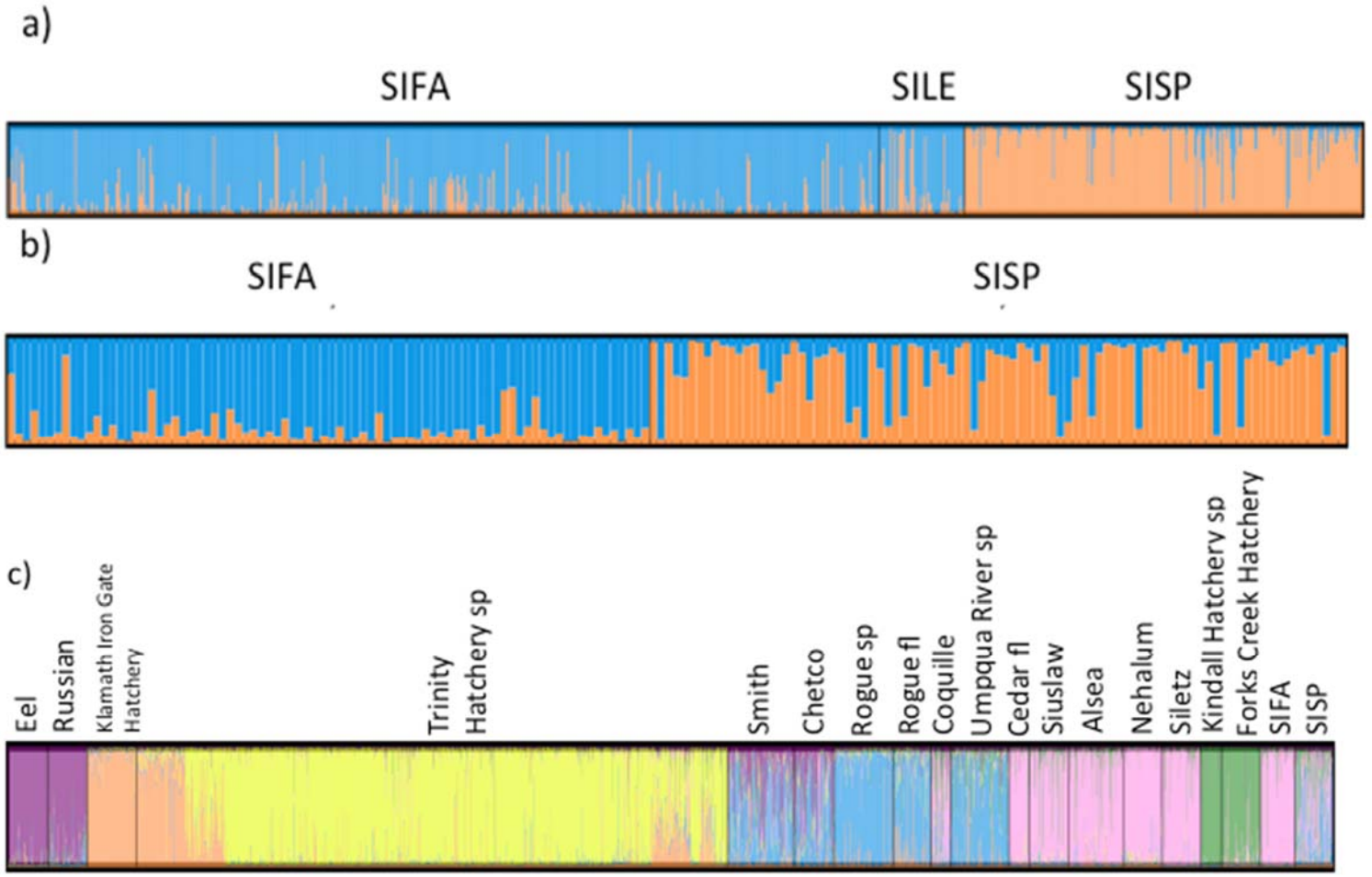

Figure 3.2: Fractional ancestry plots obtained from STRUCTURE for a) microsatellite markers, b) SNPs, and c) SNPs using Clemento *et al.* (2014) genotypes for the North Oregon Coast Reporting Unit. Each vertical bar represents an individual's genotype and the probability of being assigned to one of k (k= 2 or 6) genetically distinct clusters. Spawning groups of Chinook salmon in Siletz River are identified as follows: SIFA (fall run), SILE (early fall run), and SISP (spring run).

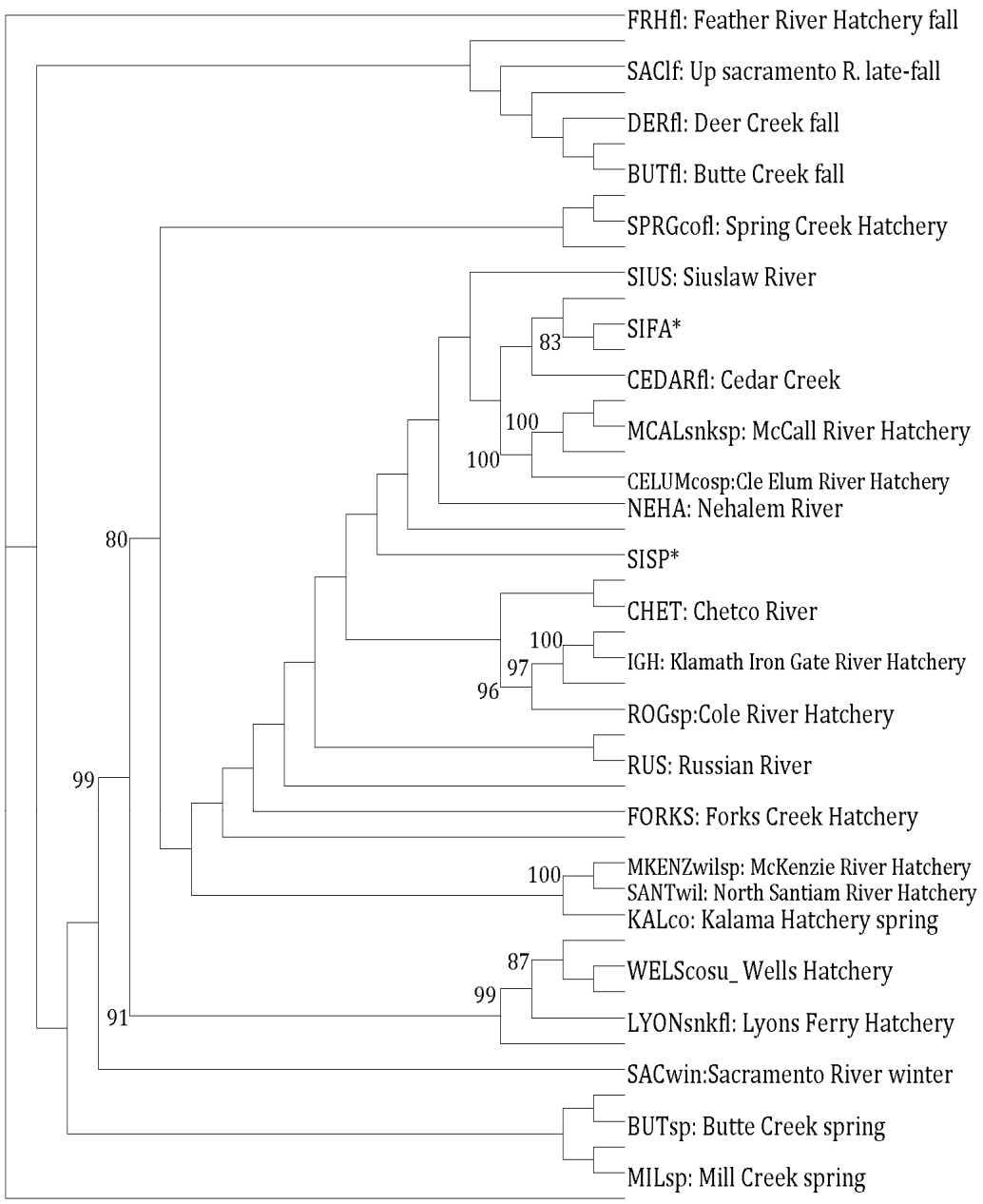

Figure 3.3: Mid-point rooted neighbor-joining dendrogram constructed with chord distances calculated from SNP data. All SNP data other than those from SIFA and SISP are from Clemento *et al.* (2014). Bootstrap values greater than 80% (out of 1000 bootstrap resamplings) are reported

Chapter 4:

Riverscape genetics of Chinook salmon in Siletz River

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Abstract

The research presented here investigated spatial genetic relationships of an individual Chinook salmon spawning run throughout the extent of its spawning habitat and linked hydrogeomorphic processes that drive functional connectivity to observed genetic variation. Habitat fragmentation, land use practices, and water impediment modify the natural course of major rivers, disrupting connectivity and subsequently affecting dispersal and gene flow. However, many of the relationships between the physical river network and population structure are not well understood across multiple spatial scales. Here I characterized the effects of hydrology in shaping the genetic structure of a highly migratory fish within a small coastal watershed. I evaluated whether gene flow was limited by 1) site-specific hydrologic features occurring within spawning habitat using a dissimilarity matrix and 2) costs associated with traveling between habitats using a novel riverscape genetic method. It was evident that even within a small watershed the combined effects of hydrologic riverscape features limited dispersal. Salmon that spawned at higher elevations after traversing steeper gradients were more genetically distinct from individuals that traversed shallower gradients and spawned at lower elevations. This effect (isolation by resistance) was distinguishable from isolation by distance (IBD), which was not detected among spawning groups. The riverscape genetics approach applied here enhanced interpretation of habitat heterogeneity in shaping gene flow and spatial genetic structure at a fine spatial scale.

Introduction

Expanding quantitative genetic research in river systems to explicitly consider the physical configuration of the river would help develop a clear understanding of its importance in terms of population persistence. In aquatic ecology, rivers and their drainages have come to be called riverscapes (Wiens, 2002). Habitat fragmentation, land use practices, and water impediment modify the natural course of major rivers, disrupting connectivity and subsequently affecting dispersal and gene flow (Brenkman et al., 2012; Nehlsen et al., 1991; Yoshiyama et al., 1998). A biological response resulting from the inability of individuals to disperse may include loss of genetic variation and reductions in life-history variation (Yoshiyama et al., 1998). The ability to assess these

genetic changes at the riverscape scale is necessary in order to understand how functional connectivity will affect fish assemblages in the future.

Linking genetic variation to riverscape scale processes that alter functional connectivity requires consideration of the spatial configuration of the network (Blanchet et al., 2011; Ganio et al., 2005; Peterson et al., 2013; Thorp et al., 2006) and the functional connectivity within the network (Auerbach and Poff, 2011; Cote et al., 2009; Flitcroft et al., 2014). Riverscape scale analysis is generally considered to be analysis that encompasses processes occurring throughout the system, rather than in small, discrete sample locations or river reaches (Falke et al., 2010; Fausch et al., 2002). Advances in geospatial tools and availability of GIS data allow for evaluation of hydrologic, geomorphic, and ecological change throughout entire stream networks at the scale of a riverscape (Benda et al., 2007; Peterson and Ver Hoef, 2014; Roux et al., 2015). Riverscape genetics is a developing field that uses population genetic metrics to assess spatial genetic structure within the context of environmental variables that drive functional connectivity across the river network (Davis *in review*, (Selkoe et al., 2016).

Although the distribution of riverine habitat is driven by hydrology, stochastic (*e.g.,* fires and floods) and geomorphic processes also maintain attributes of habitats by providing physical disturbances over time (Reeves, G. et al., 1995). Physical habitat diversity within riverscapes is characterized by differences in bed type, channel morphology, patchiness, biological communities, and physical configuration; processes that are driven by natural and anthropogenic disturbance processes (Borrett et al., 2014; Fausch et al., 2002; Frissell et al., 1986; Montgomery, 1999). Research has begun to consider spatial structure of the physical network in conjunction with disturbance and geomorphic processes (Ganio et al., 2005; Wipfli et al., 2007). The spatial structure of riverine systems influences connectivity, but many of the relationships between the physical network and population structure are not well understood (Benda et al., 2004; Frissell et al., 1986; Peterson et al., 2013).

A riverscape genetic approach was applied to characterize the effects of hydrology in shaping the genetic structure of Chinook salmon, a highly migratory fish,

within a small coastal watershed. Life history diversity of Chinook salmon in the Pacific northwest is characterized by differences in return timing (O'Malley and Banks, 2008; O'Malley et al., 2013), homing fidelity to natal spawning rivers (Waples, 2001) and freshwater rearing strategies (Groot and Margolis, 1991). Genetic structure of anadromous salmon is enhanced by homing behavior, an inherited life history characteristic that enables mature and maturing adults to return to natal spawning streams (Neville, H. M. et al., 2006; Quinn, 2004). Additionally, changes in genetic structure that reflect restricted gene flow resulting from decreased functional connectivity among essential habitat have been well documented in Pacific salmon (Benavente et al., 2015; Bradbury et al., 2014; Dionne et al., 2008; Fraser et al., 2011; Kanno et al., 2011).

Here, in Siletz River, a population of fall run Chinook salmon that spawns downstream of a natural waterfall was investigated (Clemento et al., 2014; Davis et al., 2017; Moran et al., 2013). The fall run spawns in the main stem, downstream of the waterfall, returning from September to December and spawning from late October through January with the majority of spawning occurring in November (Davis et al., 2017). The primary objective of this study was to assess whether indicators of hydrology (*e.g.*, gradient, elevation, stream width, or stream depth) influenced observed genetic structure in fall run Chinook salmon, after accounting for the influence of geographic distance and network structure. Chinook salmon have specific requirements for spawning habitat that are characterized by stream flow, water depth, gradient, and other variables (Geist and Dauble, 1998; Moir and Pasternack, 2010). Geomorphic processes that affect spawning habitat are constrained by the hierarchical properties of nested catchments and drainage basins within the river network (Miller et al., 2008), therefore site-specific variation is associated with the position of habitat in the network and may be a predictor for genetic structure at these fine spatial scales. However, because salmon may undergo different costs when dispersing among reaches within the network, the cumulative effect of riverscape variables on spatial genetic structure should be considered. Furthermore, homing behaviors enable navigation to specific

natal spawning habitat (Quinn, 2004; Waples, 2001) and individuals spawning in different tributaries might be less likely to exchange genes, therefore the influence of movement among tributaries within the network also should be considered. The different drivers of fine-scale genetic structure were assessed by first testing if distance or movement among tributaries predicted spatial genetic structure. Then, the effect of hydrologic features on genetic structure was assessed using both site-based and path-based methods to characterize differences in hydrologic features.

Methods

Study area

The drainage area of the Siletz River system is 523 km^2 with average yearly streamflow velocity is 1,500 m^3/s (https://waterdata.usgs.gov/nwis/inventory?). The river extends ~ 120 km from the Polk-Lincoln county border in the Central Oregon Coast Range and exits into the Siletz Bay at the coastal town of Kernville. The river flows over principally volcanic terrain and is located in a Mediterranean climate where water flow is sourced by precipitation in the form of rain. Five anadromous salmon populations spawn in Siletz River, spring and fall Chinook salmon, summer and winter steelhead, and coastal cutthroat trout (Davis et al., 2017). A natural waterfall at river kilometer 103 may be a barrier to dispersal by anadromous fish. In addition to natural disturbances, historic logging and damming has had substantial and long lasting alterations to water flow and habitat availability throughout the watershed (Miller, 2010).

Spawning surveys and genetic data collection

Tissue samples were removed from carcasses of fall run Chinook salmon by the Oregon Department of Fish and Wildlife (ODFW) Coastal Chinook Research and Monitoring Program (CCRMP) during yearly spawning surveys in 2011 and 2012. The survey established 34 sample reaches throughout the mainstem Siletz River and Rock Creek (Figure 4.1). Upstream and downstream GPS coordinates were collected to identify reach boundaries. Tissue samples were assigned a reach according to the downstream boundary of the reach where the carcass was collected. Samples from reaches that had low sample sizes ($N < 9$) were combined with samples from the nearest

neighbor where appropriate but samples from reaches were not combined if they spanned a confluence. Spatial locations for combined reaches were assigned the downstream reach boundary of the upstream reach.

Environmental layers

Relevant riverscape variables were selected based on review of the ecological literature and expert knowledge of habitat characteristics that were most likely to affect gene flow and dispersal of fall run Chinook salmon. Dispersal requires sufficient water velocities and depths during migration to pass obstacles (*e.g.,* waterfalls and log jams). Spawning habitat requirements differ substantially from migration because they serve reproductive needs. Investigators have identified several important elements of suitable salmon spawning habitat including stream depth, stream flow, gravel size, and habitat area (Bjornn and Reiser, 1991; DeVries, 1997; Geist and Dauble, 1998). For Chinook salmon in the Pacific Northwest, stream flow velocity and stream depth are important factors that influence formation of suitable spawning habitat (Hamann et al., 2014; Isaak et al., 2007). Stream flow, in conjunction with depth, affects gravel deposition on spawning sites. Low streamflow allows fine sediment deposition that may smother redds while suitable gravel sizes do not settle if stream velocities are too great (Miller et al., 2008; Reiser and White, 1988). For Chinook salmon in the Pacific Northwest, stream flow velocity and stream depth are examples of factors that contribute to the formation of suitable spawning habitat (Hamann et al., 2014; Isaak et al., 2007)

Combined utilities in ArcGIS 9.3. ArcMap and NetMap were used to develop a synthetic stream layer in vector format of the Siletz River. A National Hydrography Database (NHD; https://nhd.usgs.gov) stream layer at 1:100,000 scale was clipped to HUC8: 17100204 representing Siletz and Yaquina watersheds. Using the Network Analyst extension in ArcMap, pairwise waterway distance was calculated using the downstream boundary for each reach. This measure of distance follows the path of the stream and is considered to be an analogue to Euclidean distance that represents distance “as the fish swims”, a more biologically meaningful measure for freshwater aquatic organisms.

NetMap is a community supported geographic analysis platform, containing standardized digital watershed data that are commonly used for analysis of freshwater systems (Benda et al., 2007) including fluvial processes (*i.e.*, reach gradient, stream depth, stream width) and distance (*i.e.,* distance to mouth). Channel depth (m) and channel width (m) were modeled as a power function of mean annual flow, drainage area or precipitation. Gradient (m/m) was calculated from 10 m digital elevation models (DEMs). Spatial resolution used in NetMap was at a finer resolution than the spatial scale used during genetic sampling. Therefore, to develop reach-scale descriptors of physical habitat conditions, stream depth, stream width, and elevation were averaged within each reach. To facilitate modeling of riverscape resistance (see below) stream gradient (m/m) was first standardized by dividing by the smallest observed gradient and then converted into degree (hereafter, "standardized gradient"), resulting in the lowest gradient having a value of "1".

Genotyping

Genetic Analysis

Sample sizes ranged from 9 -70 individuals per reach (Figure 4.1). Genotyping and genetic differentiation of Chinook salmon in the Siletz River was previously described using 11 neutral microsatellite markers, 96 single nucleotide polymorphisms, and candidate loci for spawn time (Davis et al., 2017). Neutral microsatellite markers are not known to be associated with phenotypic expression and are considered to be selectively neutral; therefore, they may be used to infer demographic processes that shaped population structure (Holderegger et al., 2006; Kirk and Freeland, 2011). The following riverscape genetics analysis was conducted using the publically available neutral microsatellite marker genotype dataset from Davis et al., (2017) containing 540 fall run Chinook salmon.

Characterization of genetic diversity among loci was evaluated using allelic richness (A_r) a measure of allelic number that corrects for unequal sample sizes using a rarefaction method with HP_RARE (Kalinowski, 2005). Observed (H_o) and expected (H_e) heterozygosity were calculated in GENALEX (Peakall and Smouse 2012).

Population Genetic Structure

Genetic differentiation is popularly estimated using the F_{ST} fixation (Weir and Cockerham, 1984) index but F_{ST} interpretation of the index becomes difficult because it relies on estimates of heterozygosity, therefore maximum values are strongly constrained to near zero when number of alleles per locus is high (Meirmans and Hedrick, 2011). Several alternate models have been proposed, including Jost's D (D_{est}) that does not rely on heterozygosity (for details see (Gerlach et al., 2010; Jost, 2008; Merimans and Hedrick, 2011; Whitlock, 2011). Analysis was conducted at the spatial resolution of a stream reach and genetic differentiation was calculated using F_{ST} and D_{est}. Pairwise genetic distance values were calculated in the program GENALEX and the data set was permuted 1000 times to determine if the values differed significantly from zero, an indication the populations may be genetically distinct (Peakall and Smouse, 2012).

Riverscape genetics of Chinook salmon

Hypothesis 1: Isolation By Distance (IBD)

Within the IBD framework it was predicted that genetic distance would increase with increased waterway distance, similar to relationships found in larger watersheds (Dionne et al., 2008; Petrou et al., 2014). Significance was assessed by Mantel test and Multiple Regression on Distance Matrices (MRDM) using the ECODIST package in R (Oksanen et al., 2015). MRDM was applied using the ECODIST package in R (Lichstein, 2006) with 10,000 permutations. MRDM tested for significant relationships between a dependent distance matrix (here, F_{ST} and D_{est}) and one or multiple predictor matrices (Lichstein, 2006). To identify the contribution of each explanatory variable to the overall fit of the model each distance matrix was unfolded into vectors that represented pairwise distances and a regression was performed between the response variable and each predictor. Statistical significance was interpreted through permutations. Mantel tests also have been used to compare distance matrices but heavy criticism in landscape genetics due to elevated risk of type 1 error has lead to a decrease in their use (Castellano and Balletto, 2002; Diniz-Filho et al., 2013; Guillot and Rousset, 2013; Legendre and Fortin, 2010). Here, Mantel tests were used as an exploratory tool and to

provide a comparison among analytical methods. In a Mantel test, correlation (r_M) was calculated between two square matrices that have been unfolded to form distance vectors. Significance was evaluated through permutation by holding one matrix constant and resampling the other.

Hypothesis 2: Isolation by resistance (IBR)

The hypothesis that gene flow is limited by site-specific hydrologic features occurring within spawning habitat was evaluated by examining the differences among sites for riverscape variables (RV): elevation, stream width, and stream depth. Dissimilarity matrices were calculated as the difference between measurements, $x_i - x_j$, where x represents an environmental predictor variable (*i.e.*, stream depth) and sample sites were represented by i and j. The relationship of genetic distance (*i.e.*, F_{ST} and D_{est}) by with waterway distance was quantified using pairwise measures of genetic distance calculated as linearized (index * (1-index)$^{-1}$) values. The effect of genetic distance by riverscape variable was quantified individually in three univariate models (Table 4.1). Significance among models was assessed as described above using MRDM and Mantel tests. Next, the model(s) with significant Mantel's r (r_M), and largest significant R^2 (MRDM) were re-evaluated for significance after accounting for effect of WD. For this analysis, the partial Mantel test (Guillot and Rousset, 2013; Smouse et al., 1986) was used to evaluate the effect of one predictor while holding an alternate predictor (*e.g.,* WD, but can be any predictor) constant. The analysis produces an r_M for each predictor variable and can be used to assess significance of individual variables on genetic distance.

Besides site-specific variation that may affect genetic distance, costs associated with traveling between habitats may also be functionally important components of genetic structure. Increased travel costs were hypothesized to be associated with steeper gradients because steeper gradients maintain faster water flow and therefore require more energy to navigate than a stream reach with shallow gradients. Model optimization techniques were employed that were similar to approaches used in landscape genetics analysis (Bowlby et al., 2016; Castillo et al., 2014; Epps et al., 2007)

in which the appropriate biologically relevant range or cut off values for individual variables are determined by testing a wide range of parameters for each model against genetic structure. Therefore, five candidate models (Table 4.2) were developed to evaluate hypothesis that steeper or shallower gradients increased resistance to dispersal. A power function modeled as x^y, where x = standardized gradient and y = 0.001, 0.01, 0.1, 1.0, 1.5, was used to produce transformations of gradient. These transformations represented a linear increase (Figure 4.2a), an exponentially increasing relationship between resistance and gradient (Figure 4.2b), and a steep initial increase in resistance with increasing gradient followed by a plateau (Figure 4.2c). Transformed gradient was multiplied by the length of stream segment and summed along the shortest path to quantify effective distance (Shirk et al., 2010); Figure 4.3). Shortest path was identified from a river network that was designed using the Network Analysis and Visualization library and package IGRAPH (R core team 2016; Figure 4.3). A pairwise matrix representing cumulative gradient along the shortest path between sites was compared against pairwise genetic distance. Significance among models was assessed as described above using MRDM, Mantel and partial Mantel tests. Univariate tests identified which of the five gradient models were the best predictors of genetic distance. These models were re-evaluated to identify if correlations changed after accounting for WD as described above.

Hypothesis 3: Isolation by directional movement

Additional energetic costs may be incurred for salmon when a change in swimming direction is associated with navigation between reaches (*e.g.,* moving from one tributary into another tributary upstream). The hypothesis that gene flow may be correlated with movement was evaluated by creating a simple categorical matrix. For each pair of sampling locations, movement between reaches that consisted solely of travel upstream or downstream was scored as zero, while reach comparisons that required navigating a change in river direction were scored as one (Table 4.3). For example, movement between reach FAP and any reach upstream was scored zero, where no directional changes were required during dispersal (Figure 4.3). Whereas,

dispersal between reach FAM to reaches FAB, FAC, FAD or FAE required travel downstream and then a change in direction to head upstream, therefore these comparisons were scored one. Significance was assessed by Mantel test and MRDM. Then significant models from IBD and IBR analysis were reassessed for significance after accounting for effects of direction using partial Mantel tests and MRDM as with previous analysis.

Results

Genetic survey

Multi-locus genotypes from 540 samples of fall run Chinook salmon were genotyped. Allelic richness had a mean value of 7.5 that ranged from 7.3 (FAJ) to 7.8 (FAH). Allelic richness was not influenced by distance from the river mouth (Figure 4.4a). Genetic diversity (expected heterozygosity) had a mean value of 0.85, and ranged between 0.83 (FAJ) and 0.87 (FAQ, FAF) but was not significantly influenced by distance from mouth (Figure 4.4b). Pairwise comparisons of genetic distance for both indices ranged between < 0.01 – 0.02 ($p < 0.05$; Table 4.4, Appendix A1).

Isolation by distance (IBD) and resistance (IBR)

Genetic structure was not influenced by waterway distance (Table 4.1). However, genetic distance was affected by site-specific differences in river characteristics. Specifically, differences in elevation of sampling location influenced D_{est} (MRDM, $R^2 = 0.11$, $p < 0.01$) but not F_{ST} (MRDM, $R^2 < 0.001$, $p = 0.90$), both as a univariate model and after controlling for waterway distance in a bivariate model (Table 4.1).

Path-based measures of river characteristics also influenced genetic distance. Model optimization by MRDM showed that two gradient models characterizing steeper channel gradients as more resistant to gene flow were significantly correlated ($p = 0.01$) with D_{est}: $grad^{1.0}$ ($R^2 = 0.12$, $p = 0.01$), $grad^{1.5}$ ($R^2 = 0.14$, $p = 0.01$) in univariate models (Table 4.2). Results of partial Mantel tests showed similar relationships among models (Appendix A2). When the two significant gradient models were re-evaluated for correlation with genetic distance after accounting for waterway distance, they remained significantly correlated with D_{est} (Table 4.2). The model $grad^{1.5}$ displayed strong

correlation after accounting for waterway distance and also reported a slightly larger R^2. Similarly, the point at which the mantel correlation (r_M) among significant models began to plateau suggested $grad^{1.5}$ was the optimum model (Appendix A2). Therefore, $grad^{1.5}$ was selected as the optimum model to describe the relationship between gradient and genetic distance (D_{est}). As with results from site-specific comparisons, linearized F_{ST} did not show significant relationships in any model.

Isolation by direction

There were no significant relationships between direction and genetic distance. Relationships between significant models of IBR remained the same after accounting for direction (Tables 4.1 and 4.2; Appendix A2).

Discussion

This study demonstrated that even within a small coastal watershed, gene flow among spawning locations for fall-run Chinook salmon was influenced by elevation and gradient. Salmon spawning at higher elevations and after traversing steeper gradients were more genetically distinct from those spawning at lower-elevation and lower-gradient areas. This effect (isolation by resistance) was distinguishable from IBD, which was not detected within this system. Although this research has illustrated the potential of a riverscape genetics (RG) approach to better understand structural and functional connectivity in freshwater rivers, it is worth noting that the interpretation of spatial genetic variation differed between the two indices of genetic diversity. F_{ST} did not resolve spatial genetic variation within the fall run and although D_{est} was able to identify significance among riverscape features, this inconsistency between metrics highlights the importance of selecting appropriate metrics for analysis.

Several options now exist for calculating genetic distance, although agreement on the best choice has not been reached (Gerlach et al., 2010; Jost, 2008; Merimans and Hedrick, 2011; Whitlock, 2011). Often convention and the need to make comparisons across watersheds or among taxa dictate whether one or a several metrics are employed. The use of F_{ST} and D_{est} together in this study provided measures of genetic

distance that were based on different components of genetic diversity; F_{ST} was calculated from heterozygosity (Weir and Cockerham, 1984) while D_{est} was calculated from the effective number of alleles (Jost, 2008) thus allowing a slightly different interpretation of genetic distance. However, there was an abundance of low D_{est} values that approached zero, which may have affected the interpretation and inference of spatial genetic relationships from a mathematical perspective.

On the use of IBD and IBR models in riverscape genetics

Investigations that have assessed spatial genetic structure of salmonids within and among watersheds using the classical IBD framework established waterway distance as a consistent predictor for observed genetic isolation (Gomez-Uchida et al., 2009; Harris et al., 2015; Meeuwig et al., 2010; Petrou et al., 2014). However, these relationships are commonly identified among groups at broad spatial scales that spanned thousands of kilometers and included multiple watersheds (Bowlby et al., 2016; Castric et al., 2001; Ozerov et al., 2012). Olsen, J. B. et al. (2010) determined broad-scale spatial structure of Chinook salmon in the Yukon River was influenced by the number of major drainages and flow velocity. The authors also found that these relationships were not maintained at intermediate spatial scales and indicators of hydrology did not explain genetic structure better than distance (Olsen, J. B. et al., 2010). Likewise, among non-Salmonids, similar patterns between IBR and IBD have been identified at broad and fine spatial scales among Rocky Mountain Sculpin populations in Alberta Canada**.** Ruppert et al. (2017) described genetic structure among drainages within the species' eastern range. The authors found significant IBD was identified across multiple watersheds, but at a fine spatial scale (*i.e.*, within Lee Creek, or St. Mary River), the difference in elevation among sites (IBR) was also a predictor of spatial genetic variation in some but not all, watersheds (Ruppert et al., 2017). The complexity of interactions between IBD and IBR highlight the need for continued investigation of specific riverscape features on spatial genetic variation at multiple spatiotemporal scales. Tools and methods being developed for riverscape genetics analysis are able to identify how riverscapes facilitate genetic

exchange and resolve patterns of genetic structure (Davis et al., in review; Landguth et al., 2012b).

Incorporating network and scale in riverscape genetics

Although the spatial structure of riverine systems influences connectivity and consequently affect dispersal, many of the relationships between the physical network, population distribution and genetic structure are not well understood (Le Pichon et al., 2006; Leps et al., 2015). Thinking about the riverscape as a branched network of interconnected tributaries is a relatively recent advancement in riverine ecology (Altermatt, 2013; Borrett et al., 2014; Campbell Grant et al., 2007; Thorp et al., 2006; Wiens, 1989). Theoretical literature discussing connectivity in freshwater river systems has provided models and frameworks through which this scale of analysis may be accomplished (Benda et al., 2004; Borrett et al., 2014; Campbell Grant et al., 2007; Frissell et al., 1986; Montgomery, 1999; Wipfli et al., 2007). Here, the effect of movement among the branched network was tested by incorporating a model that placed a penalty on movement between reaches that required a change in the direction of travel. Although there was no relationship using the movement model applied in this study, the approach may be worth attempting in systems that have a more complex network. Efforts, like the current research, to incorporate these concepts into analysis are ongoing but continued development and refining of methodologies are still needed (Dyer, 2015; Selkoe et al., 2016).

The research presented here is the first to investigate spatial genetic relationships of an individual salmon run throughout the extent of its spawning habitat and link hydrogeomorphic processes that drive functional connectivity to the observed genetic variation. Emerging riverscape genetics research prior to this work has investigated IBR at broad spatial scales (Bradbury et al., 2014; Faulks et al., 2010; Kanno et al., 2011). However, at intermediate scales, waterway distance alone does not consistently describe these spatial genetic relationships. Dionne et al. (2008) investigated 51 spawning groups of Atlantic salmon in Québec, New Brunswick and identified temperature and coastal distance as factors that shaped seven spatially

structured genetic clusters. Hand et al. (2016) showed winter precipitation, summer maximum temperature, and summer mean flow significantly affected spatial genetic structure among 79 spawning groups of *Oncorhynchus mykiss* in the Columbia River Basin. Riverscape genetics studies have yet to assess finer nuanced patterns that occur in watersheds < 200 km, which are likely responsible for long-term persistence.

Conclusion

Here I have demonstrated how IBR is detectable within fall run Chinook salmon from a 109 km^2 watershed. I also show that incorporating path analysis in a riverscape genetics framework helped expand interpretation of IBR by considering the path between habitat. Habitat fragmentation and changes to water flow that alter functional connectivity within riverscapes have caused increased resistance to dispersal and loss of access to suitable spawning habitat. For Pacific salmon such physical changes have contributed to population decreases, restructuring of source-sink dynamics (Schick and Lindley, 2007), and reduced range distributions (Yoshiyama et al., 1998). To combat these effects at state levels, entities like the Pacific Fishery Management Council have developed Pacific Coast Salmon Fishery Management Plans (FMP;((PFMFC), 2016). These plans list management objectives for ESA listed species, escapement goals, catch limits, and describe essential fish habitat for the management of commercial and recreational salmon fisheries. However, management at this level is based upon status of larger, regional management units (*e.g.*, the North Oregon Coast Chinook salmon ESU). These management units are combinations of unique spawning groups from multiple, smaller river basins and may not account for fine-scale genetic and phenotypic variability that is present within smaller watersheds (Davis et al., 2017).

Within riverscapes, geographic and hydrologic characteristics are often correlated and identifying how each or both impact genetic structure is difficult at best. Nonetheless, the ability of conservation and management to ensure long-term viability of salmon populations or establish effective recovery strategies for any threatened species is dependent on understanding how these processes affect dispersal and subsequent gene flow. Riverscape genetics methods that include network relationships

would enable more informed interpretation of these fine-scale relationships, thereby ensuring the design of effective recovery strategies for pacific Salmonids.

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Table 4.1 Models of pairwise genetic distance (Linearized D_{est}, and F_{st}) as a function of pairwise difference between riverscape variables measured at each reach, using Multiple Regression on Distance Matrix (MRDM). Riverscape variables
Significa

	Dest				Fst			
	coefficient	**pvalue**	**R^2**	**pvalue**	**coefficient**	**pvalue**	**R^2**	**pvalue**
Index ~ WD	2.41E-08	0.65	2.77E-03	0.65	1.75E-08	0.60	4.32E-03	0.60
Index ~ Dir	2.39E-04	0.94	1.26E-04	0.94	-7.35E-05	0.97	3.47E-05	0.97
Index ~ depth	3.28E-03	0.35	1.03E-02	0.35	5.19E-05	0.99	7.55E-06	0.99
Index ~ width	4.96E-05	0.58	4.12E-03	0.58	-1.04E-07	1.00	5.30E-08	1.00
Index ~ elev	5.28E-05	4.90E-03	**1.06E-01**	**4.90E-03**	2.11E-06	0.90	4.91E-04	0.90
Index~ elev(dist)(dir)			**1.16E-01**	**0.03**			4.52E-03	0.97
Int	1.67E-03	0.94			9.81E-03	0.75		
WD	-5.21E-08	0.33			1.88E-08	0.64		
dir	-1.09E-04	0.97			-1.49E-04	0.94		
elev	6.11E-05	3.30E-03			-7.96E-07	0.97		

Table 4.2 Models of pairwise genetic distance (Linearized D_{est}, and F_{st}) as a function of cumulative gradient using Multiple Regression on Distance Matrix (MRDM). Waterway distance (WD), direction (dir), gradient (grad). Significant relationships are reported in bold.

	Dest				Fst			
	coefficient	**pvalue**	**R^2**	**pvalue**	**coefficient**	**pvalue**	**R^2**	**pvalue**
Index ~ $grad^{0.001}$			0.03	0.12			0.01	0.51
	1.70E-03	0.93			1.09E-02	0.26		
	1.03E-07	0.12			-3.21E-08	0.51		
Index ~ $grad^{0.01}$			0.03	0.12			0.01	0.50
	1.73E-03	0.92			1.09E-02	0.26		
	1.02E-07	0.12			-3.21E-08	0.50		
Index ~ $grad^{0.1}$			0.04	0.09			0.01	0.53
	1.57E-03	0.94			1.08E-02	0.27		
	8.52E-08	0.09			-2.35E-08	0.53		
Index ~ $grad^{1.0}$			**0.11**	**1.90E-03**			4.99E-06	0.99
	8.53E-04	9.99E-01			8.53E-04	9.99E-01		
	5.22E-09	1.90E-03			5.22E-09	1.60E-03		
Index ~ $grad^{1.5}$			**0.13**	**6.00E-04**			2.33E-03	0.78
	1.44E-03	1.00			1.00E-02	0.62		
	2.88E-10	6.00E-04			2.28E-11	0.79		
Index ~ $grad^{1.5}$+ dist + dir			**0.14**	**0.01**			0.01	0.93
Int	2.63E-03	0.76			9.47E-03	0.84		
Pwr1.5	3.30E-10	1.10E-03			1.16E-11	0.90		
WD	-5.74E-08	0.35			2.91E-08	0.54		
dir	-1.81E-03	0.51			-1.94E-04	0.92		

Table 4.3 Pairwise matrix representing movement among tributaries within the network. Movement between reaches that consisted solely of upstream or downstream travel was scored as zero. Comparisons that required a change in direction of travel were scored as one.

Pop	FAB	FAC	FAD	FAE	FAF	FAH	FAJ	FAK	FAM	FAO	FAP	FAQ	FAS	FAT	FAU	FAV	FAW
FAB	NA	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
FAC	0	NA	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
FAD	0	0	NA	0	0	0	1	1	1	0	0	0	0	0	0	0	0
FAE	0	0	0	NA	0	0	1	1	1	0	0	0	0	0	0	0	0
FAF	0	0	0	0	NA	0	1	1	1	0	0	0	0	0	0	0	0
FAH	0	0	0	0	0	NA	1	1	1	0	0	0	0	0	0	0	0
FAJ	1	1	1	1	1	1	NA	0	0	0	0	0	0	0	0	0	0
FAK	1	1	1	1	1	1	0	NA	0	0	0	0	0	0	0	0	0
FAM	1	1	1	1	1	1	0	0	NA	0	0	0	0	0	0	0	0
FAO	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0	0
FAP	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0	0
FAQ	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0	0
FAS	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0	0
FAT	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0	0
FAU	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0	0
FAV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	0
FAW	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA

Table 4.4 Pairwise genetic distance (Linearized D_{est}) between fall run Chinook salmon spawning groups. Pairwise Estimates of Linearized D_{est} (below diagonal) and p-values (above diagonal) for all population pairs. Significant D_{est} values are in bold.

Pop	FAB	FAC	FAD	FAE	FAF	FAH	FAJ	FAK	FAM	FAO	FAP	FAQ	FAS	FAT	FAU	FAV	FAW
FAB	-	0.07	0.75	0.49	0.43	0.91	0.80	0.75	0.31	0.73	0.90	0.77	0.61	0.15	0.35	0.70	0.47
FAC	0.020	-	0.03*	0.03*	0.04*	0.71	0.79	0.16	< 0.01*	0.18	0.27	0.01*	0.09	0.01*	0.38	0.16	0.04*
FAD	0.000	**0.030**	-	0.86	0.52	0.99	0.30	0.58	0.56	0.19	0.80	0.38	0.87	0.33	0.28	0.56	0.26
FAE	0.000	**0.021**	0.000	-	0.13	0.96	0.83	0.65	0.16	0.37	0.84	0.15	0.13	0.01	0.91	0.39	0.37
FAF	0.001	**0.018**	0.000	0.012	-	1.00	0.37	0.99	0.31	0.71	0.91	0.62	0.51	0.75	0.67	0.97	0.24
FAH	0.000	0.000	0.000	0.000	0.000	-	0.95	0.97	0.98	0.98	1.00	0.85	0.98	0.96	0.93	0.99	0.97
FAJ	0.000	0.000	0.013	0.000	0.006	0.000	-	0.96	0.52	0.99	0.91	0.80	0.66	0.71	0.96	0.97	0.79
FAK	0.000	0.009	0.000	0.000	0.000	0.000	0.000	-	0.72	0.94	0.90	0.99	0.65	0.97	0.60	0.99	0.63
FAM	0.007	**0.025**	0.000	0.010	0.004	0.000	0.000	0.000	-	0.09	0.64	0.05	0.50	0.26	0.55	0.86	0.06
FAO	0.000	0.007	0.014	0.003	0.000	0.000	0.000	0.000	0.012	-	0.91	0.47	0.60	0.64	0.92	0.97	0.95
FAP	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	0.99	0.86	0.94	0.99	0.95	0.90
FAQ	0.000	**0.019**	0.005	0.009	0.000	0.000	0.000	0.000	0.011	0.001	0.000	-	0.46	0.84	0.49	0.90	0.65
FAS	0.000	0.018	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	-	0.90	0.33	0.96	0.43
FAT	0.019	**0.029**	0.008	0.030	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	-	0.41	0.96	0.33
FAU	0.007	0.005	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.004	-	0.57	0.90
FAV	0.000	0.011	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	0.68
FAW	0.000	**0.018**	0.013	0.004	0.008	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.002	0.004	0.000	0.000	-

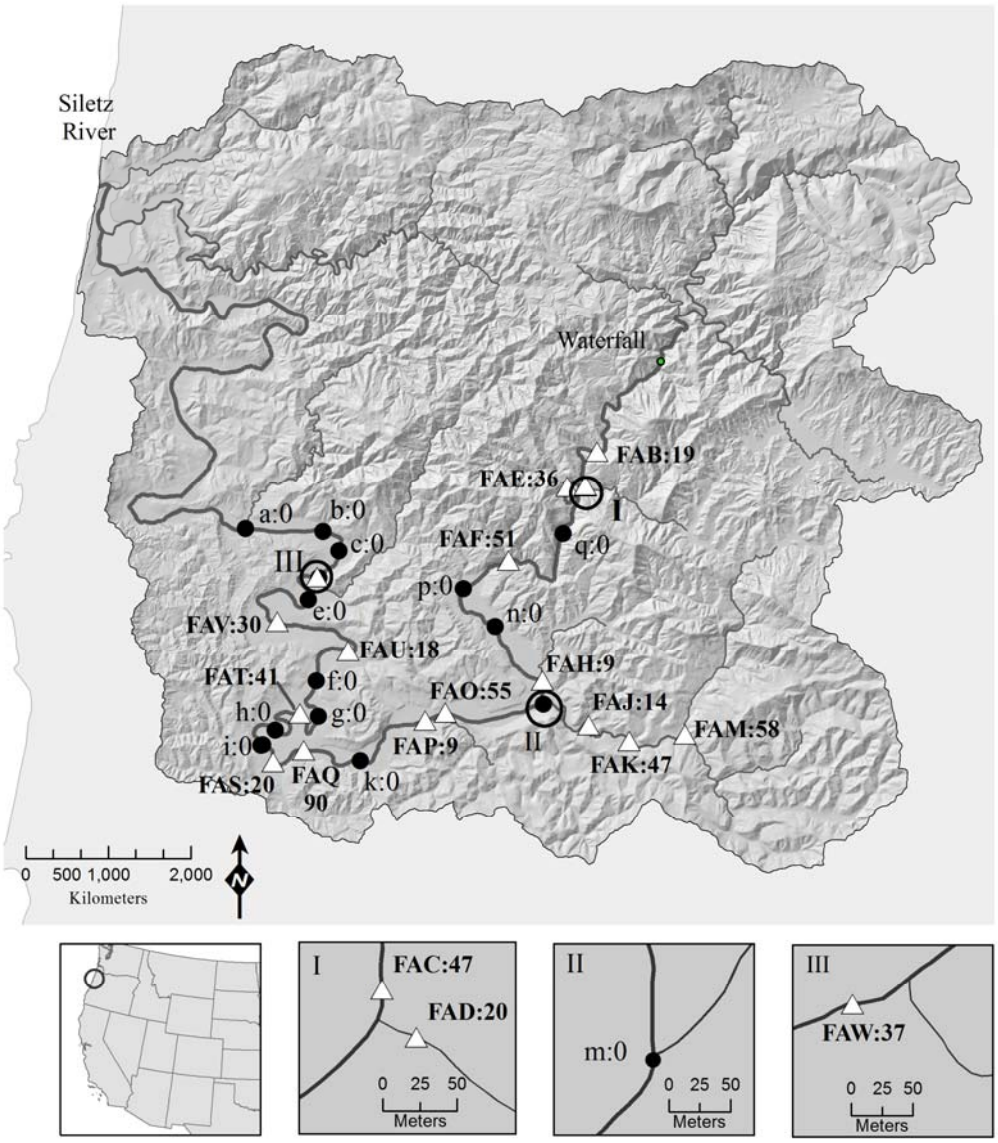

Figure 4.1 Map of Siletz River, Oregon. Grey lines represent the main stem and tributaries of the river. Downstream boundary for each reach is represented by black filled circles with lower case letters (no samples were collected) and triangles with capital letters (sample numbers reported). Roman numerals identify sites that were located around river confluences; each inlay expands the area at these sites.

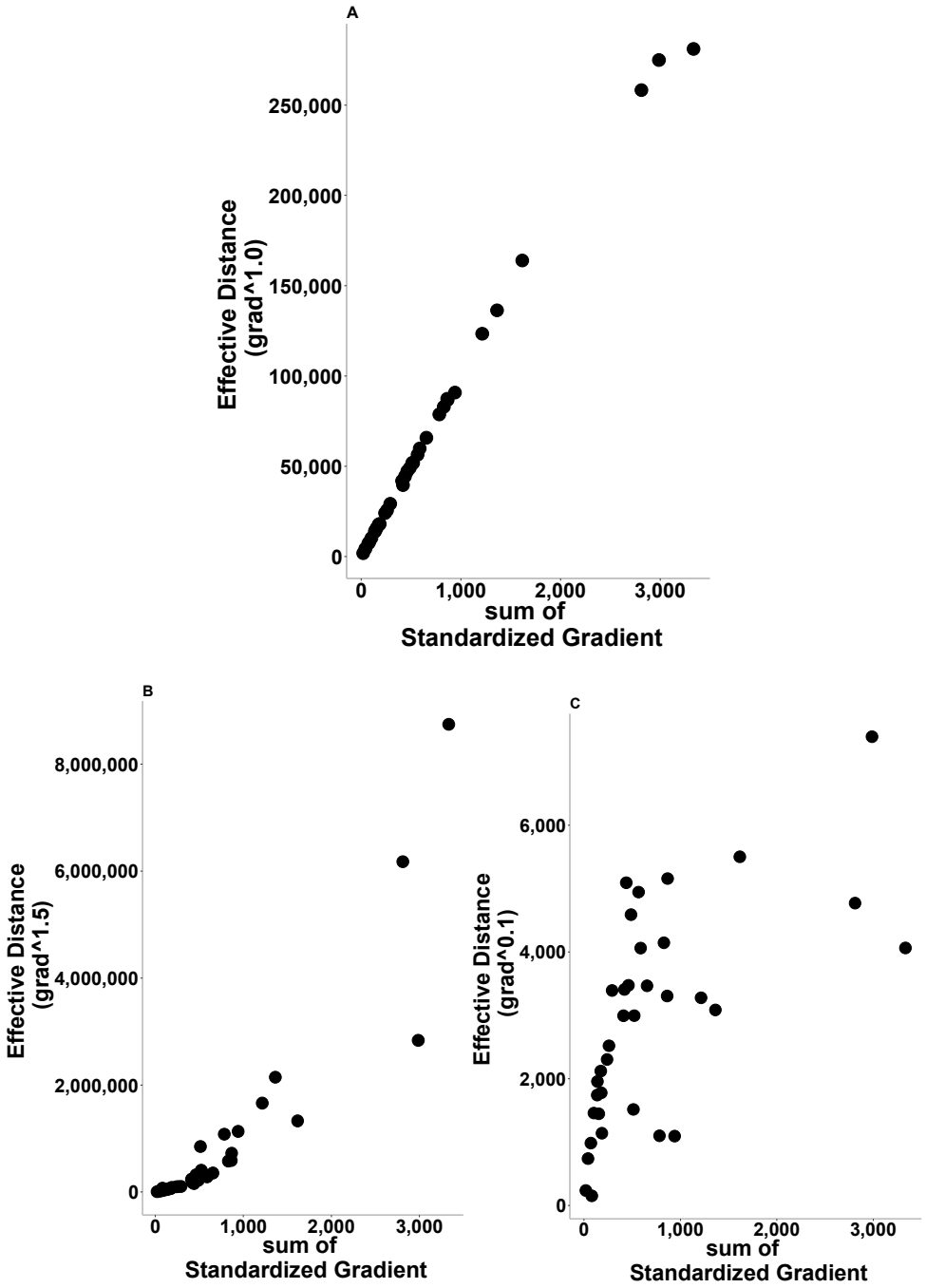

Figure 4.2 Effective distance is plotted against standardized gradient. Effective distance is a power function transformation of standardized gradient $x^{1.0}$ (A), $x^{1.5}$ (B), and $x^{0.1}$ (C) multiplied by the length of stream segment and summed along the shortest path between paired reaches. Data transformations reflect hypothesis that steeper (B) or shallower (C) gradients increased resistance to dispersal relative to non-transformed gradient (A).

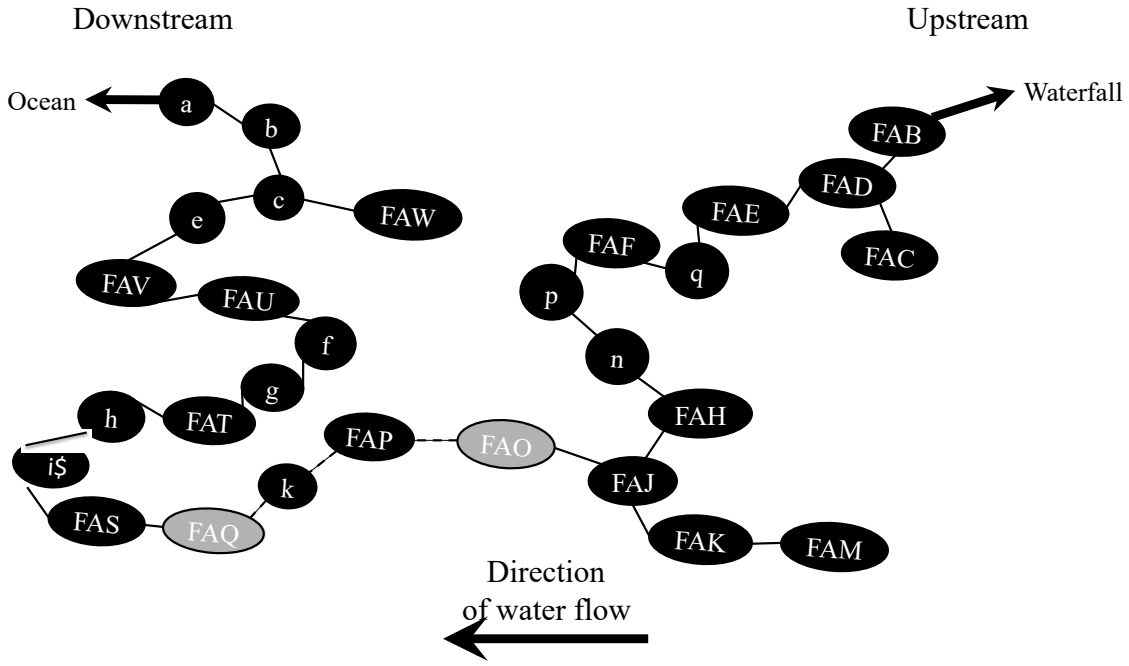

Figure 4.3 A river network where graph vertices represent a reach and corresponding edges reflect the organization of reaches at the extent of the watershed. Arrows identify water flow and the location of upstream and downstream river features to orient the river relative to the true river network (figure 4.1). Effective distance was calculated as the sum of riverscape features along the shortest path (*e.g.,* dashed lines reflect the shortest path between paired reaches FAQ and FAO (grey ovals)). Analysis was carried out using R package IGRAPH (R core team 2016).

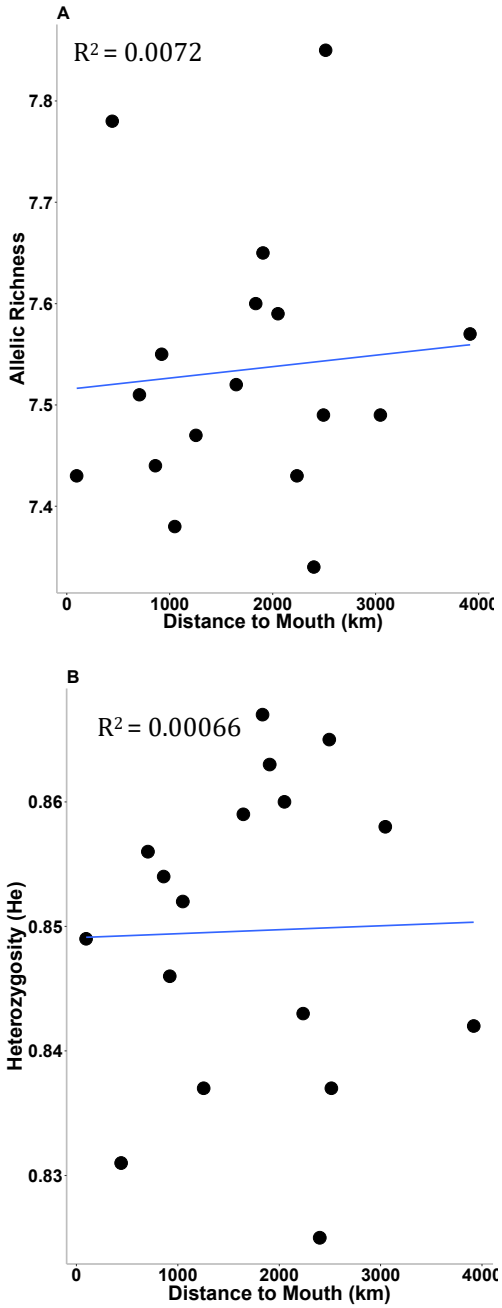

Figure 4.4 Plot of allelic richness (A) and expected heterozygosity (B) with distance from Siletz river mouth for each group.

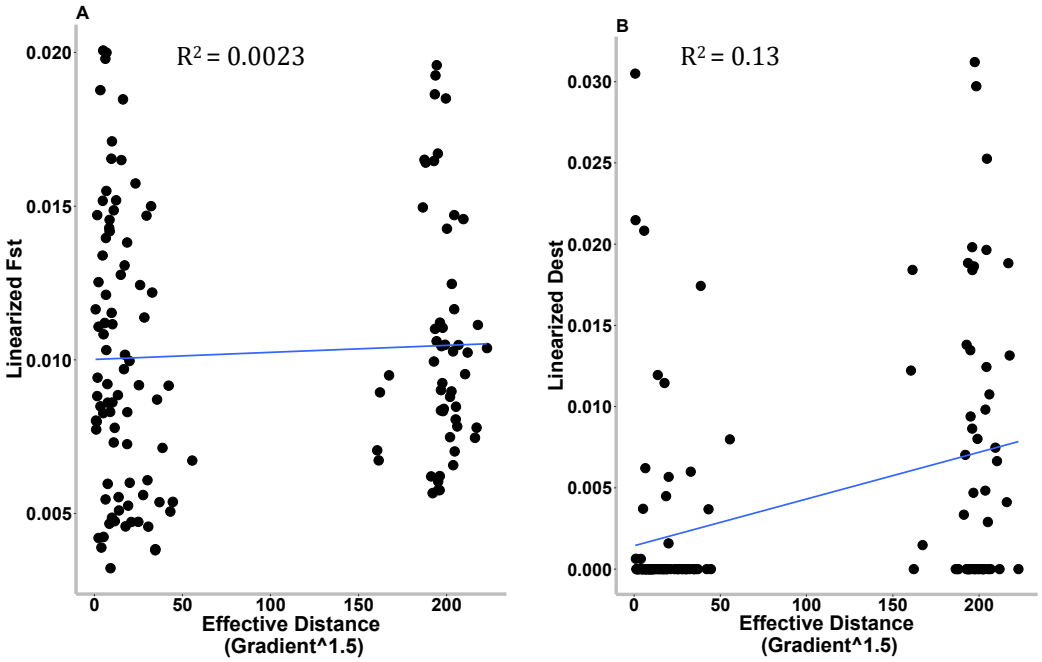

Figure 4.5 Isolation by resistance (IBR) plots of genetic distance as pairwise linearized F_{st} (A) and D_{est} (B). Effective distance is a power function transformation of standardized gradient multiplied by the length of stream segment and summed along the shortest path. The gap within each figure results from data transformation where effective distances are not present within the river

1

Chapter 5:

General Conclusion

I investigated the utility of riverscape genetics to identify fine-scale (*i.e.*, stream reach) spatial genetic structure in a freshwater river. In the preceding chapters I defined riverscape genetics (RG), and provided a perspective on how RG could provide a more comprehensive conceptual and applied understanding of connectivity and dispersal in freshwater systems. I used standard population genetic metrics to assess the genetic structure of Chinook salmon in a small coastal watershed and then used RG analysis to identify spatial resolution among the unique spawning groups. Throughout the literature I noted that there were fundamental challenges in previous studies in translating the approaches used in land and seascape genetics into freshwater river networks. These challenges resulted from the functional differences in riverscape topography that created constrained pathways for fish movement and directional water flow. I identified a method using network theory and calculations of effective distance would have great potential for freshwater application.

I selected Chinook salmon (*Oncorhynchus tshawytscha*) for this analysis because these culturally important and economically valuable fish express diverse life histories characterized by the season of their return migration to spawning habitat (called a "run") and populations have been significantly affected by physical changes in freshwater habitats (Fullerton et al., 2010; Miller, 2010; Schick and Lindley, 2007; Yoshiyama et al., 1998). Identifying features of the landscape that promoted or impeded dispersal would provide an ecological context for understanding how these populations are structured and help to prevent further decline in population abundances or extinctions (Manel et al., 2003). Using traditional population genetic metrics I found strong support for the existence of two genetically and phenotypically distinct salmon populations in a watershed where only one is currently recognized. The fall-run population spawned downstream of the waterfall, and a genetically distinct population of the spring-run ecotype spawned upstream of the waterfall. This study was the first analysis of spring run in Siletz River, and has provided knowledge that a greater diversity of life histories may exist in small watersheds for these highly migratory species. This finding also highlighted the need for research that is conducted at multiple

spatial scales because research conducted only at large spatial scales may miss finer nuanced patterns that occur in smaller watersheds and are likely responsible for long-term persistence.

Separating and identifying the effects of waterway distance and riverscape features to better understand the effects of dispersal on population structure is a major challenge facing conservation and management of highly migratory species like Pacific Salmon. I used two approaches to further investigate spatial genetic structure of the fall run: a path analysis RG approach that incorporated network position into analysis, and the more commonly applied approach of calculating site-specific differences among patches that does not incorporate the river network. The results of this final study provided evidence that indicators of hydrology, elevation and gradient defined spatial genetic structure. The cumulative "cost" of movement between sampling locations or "effective distance" was based on changes in gradient. The results suggested that increased resistance was generated by steeper gradients and this influenced dispersal of individuals within the network. Additionally, within specific habitats the elevation at which reaches were positioned in the watershed were significantly correlated with spatial genetic structure. Dissimilarity matrices provided some context of the differences among freshwater habitat patches that contributed to overall genetic variation, but cannot easily capture the continuous exposure to environmental selection and resistance experienced by organisms moving through the riverscape.

Distance was not a predictor of spatial genetic variation within this study although in larger systems across greater spatial scales that approach > 1000 km, distance is a consistent predictor of spatial variation. Given that smaller watersheds may hold life history variation that is important to long-term population persistence, there is need to begin to understand the relationships that maintain this diversity. IBR played a greater role than IBD in predicting spatial genetic variation of fall run Chinook salmon in Siletz River. The combined context of site specific and path analysis placed emphasis on the utility of IBR model to provide a better understanding of the relationship among groups within the river. Continued investigation using RG at fine spatial scales and

incorporating network relationships have the potential to better understand the effects of dispersal on population structure for the conservation and management of highly migratory species.

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Appendix

Appendix A.1 Pairwise genetic distance (Linearized FST between Fall run Chinook salmon groups among reaches. Pairwise Estimates of Linearized F_{ST} (below diagonal) and p-values (above diagonal) for all population pairs. Significant F_{ST} values are in bold.

Pop	FAB	FAC	FAD	FAE	FAF	FAH	FAJ	FAK	FAM	FAO	FAP	FAQ	FAS	FAT	FAU	FAV
FAB	-	0.07	0.75	0.50	0.46	0.88	0.81	0.78	0.32	0.75	0.87	0.83	0.62	0.17	0.35	0.70
FAC	0.011	-	0.03*	0.03*	0.04*	0.65	0.82	0.16	<0.01*	0.18	0.23	0.01*	0.08	0.01*	0.38	0.16
FAD	0.012	**0.012**	-	0.86	0.52	0.98	0.31	0.59	0.55	0.20	0.77	0.43	0.86	0.37	0.29	0.55
FAE	0.010	**0.008**	0.009	-	0.13	0.94	0.86	0.66	0.16	0.37	0.79	0.17	0.11	0.01	0.92	0.40
FAF	0.009	**0.007**	0.009	0.007	-	0.99	0.40	0.99	0.30	0.71	0.89	0.63	0.45	0.74	0.70	0.97
FAH	0.018	0.016	0.016	0.015	0.012	-	0.93	0.96	0.97	0.97	1.00	0.85	0.98	0.97	0.91	0.98
FAJ	0.014	0.010	0.016	0.011	0.012	0.020	-	0.98	0.57	0.99	0.89	0.89	0.71	0.81	0.97	0.98
FAK	0.009	0.006	0.009	0.006	0.004	0.014	0.009	-	0.72	0.93	0.88	0.99	0.60	0.97	0.64	0.99
FAM	0.009	**0.007**	0.008	0.007	0.005	0.013	0.011	0.005	-	0.09	0.59	0.05	0.43	0.24	0.56	0.86
FAO	0.008	0.006	0.010	0.006	0.005	0.013	0.008	0.004	0.006	-	0.89	0.48	0.55	0.62	0.94	0.97
FAP	0.018	0.019	0.019	0.016	0.015	0.019	0.020	0.015	0.016	0.014	-	0.99	0.88	0.96	0.98	0.93
FAQ	0.007	**0.006**	0.008	0.006	0.004	0.014	0.009	0.003	0.005	0.004	0.012	-	0.42	0.83	0.58	0.93
FAS	0.012	0.010	0.011	0.011	0.009	0.016	0.014	0.009	0.008	0.008	0.018	0.008	-	0.90	0.34	0.95
FAT	0.012	**0.008**	0.010	0.009	0.005	0.015	0.011	0.005	0.006	0.005	0.015	0.004	0.008	-	0.49	0.97
FAU	0.014	0.010	0.014	0.009	0.009	0.018	0.013	0.010	0.009	0.008	0.017	0.009	0.014	0.011	-	0.57
FAV	0.010	0.008	0.010	0.008	0.005	0.014	0.010	0.005	0.006	0.005	0.015	0.005	0.008	0.006	0.011	-

Appendix A.2 Mantel correlation (r_M) reported for Mantel and partial Mantel tests between pairwise genetic distance for linearized [x * (1-x)$^{-1}$] D_{est} and F_{st}. Explanatory variables are: waterway distance (WD), direction (dir), Elevation (elev), Stream width (width), Stream depth (depth) as well as five transformations of Gradient (grad$^{\#}$). Significant relationships are reported in bold.

	Dest		Fst	
	r_M	pvalue	r_M	pvalue
Mantel:				
Index ~ WD	0.05	0.66	0.07	0.62
Index ~ Direction	0.01	0.94	-0.01	0.97
Index ~ depth	0.10	0.36	2.75E-03	0.99
Index ~ width	0.06	0.59	-2.30E-04	1.00
Index ~ elev	**0.32**	**0.00**	0.02	0.89
Index ~ grad $^{.001}$	0.18	0.12	-0.10	0.51
Index ~ grad $^{.01}$	0.18	0.12	-0.10	0.50
Index ~ grad $^{.01}$	0.20	0.10	-0.09	0.54
Index ~ grad $^{1.0}$	**0.33**	**3.00E-03**	2.23E-03	0.99
Index ~ grad $^{1.5}$	**0.36**	**1.00E-03**	0.05	0.79
Patail Mantel:				
Index ~ depth(WD)	0.10	0.35	3.50E-03	0.98
Index ~ width(WD)	0.07	0.59	1.99E-03	0.99
Index ~ elev(WD)	**0.34**	**2.70E-03**	-0.01	0.96
Index ~ WD(depth)	0.05	0.648	0.07	0.62
Index ~ WD(width)	0.05	0.636	0.07	0.61
Index ~ WD(elev)	-0.11	0.315	0.06	0.63
Index ~ depth(dir)	0.10	0.36	2.58E-03	0.99
Index ~ width(dir)	0.06	0.58	3.53E-04	1.00
Index ~ elev(dir)	**0.32**	**3.80E-03**	0.02	0.89
Index ~ dir(depth)	0.01	0.93	-0.01	0.97
Index ~ dir(width)	4.91E-03	0.98	-0.01	0.97
Index ~ dir(elev)	-0.01	0.93	-0.01	0.97
Index ~ grad$^{1.0}$ (WD)	**0.34**	2.40E-03	-0.03	0.86
Index ~ grad$^{1.5}$(WD)	**0.37**	**1.20E-03**	0.02	0.92
Index ~ WD(grad$^{1.0}$)	-0.11	0.40	0.11	0.47
Index ~ WD(grad$^{1.5}$)	-0.11	0.36	0.09	0.53
Index ~ grad$^{1.0}$(dir)	**0.33**	**2.70E-03**	3.57E-03	0.98
Index ~ grad$^{1.5}$(dir)	**0.36**	**1.00E-03**	0.05	0.78
Index ~ dir(grad$^{1.0}$)	-0.06	0.66	-0.01	0.97
Index ~ dir(grad$^{1.5}$)	-0.08	0.54	-0.02	0.91