BIODIVERSITY OF ARCTIC CHAR (*SALVELINUS ALPINUS*): SYMPATRIC MORPHS AND HYBRIDIZATION WITH DOLLY VARDEN (*S. MALMA*) IN SOUTHWESTERN ALASKA

by

SHANNAN LEIGH MAY-MCNALLY

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Abstract

Resource polymorphism and natural hybridization are evolutionary phenomena that play an important role in the development of reproductive isolation during speciation. The Arctic char (Salmonidae: *Salvelinus alpinus*) exhibits substantial phenotypic and genetic diversity across its Holarctic range, making it an ideal species to examine the role of intraspecific trophic polymorphism in driving reproductive isolation and the interactions between hybridizing species in sympatry. In one southwestern post-glacial lake previously analyzed for resource polymorphism (Lower Tazimina Lake), I found evidence for two genetic groups of char and for significant differences in the distribution of microsatellite variability among at least two of the three previously described body-size morphotypes ('large', 'medium', and 'small'–bodied char; maximum F_{ST} = 0.09). I also found significant associations between genetic type and gill raker counts among body-size morphs (r = -0.73, P < 0.001). These data represent the first record of genetically distinct sympatric Arctic char morphs in Alaska and provides further evidence that morphological differences associated with feeding and growth trajectories reflect niche diversification and may promote genetic divergence. Furthermore, I evaluated the level of natural hybridization and ecological segregation between two sympatric sister species of char, Arctic char and Dolly Varden (*Salvelinus malma*), from two southwestern Alaskan lakes. A total of N = 725 char were collected from 44 microhabitats across two lakes (Lake Aleknagik and Lake Nerka) and genotyped at 13 microsatellite loci. Using genetic admixture (Q)-values generated through Bayesian-based clustering, hybridization levels between Arctic char and Dolly Varden were found to be minimal; less than 0.5% of samples were classified as hybrids. Concurrent analyses with NEWHYBRIDS, however, supported the presence of late-generation hybrids in Lake Aleknagik with up to 7% of all samples representing F_2 hybrids or backcrossed Dolly Varden. The existence of two discrete gene pools in sympatry (F_{ST}= 0.172–0.187 across the two lakes) which were generally spatially separate and low levels of contemporary hybridization between Arctic char and Dolly Varden helps to solidify their taxonomic status as

distinct biological species. Moreover, high levels of reproductive isolation between these sympatric species may be driven largely by strong pre-mating spatial segregation.

Preface

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Chapter 2. A version of this chapter has been published as [May-McNally, S.L., Quinn, T.P., Woods, P.J. and Taylor E. B. 2014/ Evidence for genetic distinction among sympatric ecotypes of Arctic char (*Salvelinus alpinus*) in south-western Alaskan lakes. *Ecology of Freshwater Fish*, available online, doi: 10.1111/eff.12169]. I was responsible for concept formation, genetic analyses, data synthesis, and manuscript composition. Tissue samples used in this study were collected by P.J. Woods (School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA, USA). Sample collection in Lake Clark National Park was facilitated by D. Young (U.S. National Park Service). J.S. Moore and E.B. Taylor developed the genetic methods that I employed in this study. T.P. Quinn and P.J. Woods were involved in concept formation and refinement of the manuscript. E. B. Taylor was the supervisory author on this project and was involved throughout the project in statistical analysis and manuscript preparation.

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Dedication

To my family and all fish enthusiasts.

Chapter 1:

General introduction

Understanding the origins and maintenance of reproductively isolated populations is a central focus of speciation biology. Reproductive isolation can arise indirectly when divergent natural selection acts upon morphological, behavioural or physiological traits associated with differential resource use in a population or through direct reinforcement of pre-mating isolation (Schluter 1996). For instance, divergent selection has been shown to occur through resource polymorphisms in sympatric populations when an ancestral population expands to exploit novel resources (niche invasion) or through subdivision of resources from a broad niche into two narrower niches (niche specialization) (Schluter 2000; Knudsen *et al.* 2006). In such cases, reproductive isolation may arise indirectly because traits that are the focus of divergent selection also influence mate choice (e.g., body size –Foote & Larkin, 1988; Conte & Schluter, 2013). Alternatively, reinforcement can occur through direct selection against hybrids causing selection for divergent mate-selection traits (Schluter 1996; Servedio & Noor 2003; Mallet 2005).

Hybridization is defined as the successful interbreeding of individuals from two populations, or groups of populations, that can be distinguished on the basis of one or more heritable characters (Harrison 1990; Arnold 1997; Dowling & Secor 1997). Hybridization was once considered to be both a source of evolutionary “noise” and a rare phenomenon (Arnold 1997). This view, however, is becoming increasingly challenged as modern molecular techniques facilitate the identification of hybrid individuals (Seehausen 2004; Aboim *et al.* 2010). Although botanists have long recognized the creative role of hybridization in evolution, the evolutionary role of hybridization as a process that can promote or limit gene flow, reinforce or break down reproductive barriers and/or generate novel genotypes on which natural selection can act has only been more widely appreciated by zoologists (Arnold 1992; Mallet 2005).

Post-Pleistocene glacial populations of fishes have provided key study systems in research designed to better understand the roles of resource polymorphism and hybridization in speciation (Schluter & Rambaut 1996). The rapid evolution of temperate fishes in post-glacial lacustrine systems younger than < 15 000 years old provide unique opportunities to examine the first steps in divergence (Schluter & Rambaut 1996). Secondary contact and introgression of species or populations isolated during the last glaciation in refugia can also be a means of understanding the evolutionary consequences of hybridization and determining the role of pre- or post-mating reproductive barriers in constraining gene flow in sympatry (e.g., Lu & Bernatchez, 1998). Unfortunately, like many Arctic environments, post-glacial lacustrine systems are fragile and are becoming increasingly at risk from human perturbations which include climate change, environmental pollution from resource extraction and over-fishing (Reist *et al.* 2013).

Fishes from the Salmonidae family (66 Holarctic species of salmon, trout, char, grayling and whitefishes) are frequent inhabitants of post-glacial waters (Hendry & Stearns 2003; Nelson 2006). Many species of salmonids are capable of forming spatially and temporally discrete breeding units that can exhibit distinct genetic and phenotypic differentiation (Behnke 1972; Schluter & Rambaut 1996; Hendry & Stearns 2003; Taylor *et al.* 2008). These discrete breeding units are often adapted to different habitats but are not isolated by geographic barriers (Taylor 2004). Salmonids may also have the capacity to quickly evolve reproductive isolation through adaptive divergence (reviewed in Schluter, 1996; Taylor, 1999). For instance, some populations of sockeye salmon (*Oncorhynchus nerka*) released into a novel lake system were capable of founding several new, genetically-distinct populations differing in niche use, male body depth, female body size, and embryo survival rate after only 13 generations (Hendry 2001). This result suggests that ecological speciation in salmonids often closely follows the colonization of new environments containing vacant niches.

Char (salmonid fishes of the genus *Salvelinus*) show an especially wide range of variation in ecology, habitat use, life history, and phenotype. Char often differentiate into morphologically distinct forms in sympatry and hybridization amongst the

various species is common. Consequently, *Salvelinus* has been an important group for understanding factors related to ecological speciation and the divergence of fishes (Behnke 1972; Klemetsen 2010). Char are common species in many Holarctic regions and are abundant in areas throughout temperate areas of Canada and the USA. Despite being widespread and phenotypically diverse in Alaska, it is surprising how little is known about the genetic diversity and evolutionary history of char from Alaskan post-glacial lakes.

1.1 Arctic char

The Arctic char (*Salvelinus alpinus* (L., 1758), Pisces: Salmonidae) is the most northerly distributed freshwater salmonid and is one of five recognized North American, and one of approximately 22 Eurasian, species of char (Johnson 1980; Maitland 1995; Reist *et al.* 2013). Generally considered to be a single taxon, Arctic char can display substantial variability in phenotype and ecology, leading to the assignment of species-level status of multiple forms or the grouping of diverse forms into a species 'complex' (Snorrason *et al.* 1994; Maitland 1995; Jonsson & Jonsson 2001; Klemetsen 2013).

The native distribution of Arctic char extends across freshwater and near-shore marine habitats in North America, northern Britain, Scotland, Iceland, Europe and Russia (Johnson 1980). The species' southern limit extends to latitudes as low as 43-45º N in North America and Europe and corresponds closely to the maximum extent of the last glaciation (Weichsel-Wisconsinan) in the Holarctic (Klemetsen *et al.* 2003; Power *et al.* 2009). Perhaps limited by its physiological preference for low temperature water, Arctic char are especially common in many northern ecosystems, especially in isolated post-glacial lakes with low interspecific competition (Johnson 1980; Skúlason & Smith 1995; Klemetsen *et al.* 2003). The capacity for Arctic char to specialize in a variety of habitats across its range such as lakes (including profundal, littoral or pelagic regions), rivers, streams, and oceans and exhibit different life histories (e.g. anadromy or non-anadromy) has been well established (Skúlason *et al.* 1996; Bernatchez *et al.* 1998; Jonsson & Jonsson 2001;

Klemetsen 2010; Woods *et al.* 2013). In fact, Klemetsen (2013) compared variation across a number of northern fishes and declared the Arctic char to be the “most variable vertebrate on Earth”. A combination of plasticity and adaptive variation in life history and various phenotypic traits are two key reasons why Arctic char are so popular for evolutionary studies of trophic polymorphism and speciation (Power *et al.* 2009).

Although thoroughly researched throughout Eurasia (Snorrason *et al.* 1994; Jonsson & Jonsson 2001; Adams & Huntingford 2002b) and, increasingly, in Canada (Reist *et al.* 1995; Gomez-Uchida *et al.* 2008; Power *et al.* 2009), Arctic char have been largely understudied in Alaska (USA). In Alaska, Arctic char are abundant along the Bering Sea and Arctic coast from the northern slope to the southwestern portion of the state where they are common in freshwater lakes and streams (McPhail 1961; McPhail & Lindsey 1970). McPhail (1961) described two forms of Arctic char in Alaska including a “western” form characterized by lower gill raker and pyloric caeca counts (13-14, 27-31, respectively) found in rivers in northern and western Alaska, and an “eastern” form with higher gill raker and pyloric caeca counts (15-17, 36-53, respectively) found east of the Mackenzie River, but also in southwestern Alaska near Bristol Bay. The current distribution of the western and eastern forms is postulated to have arisen due to isolation of various populations during the late Pleistocene (McPhail 1961). Arctic char in southern areas of Alaska tend to be lake-resident fish that complete their entire life-cycle in freshwater (i.e., non-anadromous). Colouration is often variable, but in these regions the body is typically silver in non-spawning periods (see Fig.1.3) or orange-red when sexually mature and is marked year-round with large violet, pink or white spots (McPhail & Lindsey 1970). The species generally spawns in September and October on gravel bottom beaches in lakes and is a repeat spawner (i.e., Arctic char can spawn each year over multiple years) (McPhail & Lindsey 1970; Johnson 1980). The eastern form of Arctic char is, by contrast, often sea-run (anadromous) and can also vary phenotypically. Throughout their Arctic and sub-Arctic range, Arctic char are valuable subsistence and sport fish for local Aboriginal peoples (Johnson 1980).

1.2 Sympatric Arctic char morphs

Arctic char can rapidly diverge into discrete resource-based forms following colonization of young, depauparuate post-glacial lakes (Griffiths 1994; Skúlason *et al.* 1999; Jonsson & Jonsson 2001; Klemetsen *et al.* 2003). Sympatric Arctic char morphotypes, or morphs, have been described from lacustrine systems across the species' Holarctic range and have received significant attention (especially in European lakes) as excellent study organisms for investigating the factors related to the ecological and genetic basis of resource polymorphism. Across relatively short evolutionary timespans, Arctic char have the capacity and tendency to diverge into alternative forms which exhibit variation in traits including: colouration, body morphology, growth rates, parasite load, and size and age at maturity (e.g., Gíslason *et al.* 1999). This variation is thought to be associated with differential use of trophic resources and habitats and is often associated with reproductive isolation between the forms (Malmquist *et al.* 1992; Power *et al.* 2009). The most common pathway of divergence in Arctic char appears to occur along the benthic (typically benthivorous feeders) and limnetic (planktivorous or piscivorous feeders) resource axis which highlights the potential role of habitat diversity and resource allocation during adaptive radiation and speciation in fishes (Gíslason *et al.* 1999). The magnitude of phenotypic divergence between these forms has been well studied; however, the connection between degree of genetic isolation and phenotypic or ecological divergence is often unknown.

Although many deep post-glacial Alaskan lakes contain landlocked Arctic char, it was only more recently that sympatric Arctic char morphs were discovered from one Alaskan system, Lower Tazimina Lake in southwestern Alaska, which was found to contain at least two morphs (Woods *et al.* 2013). The morphs, classified as 'large' and 'small' differed significantly in terms of colouration, age at maturity and body shape and size (Fig. 1.1).

Figure 1.1: Lower Tazimina Lake sympatric Arctic char (*Salvelinus alpinus*) morphs. **A)** Large growth form **B)** Small growth form; note the differences in colouration, morphology and size. *Photo credit*: Pamela J. Woods

To differentiate the morphs and to detect signatures of resource polymorphism, Woods *et al.* (2013) tested for the presence of multiple Von Bertalanffy growth curves among samples using a Von Bertalanffy three-parameter non-linear growth model. Von Bertalanffy growth curves model individual growth rate and are mathematically derived from measures of asymptotic body length and curvature as a function of age (Woods 2011; Woods *et al.* 2012, 2013). The authors found evidence for multiple Von Bertalanffy curves supporting the presence of two, or possibly three, Arctic char growth forms in Lower Tazimina Lake with differing growth trajectories. Furthermore, the large small and growth forms exhibited differences in gill raker counts, gonado-somatic indices, stable isotope levels of δC^{13} and δN^{15} and food consumption in benthic and limnetic regions of the lake (Woods *et al.* 2013). Collectively, this work provided evidence for a resource polymorphism in Lower Lake Tazimina due to divergence along the epipelagic-littoral resource axis, yet there has been no study of the genetic relationships among forms. To further explore this phenomenon, in Chapter 2 of my thesis, I addressed the question of whether pronounced ecological divergence and niche specialization is associated with genetic divergence and perhaps reproductive isolation among these sympatric morphs in Lower Tazimina Lake.

1.3 Natural hybridization in fishes

Arnold (1992, 1997) defined natural hybrids as individuals derived from matings between individuals from populations in nature that are distinguishable from each other by at least one heritable character. Natural hybrids can potentially backcross with their respective parental populations, leading to introgression (Arnold 1992; Dowling & Secor 1997; Arnold & Martin 2009). Genetic exchange between parental populations may increase phenotypic variation, potentially altering the ecology and behaviour of hybridizing populations (Arnold 1997).

The evolutionary importance of natural hybridization and introgression has been long been established in plants, and has only more recently been accepted as a fundamental and pervasive process in animals (Rieseberg *et al.* 1990; Jiggins &

Mallet 2000; Roques *et al.* 2001). The primary reason zoologists have had difficulty accepting that natural hybridization was not an evolutionary dead end has mostly been due to a sometimes overly zealous adherence to the Biological Species Concept (Mayr 1942). Although most biologists at the time believed in the existence of species as distinct biological units, evolutionists could not (and many still can not) come to a consensus on what constituted a species (Coyne & Orr 2004). Many species concepts were created to answer this issue, but none were as influential or controversial as Ernst Mayr's Biological Species Concept (BSC) (Mayr 1942; Dowling & Secor 1997; Coyne & Orr 2004). The original BSC states that: "*Species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from each other*" (Dobzhansky 1937; Mayr 1942). Although the BSC is perhaps the best explanation we have of what constitutes a species, there are some inherent issues with the original definition, especially when applied to fishes (see Turner, 1999). The original BSC defines species on the basis of reproductive isolation caused by the development of barriers to hybridization (Arnold 1997). In the strictest sense, populations that hybridize are in direct violation of the BSC because they exhibit incomplete reproductive isolation. Despite increasing evidence that hybridization was a prevalent and a potentially important phenomenon, Mayr countered that hybrids were rare "mistakes" and evolutionarily unimportant, at least in animal taxa, because "the majority of such hybrids are totally sterile" (Mayr 1963). Furthermore, he stated: "*Even those hybrids that produce normal gametes in one or both sexes are nevertheless unsuccessful in most cases and do not participate in reproduction. Finally, when they do happen to backcross to the parental species, they normally produce genotypes of inferior viability that are eliminated by natural selection*" (Mayr 1963). Contrary to Mayr's argument, it has been shown through modern biochemical and molecular techniques that natural hybridization occurs frequently and is evolutionarily important to many animals (Grant & Grant 1992, 2010; Arnold 1997; Jiggins & Mallet 2000; Mallet 2005). Further, approximately 25% of plant species and 10% of animal species are now known to hybridize (Table 1.1, Mallet 2005). Finally, hybrids are not always sterile

and may show equivalent or higher fitness compared to parental genotypes (Barton & Hewitt 1985; Arnold 1992).

Due to the various limitations of the BSC, many evolutionists now deviate from the strict definition and use a modified version (Arnold 1992, 1997). The modified version, which I will also adhere to, states that: "*distinct species are characterized by substantial but not necessarily complete reproductive isolation*" (Coyne & Orr 2004). Gene flow between populations is permitted with the modified BSC, yet the process of speciation (i.e*.,* the formation of reproductive barriers) is still present (Coyne and Orr 2004). This definition also allows species that occasionally hybridize, but remain genetically dissimilar in sympatry, to be considered distinct biological species (Baxter *et al.* 1997).

Many taxonomically distinct species of fishes are known to hybridize (Hubbs 1955; Schwartz 1972, 1981; Thorgaard & Allendorf 1988; Warren & Burr 1993; Scribner *et al.* 2001; Taylor 2004). In fact, fishes have some of the highest rates of hybridization across all vertebrates, particularly among species that live in or near regions that were recently deglaciated (Campton 1987; Allendorf & Waples 1996; Scribner *et al.* 2001). Moreover, the capacity for fishes to produce viable and fertile hybrids has been firmly established (Hubbs 1955; Campton 1987; Verspoor & Hammar 1991). The high rate of hybridization recorded in fishes has been attributed to various causes such as: external fertilization, the often unequal abundance of parental species, competition for limited spawning sites, weak behavioural isolation during mating, decreased habitat complexity due to natural or anthropogenic impacts, frequent secondary contact between recently evolved species, and lower susceptibility to severe developmental incompatibilities following interspecific crossing (Campton 1987; Scribner *et al.* 2001). The many cases of natural hybridization have been primarily observed in freshwater species, but has now been illustrated in many marine fishes as well (Pyle *et al.* 1994; Roques *et al.* 2001; Nielsen *et al.* 2003; Riginos & Cunningham 2007). As introgressive hybridization can occur readily in both freshwater and marine species, fishes make an excellent model to investigate the role of natural hybridization in speciation.

Table 1.1: Estimate of the percentage of species that hybridize with at least one other distinct species. Adapted from Mallet (2005).

Taxa	Number of species sampled	Number of species known to hybridize with another species	% of species hybridizing with at least one other species	Study
UK vascular plants	539	135	25%	Mallet 2005
European Butterflies (Lepidoptera: Rhopalocera)	379	47	12.4%	Mallet 2005
Passion flower butterflies (Lepidoptera: Heliconiina)	73	19	26.0% (34.8% in the genus *Heliconius*)	Mallet 2005
Swallowtail butterflies (Lepitoptera: Papilionidae; *Papilio*)	216	14-32	6.5-14.8%	Mallet 2005
Drosphila (Diptera: Drosophilidae)	~ 1750	18	1.0%	Mallet 2005
Birds (Aves)	9672	895	9.2%	Grant and Grant 1992, Mallet 2005
North American Freshwater Fishes (Pisces)	~1061[a]	>168	> 6.31%	Scribner *et al.* 2001, Warren and Burr 1993
Arroyo chub (Pices: Cyprinidae; *Gila orcutti*)	~5562	442	7.9-9%	Hubbs 1955
Freshwater Shiners (Campostoma: Notropis, *Notropis cornuta*)	608[b]	68	11%	Hubbs 1955
European Mammals	200	12	6%	Mallet 2005

[a] Estimate of the number of North American freshwater species. From Verspoor and Hammar (1991)

[b] Sympatric pairs of subgenera

In fishes and in other animals and in plants, hybridization with introgression leads to a wide array of evolutionary trajectories such as the formation of various kinds of hybrid zones, adaptive radiation, and speciation (Arnold 1997; Dowling & Secor 1997; Seehausen 2004; Aboim *et al.* 2010; Culumber *et al.* 2011). The evolutionary outcomes of natural hybridization are highly variable and can depend on other factors such as environmental features, the strength of selection, pre-zygotic (pre-mating), and intrinsic and extrinsic post-zygotic (post-mating) barriers (Mayr 1963; Barton & Hewitt 1985; Arnold 1992; Barton 2001; Latch *et al.* 2011). Pre-zygotic barriers can prevent or limit initial hybridization through gamete incompatibility and/or differences in mating behaviours leading to assortative mating and differences in the location and timing of reproduction (Howard *et al.* 1998; Rieseberg *et al.* 1998; Redenbach & Taylor 2003). Post-zygotic barriers can involve intrinsic genomic incompatibilities (endogenous selection) and/or extrinsic environment-dependent selection (exogenous selection) (Arnold 1997). Understanding the ecological and genetic basis of pre- and post-zygotic isolation may provide insight into the evolutionary forces which have led to reproductive isolation (e.g., Rogers & Bernatchez 2006).

One possible outcome of natural hybridization is the formation of hybrid zones (Barton & Hewitt 1985; Rieseberg *et al.* 1990). Hybrid zones are defined as geographic localities where individuals from genetically distinct populations interbreed and produce offspring with genetically mixed ancestry (Hewitt 1988; Arnold 1997; Baxter *et al.* 1997; Anderson & Thompson 2002; Nielsen *et al.* 2003). Hybrid zones can vary both temporally and spatially and can range anywhere from a few hundred meters to several kilometers in width, can have a mosaic-like structure, and may persist throughout evolutionary time or rapidly collapse (Barton & Hewitt 1985; Baxter *et al.* 1997; Rubidge & Taylor 2004).

From an evolutionary standpoint, hybrid zones are viewed as excellent opportunities to investigate the processes that drive speciation and adaptive divergence in natural populations (Taylor 2004; Gow *et al.* 2006; Albert *et al.* 2006). Hybrid zones can also be used to examine barriers to reproductive isolation, gene flow across species, the role of selection in speciation, and the formation of new

species with hybrid origin (Barton & Hewitt 1985; Hermansen *et al.* 2011; Brelsford 2011; Jacobsen & Omland 2011). The stability and fate of a hybrid zone is ultimately linked to the relative fitness of the novel hybrid phenotypes (Barton & Hewitt 1985; Dowling & Secor 1997; Burke & Arnold 2001; Barton 2001; Culumber *et al.* 2011).

Documenting the structure of hybrid zones can yield important insights about the pre-zygotic and post-zygotic processes that help generate and maintain hybrid zones in natural populations in a variety of taxa (Irwin *et al.* 2009; Latch *et al.* 2011; Hamilton *et al.* 2013). For instance, extrinsic post-zygotic isolation, in which hybrid fitness is determined by an interaction between hybrid genotypes and the environment, may be more common than previously hypothesized (Schluter 2009; Culumber *et al.* 2011). Thus, a fair amount of research on natural hybridization focuses on extrinsic (exogenous) post-zygotic isolation as it is central to ecologically based divergent selection and is an area where empirical tests are lacking (Moore *et al.* 2007; Gow *et al.* 2007; Culumber *et al.* 2011).

1.4 Hybridization between Arctic char and Dolly Varden

Dolly Varden char (*Salvelinus malma*), another member of the genus *Salvelinus*, share many phenotypic and genetic similarities with Arctic char. The distribution of Dolly Varden encompasses nearshore coastal marine and freshwater habitats north of the Olympic Peninsula in Washington State (USA), western Canada and Alaska, and extends across the Pacific into northern Japan and Korea and east in North America to the Mackenzie River (McPhail 1961; Reist *et al.* 2002; Dunham *et al.* 2008; Oleinik *et al.* 2011). Similar to Arctic char, Dolly Varden exist as a variety of forms including lacustrine, riverine, and anadromous char (Armstrong & Morrow 1980). The range of Dolly Varden overlaps with Arctic char in Kamchatka and Alaska, and due to their morphological similarities to one another, it is here that most of the confusion between the two species exists (Armstrong & Morrow, 1980, Fig. 1.2). In Alaska, Dolly Varden are ubiquitously distributed and sympatric with Arctic char in northern and southern regions near the Pacific coast (Fig. 1.2).

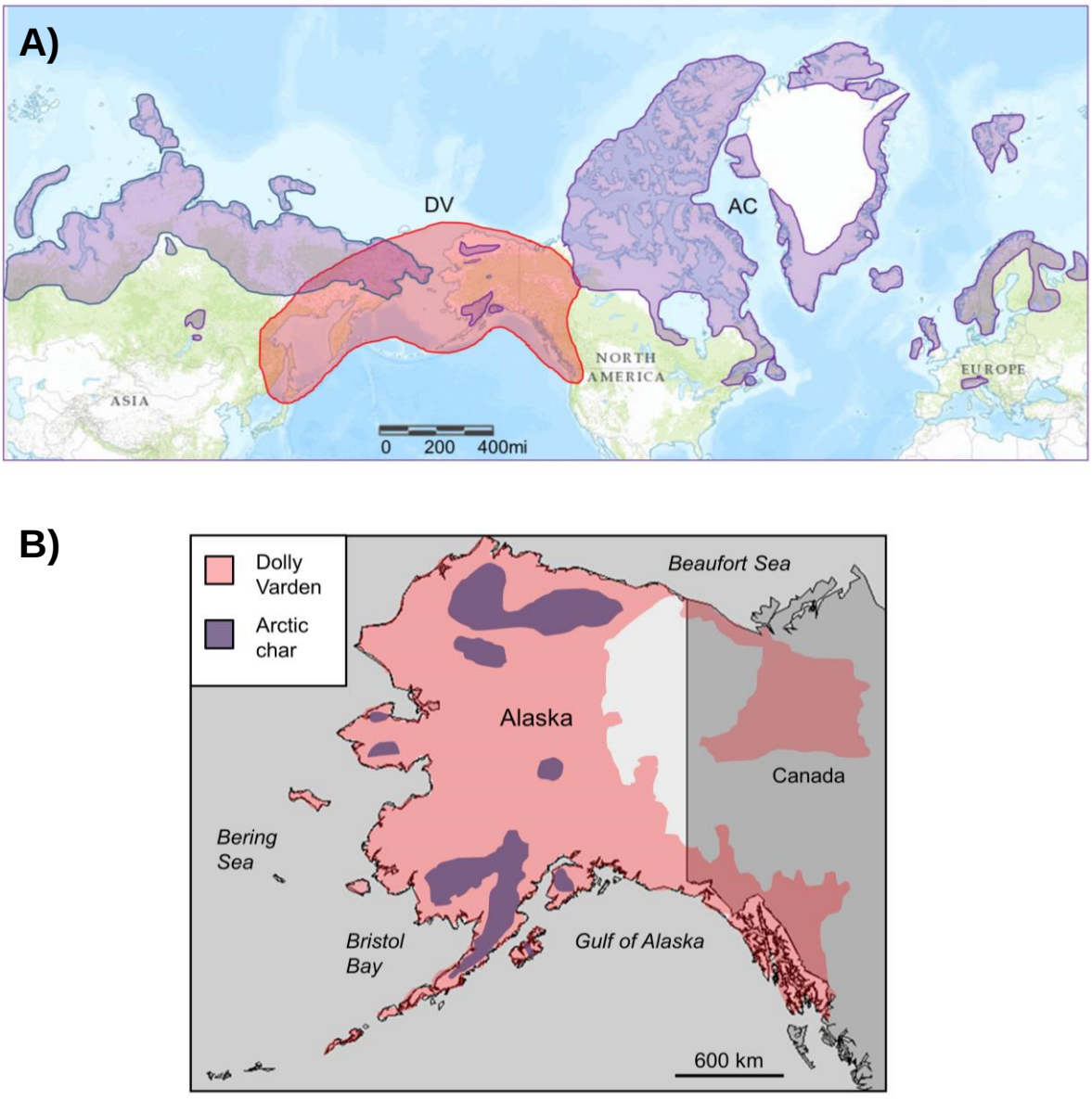

Figure 1.2: Approximate distribution of **A)** Holarctic Arctic char (*Salvelinus alpinus*), shown in purple and Dolly Varden char (*Salvelinus malma*), found around the North Pacific rim, shown in pink and **B)** Dolly Varden in Alaska (USA) including sympatric regions with Arctic char. Re-drawn and modified from Dunham *et al*. (2008), a COSEWIC (Committee on the Status of Endangered Wildlife in Canada) assessment report (2010) and Reist (2002, 2013).

In these areas, Dolly Varden can inhabit fully marine habitats, brackish estuaries or rivers, and streams and lakes. Large anadromous or sea-run Dolly Varden return to lakes and rivers annually to spawn and overwinter while small-bodied ("dwarf") stream-resident forms can remain in a single stream for their entire life-cycle (Armstrong & Morrow 1980).

Arctic char and Dolly Varden are very similar morphologically especially as juveniles, but they can usually be distinguished from one another on the basis of gill raker counts and the number of pyloric caeca (McPhail & Lindsey 1970). Gill rakers (bony protuberances on the gill arches that aid in feeding) and pyloric caeca (finger-shaped appendages that assist in digestion) are, however, highly variable characters that can be under selection depending on habitat and diet differences between populations or individuals within species (Jonsson *et al.* 1988; Skúlason *et al.* 1989; Helen 2001). In southwestern Alaska, Arctic char are typically characterized by 12-17 gill rakers on the lower limb of the first arch and 24-74 pyloric caeca, while Dolly Varden distributed north of the Alaska Peninsula generally have 11-14 gill rakers on the first arch and 19-47 pyloric caeca on average (McPhail 1961). Although these counts are normally discrete in allopatry, they can overlap when the species are found in sympatry.

Aside from gill rakers and pyloric caeca, the species may display slight differences in morphology and colouration. Arctic char in southwest Alaska have silver bodies, pale pink or white spots, delicate round heads and narrow caudal peduncles with a definite fork in the tail (McPhail 1961; Johnson 1980). Conversely, Dolly Varden char have deeper and darker olive-green coloured bodies, "blocky" parr marks in younger life stages (resembling the pattern of military camouflage) or, in stream resident forms, blunter heads, thicker caudal peduncles and paddle-shaped tails (Armstrong & Morrow 1980). The presence of orange, red or yellow spotting has also been observed in mature small-bodied, stream-resident forms (McPhail 1961; Koizumi & Maekawa 2004). Sea-run Dolly Varden are silvery along their dorsal surface with pale pink or white spots that change to bright red or orange during spawning periods (Fig 1.3).

Figure 1.3: **A)** Arctic char (*Salvelinus alpinus*) from Happy Creek, Lake Aleknagik, AK; note the silver body with white spots, narrow caudal peduncle and defined fork in the tail; **B)** Stream-resident Dolly Varden from Yako Creek, Lake Aleknagik; note the small colorful orange spots along a darker green body, thick caudal peduncle, paddle-shaped tail and small size at maturity. Mature stream-resident Dolly Varden retain parr marks which generally disappear in anadromous Dolly Varden upon their first migration seaward; **C)** Anadromous Dolly Varden from the Egegik fishing district, AK; silver bodied with light spots, thick caudal peduncle and paddle-shaped tail.

Historically, the systematic relationship between Arctic char and Dolly Varden was the subject of much debate (McPhail 1961). Prior to the development of modern genetic techniques, Arctic char and Dolly Varden were both characterized by highly plastic and often overlapping morphological features (e.g. gill rakers and pyloric caeca) and Dolly Varden were considered a sub-species of Arctic char (see Discussion in McPhail 1961). In regions of sympatry, these species are very similar in terms of mitochondrial DNA, and even more recent phylogeographic work by Brunner *et al.* (2001) could not taxonomically resolve the two species.

Taylor *et al.* (2008) conducted a microsatellite DNA survey of sympatric samples from southwestern Alaska and provided evidence that Arctic char and Dolly Varden are highly distinct in sympatry and should be designated as valid biological species. Interestingly, Taylor *et al.* (2008) also provided evidence for natural hybridization between Dolly Varden and Arctic char which hinted at the lack of complete reproductive boundaries between the species and suggested that they have not yet fully diverged.

Hybridization between Arctic char and Dolly Varden is probable given the many accounts of natural hybridization between other, more divergent species of char (Helen 2001). For example, there is evidence of historical and contemporary natural hybridization between Arctic char and brook trout (*S. fontinalis*) in northern Canada (Hammar *et al.* 1991) (Bernatchez *et al.* 1995; Wilson *et al.* 1998). Furthermore, natural hybridization has been described between Arctic char and lake trout (*S. namaycush*) in the Canadian Arctic (Wilson & Hebert 1993), Dolly Varden and bull trout (*S. confluentus*) in British Columbia (Baxter *et al.* 1997; Taylor *et al.* 2001) and Dolly Varden and white-spotted char (*S. leucomaenis*) in Japan (Yamamoto *et al.* 2006). If genetic and phenotypic similarities shared by the species reflect ongoing introgressive hybridization between Arctic char and Dolly Varden, knowledge of the level and factors which promote or limit hybridization in Alaska could provide important clues about their divergence into distinct taxa.

1.5 Research objectives

The northern Pacific and Beringian regions of Alaska represent important sources of biodiversity and have been instrumental in understanding factors relating to the diversity, taxonomy and speciation of temperate faunas (Taylor *et al.* 2008; Geml *et al.* 2010). Unfortunately, many Arctic and sub-Arctic species are rapidly being jeopardized by human activities such as the dispersal of bio-pollutants, extraction of natural resources and climate change (Berg *et al.* 2010). Diverse forms of landlocked Arctic char morphs and potentially hybridizing populations of Arctic char and Dolly Varden provide an excellent opportunity to examine the genetic relationships between the various forms and the factors promoting or limiting divergence within and between these species in regions most susceptible to environmental change. The overall aim of my research project was to better understand the basis of variation in *Salvelinus* in northern environments, and in particular, the relatively understudied area of Alaska. Specifically, I wanted to establish if the ecological divergence seen in the Lower Tazimina Lake morphs reflected divergence at the genetic level and to document the extent of natural hybridization between Arctic char and Dolly Varden.

To achieve my aims, I conducted the following studies:

1. In Chapter 2, I used microsatellite DNA markers to test whether morphological and ecological variability between body-size Arctic char morphs in Alaska was associated with genetic differentiation.

2. In Chapter 3, l evaluated the distribution and level of interspecific hybridization between sympatric Arctic char and Dolly Varden across various microhabitats in two southwestern Alaskan lakes to determine the degree of genetic and ecological segregation between the two species. Here, I also explored the possibility that spatial distribution limits opportunities for hybridization.

Chapter 2:

Evidence for genetic distinction among sympatric ecotypes of Arctic char (*Salvelinus alpinus*) in southwestern Alaskan lakes

2.1 Introduction

Resource polymorphism is the means by which alternative phenotypes exploit distinct niches within a given ecosystem (Skúlason & Smith 1995; Arbour *et al.* 2011). Resource polymorphism may promote reproductive isolation if the traits associated with differential resource use influence mate choice directly, or if hybrids between differentially adapted trophic ecotypes suffer reduced survival in parental niches (e.g., Hatfield & Schluter 1999; Corrigan *et al.* 2011; see also McPhee *et al.* 2012). Morphological diversification is often complex and can occur through environmentally driven plasticity, or it can reflect underlying genetic differences. As resource polymorphism has been proposed as a key factor behind the diversification of vertebrates, understanding if resource polymorphism is associated with genetic variation is critical to our knowledge of population divergence and speciation (Wimberger 1994; Skúlason & Smith 1995; Smith & Skúlason 1996; Schluter 1998).

Genetic data that accompany ecological measures of differentiation are especially informative, but are often lacking (Klemetsen 2002). In particular, the relationship between the degree of genetic isolation and resource-driven phenotypic segregation is often poorly understood (Gíslason *et al.* 1999; Loh *et al.* 2012). Furthermore, resource polymorphisms may signal underlying genetic differentiation within taxa; knowledge of these patterns and processes can result in the development of more nuanced conservation strategies by documenting cryptic variation within taxa (Taylor 1999; Foster *et al.* 2003; Taylor *et al.* 2011).

The Arctic char (*Salvelinus alpinus*) is a circumpolar species and the most northerly distributed freshwater fish. The Arctic char has been very successful in colonizing post-glacial lakes and river systems, and it is often the only salmonid

species present in northern freshwater environments (Klemetsen *et al.* 2003; Reist *et al.* 2013). Part of this success may be because Arctic char can vary greatly in resource and habitat utilization, growth patterns, life history traits, and morphology, resulting in a number of specialized forms inhabiting a range of habitats (e.g., Power *et al.* 2009; Klemetsen 2010). For example, the size and shape of the jaws and number of gill rakers vary among populations of Arctic char (Skúlason & Smith 1995) and these traits are typically heritable and associated with differences in trophic ecology (Skúlason *et al.* 1993; Adams & Huntingford 2002a). Because of its highly polymorphic nature and ability to rapidly undergo trophic diversification following colonization of relatively young ecosystems, the Arctic char is ideal for examining the role of trophic variation in adaptive radiation. Understanding natural genetic and ecological differentiation across sympatric populations can also provide important insights about how species may adapt to particular habitats during the early stages of divergence.

Differentiated populations from sympatric lake systems have long been used as natural laboratories to study questions regarding the ecological and genetic basis of intraspecific divergence within several fish species (e.g., Frost 1965; Bentzen & McPhail 1984; Bernatchez *et al.* 1999; Langerhans *et al.* 2007). Post-glacial environments containing alternative forms that differ in resource use, or morphotypes, are especially informative as these young systems often have low biodiversity and high resource availability (see review by Taylor 1999). As multiple unoccupied niches may be exploited by colonizing fish, resource polymorphism arising from alternative life history patterns can be maintained (Berg *et al.* 2010). Understanding the genetic basis of sympatric systems can thus provide important clues relating to the diversification of alternative forms (Gíslason *et al.* 1999). Sympatric morphotypes, or morphs, of Arctic char have been documented from European countries such as Scotland, Sweden, Norway, Switzerland and Iceland, and to a lesser extent, North America and Russia (Jonsson & Jonsson 2001; Adams & Huntingford 2002b). A well-known example that showcases the remarkable potential for divergence within Arctic char is the four morphs present in Lake

Thingvallavatn, Iceland (Skúlason *et al.* 1989, 1996; Snorrason *et al.* 1994). Within the lake, two morphs are benthivorous specialists, but differ in size at maturity, one morph is piscivorous, and the fourth is a pelagic zooplanktivore.

Alaska has one of the richest salmonid faunas in the Northern Hemisphere, including Arctic char which are found from the Kenai Peninsula in southwestern Alaska, around the Bering Sea drainages north and east to and including the North Slope (McPhail & Lindsey 1970). Despite this extensive distribution, relatively little is known about the extent of polymorphism in Alaskan Arctic char, investigation of which has been hampered by historical taxonomic confusion involving the relationship between Arctic char and Dolly Varden char (*S. malma*). Distinguishing the two species has proved challenging due to their similar morphology and life history, but their status as distinct species is supported by strong genetic differentiation in sympatry (e.g., Taylor *et al.* 2008). Woods *et al.* (2013) recently examined the extent of resource polymorphism in Arctic char from three isolated lakes in southwestern Alaska as a function of lake size, species diversity, and degree of isolation from a much larger fourth lake postulated to be the source population of Arctic char in the study area. The lakes were chosen because they exhibit many of the physical criteria consistent with the formation of alternative forms of Arctic char (e.g., long-term isolation and few available fish species). Indeed, in one of the three isolated lakes (Lower Tazimina Lake), two sympatric morphs of Arctic char were identified: ‘large’ and ‘small’, which were morphologically and ecologically distinct from each other (Woods *et al.* 2013). Large morphs had an estimated asymptotic size of > 50 cm and were gold or orange in colouration. Small morphs had an estimated asymptotic size of ≤ 20 cm and had silver, deeper bodies with light spots, and more gill rakers than large morphs. Large morphs consumed more benthic resources whereas small morphs fed mostly on zooplankton in the limnetic zone. Large morphs generally had steeper size-at-age growth curves, corresponding to higher growth rates and the small morphs had shallower growth curves and lower growth rates (Woods *et al.* 2013). It is unclear, however, to what degree the different morphs in Lower Tazimina Lake are genetically distinct from one another.

Interestingly, Woods *et al.* (2013) also suggested that a third group of fish was present whose growth rate was intermediate to those of the large and small morphs. These “medium” morphs were orange to silver in colouration, had an estimated asymptotic size about 33 cm, and foraged in the benthic regions of the lake like large morphs, but fed on slightly different prey items (i.e., relied more on terrestrial insects and less on snails) and had intermediate gill raker counts. The size-at-age growth curve for medium morphs closely resembled the growth curve of the large morph (Woods *et al.* 2013); however, it is unknown whether medium morphs are derived from admixture between large and small morphs or whether they represent their own distinct population within Lower Tazimina Lake. In order to better understand the degree of genetic differentiation between sympatric ecological and morphological forms of Arctic char from southwestern Alaska, I used microsatellite DNA markers to: 1) test whether the forms from Lower Tazimina Lake were genetically distinct from one another and; 2) place the genetic variation within Lower Tazimina Lake into the context of genetic differentiation among a series of four other post-glacial lakes in southwestern Alaska that also contain char, but do not appear to exhibit obvious resource polymorphism. The lakes were selected to represent different scales of geographical isolation; four, including Lower Tazimina Lake, are in the same drainage basin. The fifth lake, Lake Aleknagik is from an adjacent river system and is accessible from the sea by fishes. I sampled these lakes for char to understand the level to which genetic differences among morphs within a lake compare to divergence between lakes that have been isolated since deglaciation (e.g., Caribou Lakes and Summit Lake) and those that may experience some gene flow via anadromous char (Iliamna Lake and Lake Aleknagik), but that are located in separate watersheds.

2.2 Materials and methods

2.2.1 Fish collection

A total of 217 tissue samples (fin clips and dorsal muscle plugs) of Arctic char were donated to this study by Pamela J. Woods and Thomas P. Quinn (University of Washington, School of Aquatic and Fishery Sciences, Seattle, WA) and represented a subset of samples analyzed previously in Woods *et al.* (2013). These samples were collected from four lakes in the Kvichak River (Iliamna Lake) system in Bristol Bay, southwestern Alaska, and one lake (Lake Aleknagik) in the Nushagak River system of Bristol Bay (Fig. 2.1). Gene flow is likely limited between three of the lakes, Caribou Lakes, Lower Tazimina Lake, and Summit Lake, due to the presence of physical barriers that restrict the upstream migration of Arctic char and other fish species into the lakes. Summit Lake and Lower Tazimina Lake are above large barrier waterfalls, and migration to Caribou Lakes is limited by a series of rapids in the downstream Koksetna River. Caribou Lakes are at the highest elevation (550 m above sea level, ASL) whereas Summit and Lower Tazimina lakes are lower (152 and 194 m ASL, respectively). Caribou (1.2 km^2, max depth of 5 m) and Summit lakes (0.6 km^2, max depth of 20 m) are smaller, shallower and more remote than Lower Tazimina Lake, which is larger and deeper (520 km^2, max depth of 60 m). Caribou Lakes, and Lower Tazimina and Summit lakes eventually drain into Iliamna Lake (14 m ASL, 2,622 km^2, 301 m max depth), the largest lake in Alaska, which in turn empties into the southeastern portion of Bristol Bay via the Kvichak River. Iliamna Lake has a much more diverse fish community and is freely accessible to migratory fishes, in contrast to the other three lakes. Fish were also sampled from Lake Aleknagik (12 m ASL, 83 km^2, max depth of 101 m), found in the adjacent Wood River Lake system, which is open to migration from the sea. Sampling was conducted from August–September 2008 to 2011. Fin clips from char were taken from Summit Lake (n = 59), Lower Tazimina Lake (n = 91), Caribou Lakes (n = 25), and Iliamna Lake (n = 42) and stored in 95% ethanol.

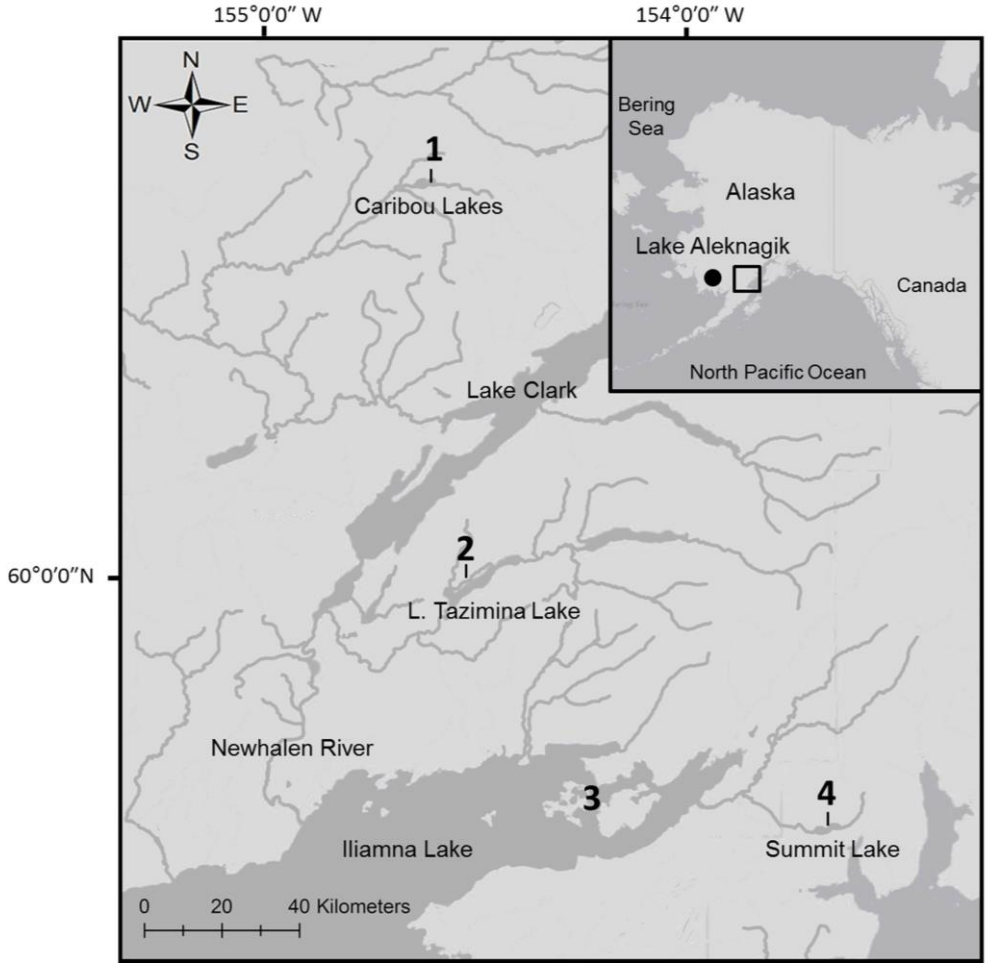

Figure 2.1: Collection sites of Arctic char (*Salvelinus alpinus*) in southwestern Alaska. **1** = Caribou Lakes, **2** = Lower Tazimina Lake, **3** = Iliamna Lake, **4** = Summit Lake. See inset for Lake Aleknagik.

For Summit Lake, Lower Tazimina Lake and Caribou Lakes, three gill nets with dimensions of 57 m length x 1.6 m depth, 38.1 m x 1.8 m, and 30.5 m x 1.8 m were set as bottom nets close to and perpendicular to shore in shallow water (1-7 m deep). An additional sinking gill net and two floating gill nets were set at 17, 9 and 10 m, respectively, in Summit Lake. Mesh sizes ranged from 10 to 102 mm (see Woods *et al.* 2013 for full details). Iliamna Lake samples were obtained through seining and angling off near-shore habitats that were approximately 1-3 m in depth at the eastern end of the lake (Fig. 2.1). Exact depth information for individual fish was not recorded for catches originating from bottom set nets, i.e., in Summit Lake, Lower Tazimina Lake, and Caribou Lakes fish caught at depths between 1 and 17 m in depth could not be distinguished.

I collected additional samples of Arctic char from Lake Aleknagik in July–August, 2012 (n = 70) using a combination of gill nets and stick seines to place the level of divergence and genetic diversity seen within the Kvichak River system in the context of differences from another basin. Lake Aleknagik is located in the Wood River Lake system, about 85 km west of the Kvichak River system, and also flows into Bristol Bay, but via the Nushagak River. The Wood River Lake system has a similarly diverse fish community with easy access to the sea by anadromous fishes (Hartman & Burgner 1972).

2.2.2 DNA extractions and microsatellite DNA analyses

I extracted genomic DNA from a total of 287 fin clip or dorsal plug samples stored in 95% ethanol using the DNeasy DNA blood and tissue extraction kit (Qiagen Inc., Valencia, CA, USA) following kit protocols. Extracted DNA samples were stored at -20 °C for later use in multiplex polymerase chain reactions (PCR) using the Qiagen multiplex kit (Cat. No. 206145). The DNA was amplified in 10-µL PCR reactions at 95 °C for 15 min, 94 °C for 30 sec, 35 cycles of 1.5 min at an annealing temperature of 55 °C followed by 72 °C for 1 min, and 60 °C for 30 min.

I assayed microsatellite variation using primers labeled with infrared fluorophores and a 3730S 48-capillary DNA Analyzer with GS 500 LIZ or 600 LIZ internal size

standards (Applied Biosystems, Carlsbad, California, USA). Alleles were manually scored using the program GeneMapper (GeneMapper v.3.7, Applied BioSystems). I genotyped fish using 11 microsatellite loci isolated from other salmonid species such as Atlantic salmon, *Salmo salar* (*SSOSL456*; Slettan *et al*. 1997), bull trout, *Salvelinus confluentus* (*Sco200, Sco215, Sco216, Sco220*; DeHaan & Ardren 2005), Chinook salmon, *Oncorhynchus tshawytscha* (*OtsG83b, OstG253b*; Williamson *et al.* 2002), Dolly Varden, *Salvelinus malma* (*Smm-17, Smm-22, Smm-24*; Crane *et al.* 2004) and rainbow trout, *Oncorhynchus mykiss* (*OMM1105*; Rexroad *et al*. 2002).

To minimize genotyping scoring errors I used MICRO-CHECKER software (van Oosterhout *et al.* 2004) to identify instances where alleles failed to amplify owing to mutations in the primer binding sites or failure to detect large alleles ("null alleles" and "large allele dropout", respectively). Genetic polymorphism was estimated from sample size (*N*), number of alleles per locus (*A*), allelic richness (A_R), and observed (*Ho*) and expected (H_E) heterozygosity using FSTAT ver 2.9.3 (Goudet 2001). The following tests were implemented using GENEPOP ver 4.2 (Raymond & Rousset 1995). Tests for departures from Hardy-Weinberg equilibrium (HWE) were performed for each locus-population combination using an exact test in which probability values were determined using a Markov chain method (*P*). Tests for genotypic linkage disequilibrium for all combinations of locus pairs within a population were also made using a Markov chain method with GENEPOP default values.

2.2.3 Population structure within and among lakes

To assess the level of population structure within and among lakes I used the Bayesian clustering analysis within STRUCTURE (Pritchard *et al.* 2000). STRUCTURE uses a Markov Chain Monte-Carlo (MCMC) to cluster individuals into *K* randomized and interbreeding groups that minimize departures from Hardy-Weinberg Equilibrium and linkage disequilibrium within the groups. I used the admixture model with correlated allele frequencies and a burn-in of 50,000 iterations proceeded by an additional 450,000 iterations, replicated five times to verify

consistency across runs. Because I had prior indications of distinct morphotypes in Lower Tazimina Lake (Woods *et al.* 2013) I analyzed the data in two ways. First, to determine the most likely number of populations (*K*) across all lakes, I ran simulations of *K* = 1 to *K* = 10 for all samples from all lakes in one analysis. Next I assessed *K* = 1 to *K* = 5 for each lake separately. For both kinds of analysis, I used STRUCTURE HARVESTER to process the results from multiple runs of STRUCTURE (Earl & vonHoldt 2012) which were visualized using DISTRUCT (Rosenberg 2004).

Pairwise genetic divergence across samples was expressed as F_{ST} and was quantified by calculating θ (Weir & Cockerman 1984). Pairwise values were then tested for significance using GENETIX ver 4.05.2 (Belkhir *et al.* 2001). A factorial correspondence analysis (FCA) was also conducted on allele frequencies in GENETIX. An FCA is a type of factor analysis that summarizes allele frequencies among all samples by finding the linear combination of variables that best describes variation between individual samples and is well-suited analysis for categorical data such as allele frequency counts. All statistical tests of differences between samples were corrected for multiple comparisons following Narum (2006).

2.2.4 Comparisons of genetic and phenotypic data

To assess the distribution of admixture values ((*Q*), the proportion of each fish's genome derived from *K* genetic groups) estimated by STRUCTURE amongst the Lower Tazimina Lake morphs, individual fish were assigned to 'large', 'medium', or 'small' morph groups according to their growth curve assignment probability as found in Woods *et al.* (2013). To obtain growth curve assignment probabilities, Woods *et al*. (2013) first generated a Von Bertalanffy growth curve for each fish,

$$\mu = L_{\infty}(1- e^{-ka})$$

using the parameters L_∞(asymptotic length), *k* (curvature) and *a* (age estimated from sagittal otoliths) to represent length predictions (μ). Using a mixed model approach to access the likelihood of multiple Von Bertalanffy curves, the authors found evidence for the presence of two or three growth curves in Lower Tazimina Lake but could not ascertain the exact number of growth forms. To access the likelihood of three growth forms in Lower Tazimina Lake, Von Bertalanffy growth curves for each fish were fit to a three-curve model corresponding to a small-, medium-, and large-growth type (based on asymptotic length, L_∞). An assignment probability value was generated for each fish and was used to assign fish to the component (growth curve, according to L_∞) whose proportion of that individual's summed likelihood was the largest. Woods *et al.* (2013) then used these assignment values to assign each fish to the large-, medium- or small- body size groups (n = 74). Using these pre-determined groupings, I then assessed the distribution of Q (defined with STRUCTURE) within each of these body size groups.

I first tested for differences in the mean values of *Q* among the three body size groups with a one-way ANOVA, with proportional *Q* values arcsine transformed, and post-hoc tests for samples with uneven variances using PAST (vers. 2.17b), a general spreadsheet-based statistical software package (Hammer *et al.* 2001).

The STRUCTURE analysis (see Results) resolved two genetic groups within Lower Tazimina Lake with the small-bodied fish tending to have admixture values < 0.2, large-bodied fish had admixture values > 0.8, and medium-sized fish tended to have intermediate admixture values. To assess whether or not the medium body size char may represent hybrids between the large and small-bodied fish I used all fish classified as large-bodied and small-bodied according to growth curve assignment and the program HYBRIDLAB 1.0 (Nielsen *et al.* 2006) to generate simulated multilocus F_1 hybrid genotypes (n = 100) between these two groups of Arctic char. The upper and lower bounds of *Q* values for putative hybrids between large and small-bodied fish were found by running all Lower Tazimina Lake samples and the simulated hybrids in STRUCTURE with 50,000 replications during the pre-simulation burn-in, proceeded by an additional 450,000 iterations. Simulated hybrids

had *Q* values that were ≤ 0.82 and ≥ 0.19. I then tested for a significant association between morph (large, medium, or small-bodied char, assigned using the growth curve assignment probabilities, and the STRUCTURE- defined genetic groupings as described above (parental = > 0.82 or < 0.19; hybrid = ≤ 0.82 and ≥ 0.19) by using a 3x3 contingency table in PAST.

Woods *et al.* (2013) also reported gill raker counts taken on the first arch (ranged from 20 – 32) for each body size morph (n = 74). I assessed the association between morphology and genetic identity by testing for a Pearson's correlation between individual gill raker count and the admixture proportions (*Q*) from the STRUCTURE analysis using the Hmisc package and the cor function in *R* (version 3.0.2, *R* Development Core Team, 2011).

2.3 Results

2.3.1 Microsatellite variation among and within lakes

MICRO-CHECKER found no evidence of a failure to resolve alleles owing to "large allele dropout" or otherwise non-amplifying ("null") alleles in any of the samples. All loci were polymorphic, except for *Sco215* in Summit Lake which was monomorphic (Appendix A Table. A.1). When examining samples within each lake (i.e., before partitioning by morph or by genetic sub-groups identified by STRUCTURE), eight of 54 tests for deviations from Hardy-Weinberg Equilibrium (four samples x 11 loci plus 1 sample x 10 loci) were significant at $P \leq 0.0171$, and three of the eight deviations were in the Lower Tazimina Lake sample owing to deficits of heterozygotes (Appendix A Table. A.1). Of the tests of linkage disequilibrium per population, 1/45 for Summit Lake, 3/55 for Lower Tazimina Lake, 1/55 for Caribou Lakes, 2/55 for Lake Iliamna, and 3/55 for Aleknagik Lake were significant. No locus pairs consistently deviated from linkage equilibrium across lakes so I retained all loci in subsequent analyses.

The greatest average allelic richness was found in Lower Tazimina Lake (17.7) and Lake Aleknagik (16.4), followed by Iliamna Lake (16.1, Appendix A Table. A.1). In Caribou Lakes and Summit Lake fish, allelic richness was lower (7.2 and 7.6,

respectively); however, a large proportion of those alleles were unique to those populations. The highest level of gene diversity was observed at the locus *Sco220* and *Smm24* (1.0) while the locus *Sco216* showed the lowest gene diversity (0.32). Across loci and populations, the highest average expected heterozygosity was seen in Lake Aleknagik fish (0.84) and the lowest heterozygosity was seen in Summit Lake fish (0.68).

2.3.2 Population structure among lakes

Pairwise F_{ST} across all five lakes ranged from 0.050 between Iliamna Lake and Lower Tazimina Lake to 0.283 between Summit Lake and Caribou Lakes ($P < 0.001$, Table 2.1). In general, F_{ST} was greatest between Caribou Lakes and Summit Lake and all other samples (minimum $F_{ST} = 0.100$, Table 2.1). The FCA showed a large degree of divergence among populations, especially between Summit Lake and the char from the other four lakes (Appendix A, Fig. A.1). The FCA also revealed that the Lower Tazimina Lake char were more similar genetically to Iliamna Lake fish when compared across all populations in the Kvichak River system (Appendix A, Fig. A.1).

Analysis by STRUCTURE, indicated that the most likely number of genetic populations was $K = 6$ among the five lakes, as evidenced by a mean log likelihood of ─13199.7 vs. ─13298.7 for $K = 7$ as the next most likely model (Fig. 2.2). In the $K = 6$ model, each lake formed a distinct genetic group, but two groups were resolved within Lower Tazimina Lake (Fig. 2.2). When the Lower Tazimina Lake samples were analyzed separately, the most likely number of clusters was $K = 2$ (mean log likelihood = ─3507.1 vs. ─3581.1 for $K = 1$ and ─3659.5 for $K = 3$). No distinct clusters were observed within any of the other lakes when they were examined together (Fig. 2.2), or when the lakes were analyzed individually; a $K = 1$ was returned in all cases (May-McNally *et al.* unpubl. data).

Table 2.1: Pairwise F_{ST} (θ) estimated by variation across 11 microsatellite DNA loci in Arctic char (*Salvelinus alpinus*) from Caribou Lakes, Summit Lake, Lower Tazimina Lake large (L), medium (M), and small-bodied (S) morphs, Iliamna Lake, and Lake Aleknagik. All comparisons except the starred values are significantly greater than 0 (P ≤ 0.0185; after adjustment for multiple tests incorporating the false discovery rate procedure of Narum, 2006).

	Caribou Lakes	Summit Lake	Lower Tazimina Lake-L	Lower Tazimina Lake-M	Lower Tazimina Lake-S	Iliamna Lake
Summit Lake	0.283	-	-	-	-	-
Lower Tazimina Lake-L	0.178	0.153	-	-	-	-
Lower Tazimina Lake-M	0.149	0.132	0.017*	-	-	-
Lower Tazimina Lake-S	0.162	0.117	0.092	0.037	-	-
Iliamna Lake	0.162	0.131	0.060	0.053	0.080	-
Lake Aleknagik	0.100	0.205	0.090	0.062	0.092	0.085

Pairwise comparisons between Caribou, Summit, Iliamna Lake, Lake Aleknagik, and the pooled sample from Lower Tazimina Lake were 0.143, 0.108, 0.050, and 0.069, respectively (all $P < 0.0185$).

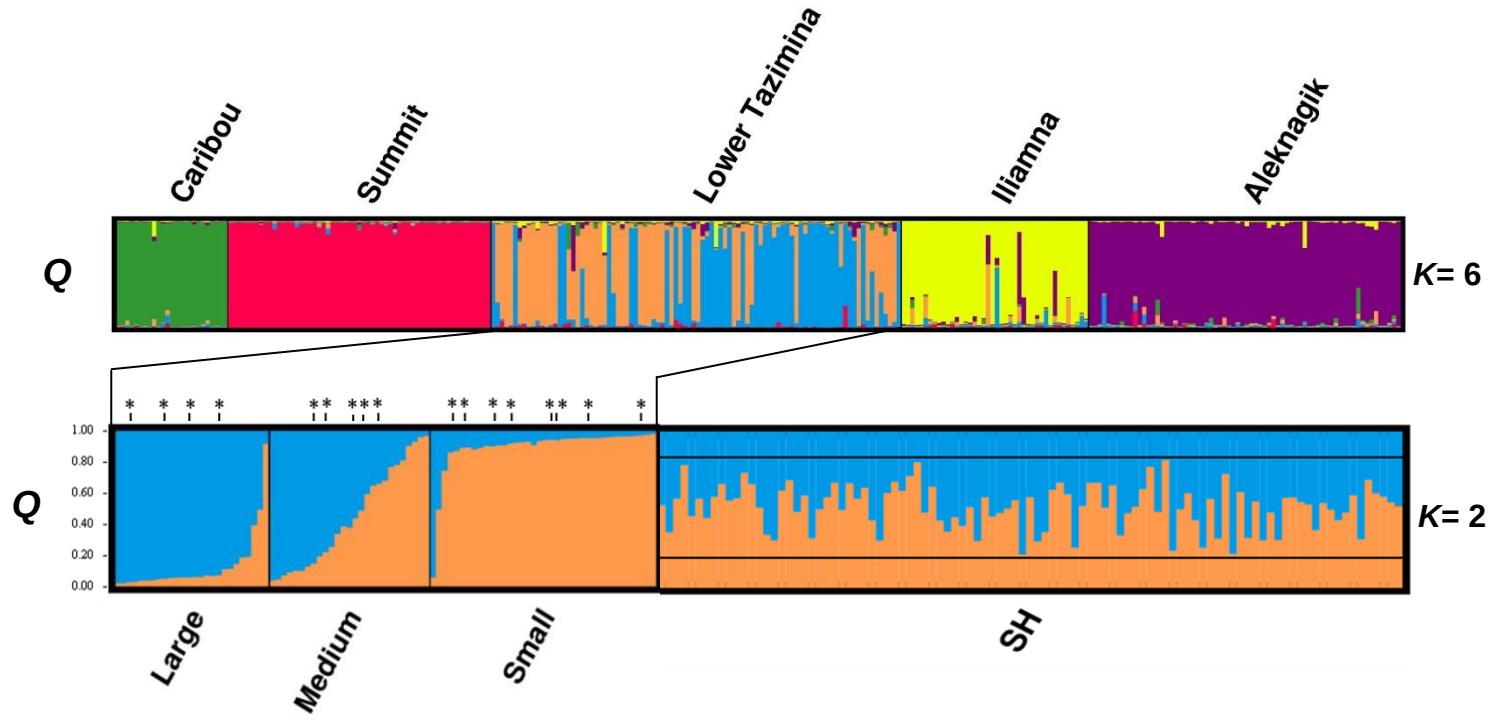

Figure 2.2: DISTRUCT plots for STRUCTURE runs of five populations of Arctic char (*Salvelinus alpinus*) from southwestern Alaska using 11 microsatellite markers: Caribou Lake (n = 25), Summit Lake (n = 59), Lower Tazimina Lake (n = 91), Iliamna Lake (n = 42), Lake Aleknagik (n = 70), and simulated hybrids (SH, n = 100). Each fish is represented by a vertical bar that denotes membership fractions (*Q*) in *K* clusters. Lower Tazimina Lake morphs (lower panel, blue and orange clusters) were defined as large-, medium- or small- based directly on growth curve assignment data from Woods *et al.* (2013) and organized according to increasing *Q* value (in reference to the orange cluster) within each group (n = 74). Asterisks denote genetic samples that lack growth curve information and were included in the appropriate genetic groups according to their *Q* values (n =18). Horizontal solid lines in the SH plot represent the upper and lower boundaries for *Q* value defined hybrids.

2.3.3 Comparisons between genetic and phenotypic data within Lower Tazimina Lake

When Lower Tazimina Lake char were grouped according to body size using (see Woods *et al.* 2013 for results), there were no deviations from Hardy-Weinberg Equilibrium (Appendix A Table. A.1) and there was only a single deviation from linkage equilibrium in the large morph sample. Further, at 9 of 11 loci, small morphs exhibited a higher A_R per locus than both large and medium morphs as well as similar or higher A_R values relative to the populations from Iliamna Lake and Lake Aleknagik (Appendix A Table. A.1).

There was a significant association between admixture values generated by STRUCTURE and body size morph; a one-way ANOVA followed by a post hoc test revealed significant pairwise differences among the mean *Q* values of each of the three morphs ($F_{2,71} = 50.9$, $P < 0.001$, all Tukey $P < 0.001$, mean $Q = 0.10$, 0.53, and 0.83 for small, medium and large-bodied morphs, respectively). Further, the mean *Q* value of the simulated hybrids (0.48) was not significantly distinct from that of the medium-sized morphs (Tukey $P = 0.72$), but was significantly distinct from the mean *Q* values of large and small-bodied morphs (both $P < 0.001$). Nine of 13 fish found in the range of *Q* values of simulated hybrids were of the medium-sized morph, while most large-bodied fish had *Q* values > 0.82 and most small-bodied fish had *Q* values < 0.19 ($X^2 = 29.6$, $P < 0.05$, d.f.= 4, Table 2.2).

Table 2.2: Contingency table analysis of *Q* value categories and growth curve assignment for small, medium and large morphs of Arctic char (*Salvelinus alpinus*) sampled from Lower Tazimina Lake, southwestern Alaska (n = 74)

Q value	Small	Medium	Large
0.0 – 0.18	28	5	0
0.19 – 0.82	2	9	2
0.83 – 1.0	0	9	19

In the FCA projection, large and small morphs showed roughly equivalent divergence from Iliamna Lake fish on the same FCA plot (Appendix A, Fig. A.2). Genetic distinction in allele frequencies was greatest between the large and small morphs (F_{ST} = 0.092, P < 0.001, Table 2.1). The medium and small morphs were also distinct from one another (F_{ST} = 0.037, P < 0.001), but there was only marginal genetic differentiation detected between the large and medium morphs (F_{ST} = 0.006, P = 0.09). Generally, Lower Tazimina Lake morphs were most divergent from Caribou and Summit lakes fish (minimum F_{ST} all > 0.108) and least divergent from char in Iliamna Lake (maximum F_{ST} = 0.080, Table 2.1). Pairwise F_{ST} averaged 0.048 among the three morphs in Lower Tazimina Lake and 0.050 between Lower Tazimina Lake morphs and fish from Iliamna Lake (Table 2.1).

I found a significant negative correlation (r =-0.73, t = -8.67, df = 72, P < 0.001) between genetic admixture value (Q) and gill raker count across individual char (Fig. 2.3). Fish with low Q values had higher average gill raker counts than fish with higher Q values reflecting the differentiation, genetically and phenotypically, between the small and large-bodied morphs, respectively. Medium-sized morphs were characterized by Q values of between 0.19 and 0.82 and had an average gill raker count, of 23.8, compared to 27.9 and 22.8 for the small and large-bodied morphs, respectively (Fig. 2.3).

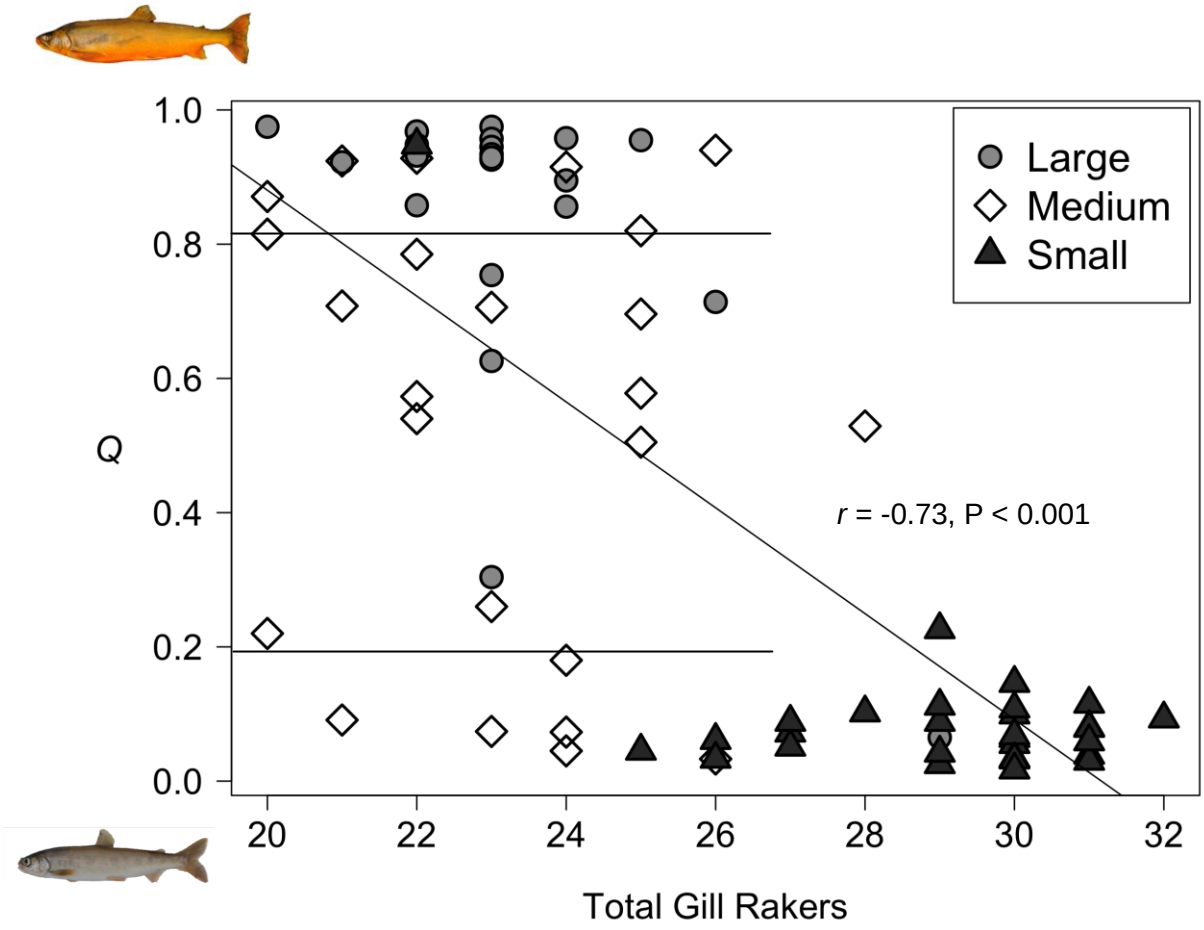

Figure 2.3: Pearson's correlation between genetic admixture coefficients (*Q*) and total gill raker counts for Lower Tazimina Lake, southwestern Alaska, morphs of Arctic char (*Salvelinus alpinus*, n = 74). Fish were grouped as large, medium or small morphs according to their growth curve assignment probability as outlined in Woods *et al.* (2013). Horizontal bars indicate upper and lower boundaries for *Q* value defined hybrids from the STRUCTURE- defined genetic groupings (parental = > 0.82 or < 0.19; hybrid = ≤ 0.82 and ≥ 0.19) Fish images on y-axis represent relative body size and colouration differences (images from Woods *et al*. 2013).

2.4 Discussion

The Arctic char is a highly polymorphic species with an impressive ability to undergo rapid trophic diversification in isolated post-glacial lakes (reviewed by Klemetsen 2010). The Kvichak River system in southwestern Alaska has phenotypically diverse forms of Arctic char, notably at least two sympatric morphs in Lower Tazimina Lake (Woods *et al.* 2013). I have provided evidence that this morphological and ecological variability is accompanied by significant genetic differentiation in that at least two of the morphs are strongly divergent from one another and their genetic identity can be predicted to some degree by growth curves and total gill raker counts.

2.4.1 Microsatellite DNA variation across remote freshwater lakes

My microsatellite data revealed substantial variability in pairwise F_{ST} across Arctic char from five lakes and is consistent with variability found in other studies involving post-glacial lake systems with Arctic char (Bernatchez *et al.* 1998; Westgaard *et al.* 2004). The high values of pairwise F_{ST} involving Summit and Caribou lakes are likely attributable to their isolation from Iliamna Lake and Lower Tazimina Lake. Arctic char from both lakes exhibited numerous private alleles as well as dissimilar morphology and Von Bertalanffy growth curves compared to fish from Lower Tazimina Lake and Iliamna Lake (see Woods *et al.* 2013).

Arctic char from Summit Lake and Caribou Lakes may represent populations that were derived from the earliest colonizing fish from Kvichak River system. Summit Lake fish, in particular, showed low heterozygosity at multiple loci which may be due to differing demographic histories (e.g., numerous generations of inbreeding and genetic drift) in this very small lake. In addition, the low values for pairwise F_{ST} between Lower Tazimina Lake and Iliamna Lake when contrasted with F_{ST} values involving Summit Lake and Caribou Lakes may indicate a more recent colonization of Lower Tazimina Lake. Alternatively, because Lower Tazimina Lake and Iliamna Lake (520 and 2,622 km^2, respectively) are many times larger than Summit Lake

and Caribou Lakes (0.6 and 1.2 km^2, respectively, Woods *et al.* 2013), they probably have had much larger effective population sizes historically that would constrain neutral divergence between these two large lakes.

The relatively low F_{ST} values between Iliamna Lake and Lake Aleknagik suggests that gene flow has occurred recently between them or is ongoing, although Arctic char in both lakes are generally regarded as non-anadromous populations because they are present in the watersheds throughout the summer when anadromous char are typically in the sea and, at least in Iliamna Lake, diet comparisons inferred from stable isotopes do not support anadromous behaviour of Arctic char (Denton *et al.* 2010). Anadromy, however, could exist as an alternative, but less common life history pattern in these systems (e.g., McBride 1980); otolith microchemistry analysis of Arctic char from these lakes would provide a definitive test of this idea. Alternatively, the low F_{ST} between Iliamna Lake and Lake Aleknagik may reflect shared ancestral genetic polymorphism in the face of relatively high historical effective population sizes and recent postglacial founding of these populations.

2.4.2 Genetic differentiation among Lower Tazimina Lake morphs

The results of the STRUCTURE analysis in Lower Tazimina Lake were consistent with other studies that have shown that morphologically differentiated forms may also be genetically distinct, especially in systems where profundal and littoral morphs of Arctic char have been identified (e.g., Westgaard *et al.* 2004; Adams *et al.* 2007; Gomez-Uchida *et al.* 2008; Power *et al.* 2009; Conejeros *et al.* 2014). The Lower Tazimina Lake morphs shared most of their alleles with fish from Iliamna Lake, which suggests that they were founded by colonists from Iliamna Lake. Moreover, Lower Tazimina Lake morphs were genetically more similar to each other than to fish from one or more other lakes, suggesting a single colonization event followed by divergence within the lake rather than two or more separate invasions of allopatrically derived forms. It is, however, impossible to discount the possibility that the large and small morphs were derived allopatrically and have become genetically similar by post-colonization gene flow without a much more extensive survey of

Arctic char throughout Alaska. A similar conclusion appears applicable to the sympatric “pale” and “dark” morphs on Gander Lake (Gomez-Uchida *et al.* 2008), and Garduño-Paz *et al.* (2010) presented genetic evidence that scenarios of allopatric and sympatric divergence of Arctic char may be lake-specific in Scotland.

In most systems with sympatric Arctic char populations, the large-bodied (and usually pelagic) morph is thought to most resemble the original anadromous colonizing individuals because anadromous Arctic char are large-bodied and commonly piscivorous (e.g., Dempson *et al.* 2002). This is likely the case for Lower Tazimina Lake morphs: the large morph is at least partially piscivorous and is more similar in terms of allelic identity, size, and morphology to Iliamna Lake fish than is the small morph (Woods *et al.* 2013). Small morphs from Lower Tazimina Lake showed greater divergence from Iliamna Lake Arctic char and they also had higher allelic richness and more private alleles than the large morphs and Iliamna Lake fish. These observations suggest that following colonization of Lower Tazimina Lake, body size divergence was accompanied by two trophic shifts: one from piscivory toward greater benthic food consumption in large morphs, and another towards foraging on small-sized limnetic prey by small morphs (cf. Woods *et al.* 2013). While the small morph represents a very distinct gene pool, the large morph population displayed greater admixture and contained more fish that were genetically intermediate between the large and small morphs. Nevertheless, mean admixture values were significantly different amongst all three morphs, but those of the medium-sized morph and simulated hybrids between large and small-sized morphs were not significantly different from one another. Further, gill raker counts of medium-sized fish were intermediate to those of the large and small-sized morphs and gill raker count typically has a genetic component in fishes (e.g., Bentzen & McPhail 1984). These observations are consistent with the idea that the medium-sized morphs are hybrids between the other two morphs of char rather than a third, less isolated population (cf. Snorrason *et al.* 1994). Also, the F_{ST} value between large and medium morphs was not significant which suggests that if medium-sized char are hybrids, they backcross preferentially with large-sized char. Despite feeding together in the benthic regions of the lake, large and medium morphs consumed

different prey items (Woods *et al.* 2013). The large morph ate more snails whereas the medium morph ate more terrestrial insects. Given the detectable genetic divergence of medium-sized morphs from at least the small-sized morph, and polymorphism in prey choice and differential size at maturity, the medium sized morph could be in the process of diverging into a third distinct population (e.g., Smith *et al.* 2003; Seehausen 2004).

2.4.3 Biogeography of sympatric morphs

While sympatric morphs of Arctic char have been described from European and Asian portions of their Holarctic range, accounts of sympatric morphs in North America are scant and restricted to the central Arctic or northeastern North America. The two morphologically distinct 'dark' and 'pale' sympatric forms in Gander Lake, Newfoundland showed strong genetic segregation (Power *et al.* 2005; Gomez-Uchida *et al.* 2008). Divergence between forms was hypothesized to have occurred when the lake became ice-free following the end of the last ice age (*c.* 12,000 years BP). Similarly, Lake Aigneau, in northern Québec, Canada contains two morphs that are largely segregated in either the pelagic or littoral zones of the lake, and with very little gene flow between forms (F_{ST} = 0.174, Power *et al.* 2009). Conversely, analysis of 'large' and 'small' sympatric and morphologically distinct morphs from Lake Hazen, Nunavut, in the Canadian High Arctic revealed no evidence of genetic distinction (Arbour *et al.* 2011). Given that the last glacial retreat only reached the Lake Hazen region *c.* 8,000 years BP and glacial ice cover of the lake was likely present up until 4,200-3,300 years BP, the lack of genetic differentiation may be attributable to a relatively recent colonization of the lake (Arbour *et al.* 2011).

While previously documented in other regions of North America, Lower Tazimina Lake morphs represent the first example of sympatric morphotypes of Arctic char found on the west coast of North America. Further, Lower Tazimina Lake morphs exhibit some genetic divergence from one another, but most medium-sized morphs also had intermediate *Q* values (0.19 - 0.82) and accounted for approximately 18% of all individuals genotyped. If the medium-sized morphs are indeed hybrids,

perhaps the char in Lower Tazimina Lake are currently at an evolutionary intermediate stage of divergence between geologically older lakes such as Gander Lake or Lake Aigneau and younger lakes like Lake Hazen. This hypothesis is consistent with the much stronger degree of divergence found between small-bodied and large-bodied morphs in Lake Aigneau (F_{ST} = 0.174, Power *et al.* 2009) compared to Lower Tazimina Lake (F_{ST} = 0.092). Lower Tazimina Lake was likely colonized by Arctic char via the Newhalen River sometime after the area became ice-free (~12 000 – 15 000 BP, Stilwell & Kaufman 1996; Ramstad *et al.* 2004), with divergence of char morphs perhaps occurring later than for the Gander Lake morphs, but earlier than for morphs in Lake Hazen. Collectively, Gander Lake and Lake Aigneau, Lower Tazimina Lake, and Lake Hazen suggest a gradation of evolutionary change, which may be dependent, in part, on the time of glacial retreat and colonization (cf. Garduño-Paz *et al.* 2010).

In addition to differences in divergence time, variation in environmental features of lakes that result in different levels of ecological opportunity probably also influence the levels of phenotypic and genetic divergence in sympatric populations of fishes (e.g., Lu & Bernatchez 1999; Landry *et al.* 2007; Siwertsson *et al.* 2010; Ormond *et al.* 2011). For instance, size-at-age growth curves were an effective means of assigning morphs into discrete populations and identifying intermediate individuals in Lower Tazimina Lake (Woods *et al.* 2013) which also differ genetically from one another as shown in our study. Further, small morphs had more gill rakers than large or medium morphs and gill raker counts were strongly associated with genetic admixture (*Q*) values. Gill rakers, which assist in feeding on small prey items from the water, are generally more numerous in forms with largely limnetic diets in a variety of fishes (e.g., Bentzen & McPhail 1984; Gowell *et al.* 2012; Roesch *et al.* 2013). Indeed, Woods *et al.* (2013) showed that the diet of the small morph was characterized by consumption of more zooplankton and fewer carbon-based prey items derived from benthic resources (fewer snails, terrestrial and aquatic insects, and fishes) and occupied a lower trophic position than either the medium or large morph. By contrast, the diets of the medium and large morphs differed only slightly from one another. Morphological adaptations such as gill rakers, diets, and growth

rates are, therefore, associated with neutral molecular differentiation which suggests that genetic changes have accompanied phenotypic shifts as the morphs diverged in Lower Tazimina Lake. Given that resource polymorphism may be the driving factor for the isolation of large and small morphs in Lower Tazimina Lake, an intriguing hypothesis is that variation in physiological and morphological traits has become partitioned across at least two different morphs and enabled optimal exploitation of available trophic resources (cf., Ohlberger *et al.* 2008; Evans *et al.* 2012). Further work should investigate whether differences in genes relevant to metabolism are also correlated with genetic identity (e.g., Bernatchez *et al.* 2010).

Past introgression of Arctic char with its sister taxa Dolly Varden (*S. malma*) could also act to complicate the evolutionary history of the Lower Tazimina morphs. Shared mitochondrial DNA haplotypes and demographic inferences led Taylor *et al.* (2008) to suggest that historical gene flow from Dolly Varden to Arctic char had occurred approximately 1100-10 900 years ago. Dolly Varden alleles may then have been introduced into certain Arctic char individuals or populations before the colonization of Lower Tazimina Lake (or after, if more than one colonizing event occurred) and perhaps contributed to a selective advantage in certain microhabitats for those individuals (hybrids) possessing Dolly Varden alleles when compared to pure Arctic char. The divergence of the morphs may then be attributable to the selectively advantageous signatures of past hybridization events and not purely to sympatric speciation.

Woods *et al.* (2013) presented evidence for two growth phenotypes in Summit Lake (although a model invoking a single growth form was not substantially less supported). These authors, however, found no evidence of diet differences (from prey occurrence or stable isotope analyses) in Summit Lake char, in contrast to the situation in Lower Tazimina Lake. Similarly, my genetic analysis found no evidence of distinct genetic groups in the char from Summit Lake so the existence of distinct forms in Summit Lake remains equivocal. In addition, there is no evidence of divergent phenotypes or gene pools within any of the other lakes that I examined (see also Woods *et al.* 2013). It is possible, however, that more extensive sampling especially with respect to depth could uncover as yet unknown diversity, especially

in a large lake such as Iliamna Lake (cf., Finstad & Berg 2004). Alternatively, why some lakes might have divergent forms and others not is an uncertainty common to many instances of postglacial adaptive radiations in fishes and may result from historical or contemporary factors and their interactions (e.g., Bernatchez & Dodson 1990; Lu & Bernatchez 1999; Taylor & McPhail 2000; Landry *et al.* 2007; Siwertsson *et al.* 2010; Ormond *et al.* 2011). Kristjánsson *et al.* (2011) and Woods *et al.* (2012) explored characteristics of lakes that might influence morphological variation in Icelandic Arctic char, not including sympatric populations, and concluded that fish community composition was one of the most important factors (see also Ormond *et al.* 2011 for *Gasterosteus aculeatus*). Fish community composition may be relevant to the potential for sympatric populations and in our system diversity ranged from two species (Summit Lake) to at least 10 (Iliamna Lake), but is confounded with many other differences amongst lakes (Woods *et al.* 2013). A model that incorporated historical factors (e.g., time since colonization, opportunities for multiple colonization) and contemporary aspects of lake physiography and fish community structure across the geographic range of lakes with sympatric char would perhaps shed light on the factors critical to the origin and persistence of sympatric populations.

The North Pacific and Beringia are increasingly being recognized as important wellsprings of biodiversity and have been instrumental in understanding biogeography and speciation in temperate faunas (Cook & Auster 2005; Ilves & Taylor 2008; Taylor *et al.* 2008). Alaska is home to a diverse and well-established assemblage of Arctic char; however, quantifying the level of biodiversity on the western regions of North America has lagged behind that in the eastern Arctic and Europe owing to taxonomic confusion with Dolly Varden, remoteness of sites and difficulty in obtaining samples. Considering that many Arctic and sub-Arctic species are rapidly becoming at risk due to anthropogenic impacts such as climate change, dispersal of bio-pollutants and the harvesting of natural resources, research into these regions is becoming increasingly critical (Berg *et al.* 2010; Reist *et al.* 2013). Moreover, the study of unique intralacustrine populations of char and other fishes can provide clues about the role of adaptive divergence in speciation. My findings

and those of Woods *et al.* (2013) suggest that ecological aspects of individuals (gill raker count and associated feeding behaviour) are correlated with genetic identity and supports the idea that the availability of divergent habitats and/or ecological opportunities within lakes may help to drive the development of specialized phenotypic traits in Arctic char and more generally (see Smith & Skúlason 1996; Jonsson & Jonsson 2001). Further, the emergence of divergent phenotypes can contribute to reduced gene flow and thus promote the evolution of reproductive isolation between sympatric populations (e.g., Schluter & Rambaut 1996; Adams & Huntingford 2002a; Klemetsen 2010; Bernatchez *et al.* 2010).

Chapter 3:

Low levels of hybridization between sympatric Arctic char (*Salvelinus alpinus*) and Dolly Varden char (*Salvelinus malma*) highlights their genetic and spatial distinctiveness

3.1 Introduction

Natural hybridization is a fundamental evolutionary process in the biology of plants and animals (Mayr 1963; Arnold 1992; DeMarais *et al.* 1992; Barton 2001). When different species or reproductively distinct populations interbreed, the level of hybridization, structure of hybrid zones, and viability of hybrids can indicate factors relating to the origin and maintenance of species differences as well as processes important in the evolution of reproductive isolation (Barton & Hewitt 1989; Arnold 1997). Moreover, as natural hybridization can occur during primary intergradation or through secondary contact, studies of hybridization can provide us with a means to evaluate the strength of isolation between evolutionarily young lineages (Schluter 1996; Jiggins & Mallet 2000). For example, contact or hybrid zones can act as a test to access the status of sympatric populations as distinct biological species (Avise 1994; Taylor *et al.* 2008) where to pass the test, sympatric species must remain genetically distinct from one another despite the potential for, or actual occurrence of, gene flow between them. Further, defining species boundaries from studies of contact zones can also be critical when designing management goals for morphologically cryptic or hybridized populations (Campton 1987; Allendorf *et al.* 2001; Bickford *et al.* 2007).

Natural hybridization is frequent in plants and several vertebrate groups; in the latter it occurs perhaps most commonly in fishes (Schwartz 1972; Campton 1987; Bernatchez *et al.* 1995; Allendorf & Waples 1996; Arnold 1997; Rieseberg 1997; Scribner *et al.* 2001). Factors such as external fertilization (Hubbs 1955), niche overlap (van Herwerden & Doherty 2006) and competition for limited spawning sites

(Campton & Utter 1985) have probably contributed to elevated hybridization in fishes. Hybridization and introgression can lead to a wide array of phenomena including the formation of hybrid zones, adaptive radiation, and reinforcement of pre- and post-mating reproductive barriers during speciation (Schluter 1996; Arnold 1997; Dowling & Secor 1997; Seehausen 2004; Aboim *et al.* 2010). For salmonid fishes (*Salmo*, *Oncorhynchus*, *Salvelinus* and related genera) hybridization is a typical phenomenon among particular pairs of species (reviewed in Taylor 2004). Evidence suggests that some species of salmonids exhibit little or no intrinsic post-zygotic barriers to interbreeding and this may be one of many contributing factors to the high levels of natural and anthropogenic induced hybridization within this group (Hendry & Stearns 2003; Rubidge & Taylor 2004). The lack of post-zygotic barriers to hybridization between some salmonid species makes them well-suited to studies on pre-zygotic isolation such as environment or ecologically dependent reproductive isolation, an important issue in research on speciation (Redenbach & Taylor 2003; Taylor 2004; Rogers & Bernatchez 2006).

Among salmonids, char (*Salvelinus*) have proved instrumental in our understanding of evolution and speciation in fishes and more generally due to their incredible diversity, polymorphism, and adaptability to different habitats (Snorrason *et al.* 1994; Gíslason *et al.* 1999; Brunner *et al.* 2001; Jonsson & Jonsson 2001; Klemetsen 2010; Reist *et al.* 2013). Of seven well-recognized char species, the Arctic char (*Salvelinus alpinus*) arguably shows the greatest adaptive potential and exhibits extensive variability in terms of morphology, colouration, ecology and genetic structure (Klemetsen 2013). Highly polymorphic, Arctic char can have anadromous or non-anadromous life histories and can be found in various marine, estuarine and freshwater habitats across their circumpolar range (Johnson 1980). Multiple (sometimes up to four) diverse morphological forms or morphs have been described from lacustrine systems in Europe, Russia and North America, especially in recently colonized post-glacial lakes with low fish taxonomic biodiversity (Skúlason *et al.* 1989; Klemetsen *et al.* 2003; Power *et al.* 2009; Klemetsen 2010; Woods *et al.* 2013; May-McNally *et al.* 2014). Depending on the system and time since colonization, morphs can show significant phenotypic plasticity and genetic

differentiation from other Arctic char populations or even other sympatric morphs which has led to the naming of multiple taxa within the Arctic char species ‘complex’ (Reist *et al.* 2013).

Historical contention over the status of the Dolly Varden char (*Salvelinus malma*) as a species distinct from Arctic char has also contributed to the taxonomic confusion within this group. Once considered to be part of the Arctic char species ‘complex’, the Dolly Varden char is now the sister species to Arctic char (Lindsey 1956; Johnson 1980; Taylor *et al.* 2008; Crête-Lafrenière *et al.* 2012). Like Arctic char, Dolly Varden also exhibit a variety of life history patterns and ecological and genetic diversity (Baxter *et al.* 1997; Koizumi *et al.* 2006; Taylor *et al.* 2008). Recently diverged from one another (*c*. 215,000–458,000 years ago) (Taylor *et al.* 2008), Arctic char and Dolly Varden have largely allopatric distributions, but within North America, their geographical ranges overlap only in central and southwestern Alaska (McPhail 1961; Fig. 1.2).

In Alaska, Arctic char are abundant in nearshore habitats on the western and southern portion of the state around the Alaska Peninsula and along the northern slope adjacent to the Canadian Arctic populations (McPhail 1961). Alaskan Arctic char are usually lacustrine and non-anadromous while Dolly Varden char are typically anadromous and reside in streams as juveniles. Although consistent morphological differences exist between Arctic char and Dolly Varden throughout this zone of sympatry (*e.g.* pyloric caeca, gill raker counts), McPhail (1961) also identified populations that had ambiguous morphology and speculated that they have resulted from hybridization between the species. Phylogeographical mitochondrial (mtDNA)-based surveys by Brunner *et al.* (2001) support the idea of recent or ongoing hybridization between the species in Alaska. Five distinct mtDNA clades across the Holarctic range of Arctic char have been identified (Bering, Siberia, Atlantic, Acadia and Arctic) and were speculated to have resulted from isolation in separate Pleistocene glacial refugia. All samples identified morphologically as Dolly Varden from the western Pacific collectively grouped in the Bering lineage and shared haplotypes with southern Alaskan Arctic char. Paraphyly in mtDNA between Beringian Arctic char and Dolly Varden resulted in a lack of

resolution between the lineages and Brunner *et al*. (2001) suggested that the separate species status of Dolly Varden was “questionable”. Shared haplotypes between the species also suggests that gene flow between the species in these areas has occurred recently or is ongoing.

Using a combination of morphology and genetics (microsatellite and mtDNA), Taylor *et al.* (2008) further examined Arctic char and Dolly Varden from lakes in western and southwestern Alaska. For the majority of systems, they found that the sympatric taxa still shared mtDNA sequence similarity, but were highly genetically distinct at nine microsatellite loci, supporting their status as valid biological species. In one southwestern Alaskan lake (Lake Aleknagik) however, 7% of fish had ambiguous genetic identity and were putatively classified as hybrids. Moreover, McPhail (1961) described fish from Lake Aleknagik with phenotypes intermediate between Arctic char and Dolly Varden and speculated that they were hybrids. Detecting putative hybrids in Lake Aleknagik is not surprising given that Dolly Varden have also been found to hybridize with other char, such as bull trout (*Salvelinus confluentus*) (Taylor *et al.* 2001; Redenbach & Taylor 2003). Given the genetic and morphological similarities between Arctic char and Dolly Varden in Lake Aleknagik, it is possible that a potential mosaic hybrid zone could exist between the species and habitats contributing to hybridization could be more diverse than expected. A combination of relatively low sample size and lack of sample site diversity in the Taylor *et al.* (2008) study, however, limited their ability to accurately access the level of ecological and genetic segregation between the char species in the Aleknagik system. Moreover, as the mode of selection structuring areas of hybridization between Arctic char and Dolly Varden has not yet been defined, sampling a variety of different microhabitats (e.g. across streams, various depths in the lake) could help establish whether environment-dependent selection may be an important factor in the spatial distribution of hybridization and perhaps yield hints to the processes involved in the divergence of the two species.

In this chapter, I report the results of a robust microsatellite survey between the two species in Lake Aleknagik, Alaska, as well as another connected lake in the same lake system to test several ideas. First, I wanted to assess the degree of

interspecific ecological segregation, in the form of species specific spatial distribution maps and age class allocations across multiple sampling sites inferred from genotyping results. Second, I used the microsatellite data to assess the degree of genetic divergence between species, hybridization levels, and assignment of individuals to different genotypic classes. The microdistribution and level of hybridization between the species was accessed across a wide variety of microhabitat types including across the length of streams, varying depths in the lake and from different beach sites in order to access whether habitat structure may influence hybrid distribution and prevalence. Determining the extent of genetic and ecological diversity will hopefully help us understand factors important in the evolution of reproductive isolation between Arctic char and Dolly Varden and have implications for the role of natural hybridization in speciation as well as our knowledge of the evolution of Arctic biodiversity.

3.2 Materials and methods

3.2.1 Sample collection

Lake Aleknagik (832 km^2, 32 km in length) is located near the southwestern coast of Alaska, in the central portion of Bristol Bay and near Dillingham, Alaska (Fig. 3.1). Lake Aleknagik is the first lake in the Wood River Lake system, a series of five interconnected lakes (425 km^2) that drain via the Wood River into the Nushagak River to the south and eventually into Bristol Bay. The lakes are oligotrophic and range from 6 to 201 km^2 in length. Lake Aleknagik is home to a diverse native fish community including pygmy whitefish (*Prosopium coulterii*), threespine sticklebacks (*Gasterosteus aculeatus*), slimy and coastrange sculpins (*Cottus cognatus* and *C. aleuticus*), and salmonids (Salmonidae), with char and sockeye salmon (*Oncorhynchus nerka*) being the most numerically dominant salmonids.

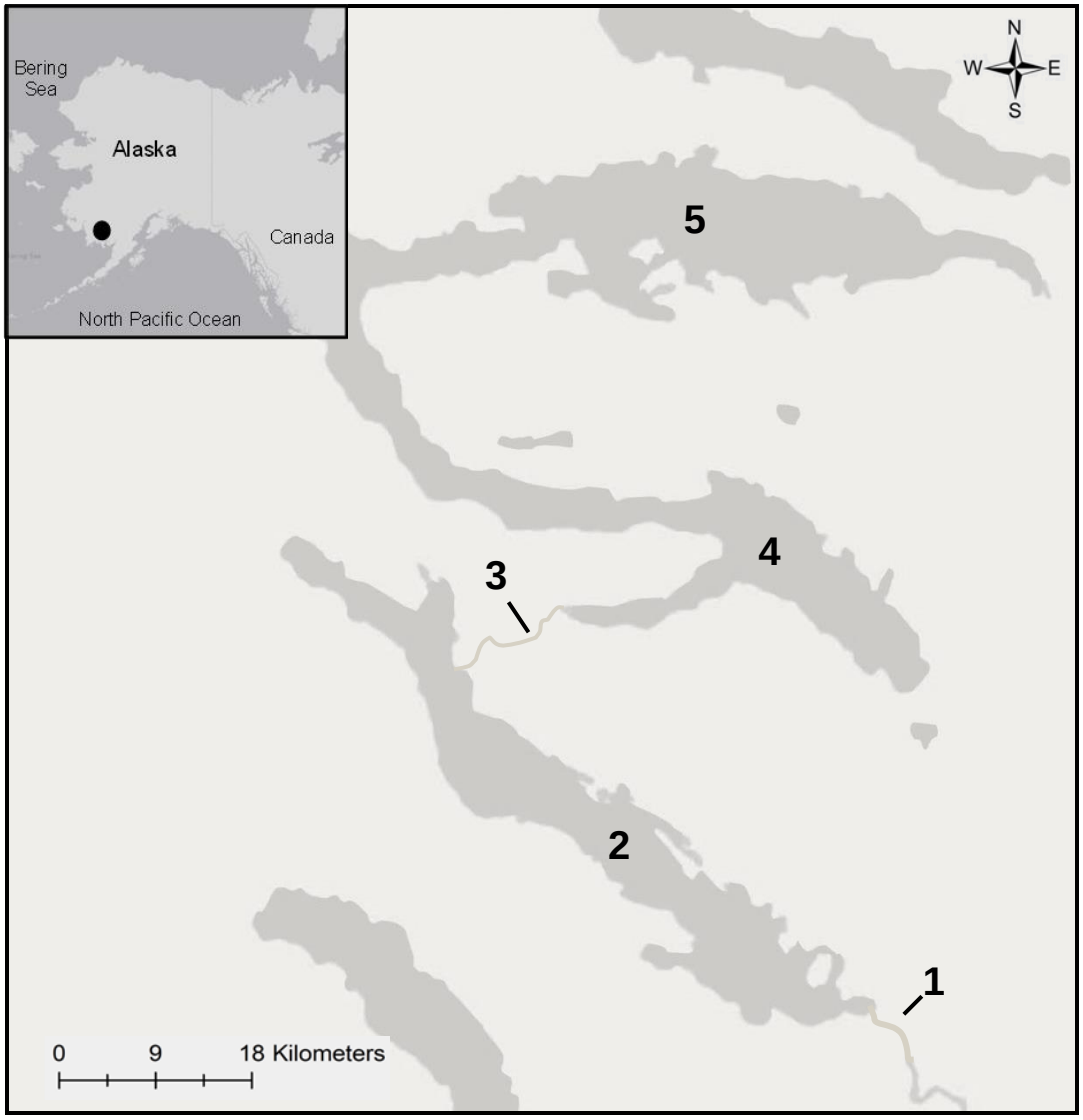

Figure 3.1: The Wood River Lake system in southwestern Alaska, USA. **1** = Wood River, **2** = Lake Aleknagik, **3** = Agulowak River, **4** = Lake Nerka (South arm), **5** = Lake Nerka (North arm)

Sympatric char were collected during the period of July to August 2012-2013 from 33 sites in Lake Aleknagik (total *N* collected = 752, Table 3.2) representing four lake, three beach, one river, and 25 stream sites by using a combination of sinking gillnets, beach seines, stick seines, minnow traps and angling (Appendix B Table. B.1). Lake, river, and lower, mid and upper streams sites were sampled in 2012 while only stream sites were sampled in 2013 in an attempt to increase the sample size of Dolly Varden. Sinking gillnets were set by boat daily for 4-12 h and suspended above the bottom with floats. Lake sites were chosen to represent various depths and regions around the lake and included a shallow (Site 7, ~5—15m deep), intermediate (Site 4, ~15—20m deep) and deep (Site 5, ~20—50m deep) site. At each of these sites, two gillnets were used in an attempt to sample different size classes of char in Lake Aleknagik, both 30 m long by 2 m high, one with 30 mm and the other with 20 mm stretched mesh. Beach sites (Sites 2, 3, 7) were selected from various regions around the lake and were sampled using a 32 m long x 5 m deep beach seine net tapered from 5 m deep in the centre 6 m wide section with 6 mm mesh to 13 mm mesh in the 2.5 m side panels and 1 m deep with 30 mm mesh at the edge panels**.** During the day it was deployed to shore by boat in a semi-circle reaching approximately 10-15 m offshore. The majority of samples originating from streams were collected using an 8 foot long stick seine with a mesh size of approximately 5 mm x 5 mm. Minnow traps baited with salmon eggs were also used in Hansen Creek sites 17-19.

Fin clips constituted the removal of the adipose fin to avoid re-captures and were stored in 95% ethanol until later laboratory analysis. Fish classified as young of the year (YOY) were euthanized immediately in a dilute solution of MS-222 (tricaine methanesulfonate) and stored whole in 95% ethanol. A visual estimate of species identity (Arctic char, Dolly Varden or hybrid), fork length, a photograph and GPS coordinates were taken for each fish sampled and microhabitat features of the sampling site noted. Samples originating from Lake Nerka, the second lake in the Wood River Lake system and just upstream of Lake Aleknagik, were collected by Daniel Schindler and Kale Bentley (University of Washington, Seattle, WA) in 2012-2013 by stick seining and angling (total *N* collected = 329). Lake Nerka sites include

one river and 14 stream sites (Appendix B Table. B.2). Additional samples of spawning Arctic char and Dolly Varden from Lake Aleknagik and the Wood River were collected from September-October, 2012 by angling and donated to this study by C. Schwanke (Alaska Department of Fish and Games, ADF&G, Dillingham, AK). The spawning Arctic char were obtained from outside the mouth of Youth Creek, located on the far north side of Lake Aleknagik. The spawning anadromous Dolly Varden return to freshwater in autumn and were obtained from the Wood River which drains Lake Aleknagik into Bristol Bay to the south.

3.2.2 Microsatellite analysis

A total of 496 Lake Aleknagik and 229 Lake Nerka fish were included in the genetic analysis. Fin clip samples were digested and total genomic DNA was extracted using the DNeasy DNA blood and tissue extraction kit (Qiagen Inc., Valencia, CA, USA). Extracted DNA samples were stored at -20 °C for later use in multiplex polymerase chain reactions (PCR) using the Qiagen multiplex kit (Cat. No. 206145). DNA was amplified in 10-µL PCR reactions at 95 °C for 15 min, 94 °C for 30 sec, 35 cycles of 1.5 min at an annealing temperature of 55 °C followed by 72 °C for 1 min, and 60 °C for 30 min. I assayed microsatellite variation using primers labeled with infrared fluorophores and a 3730S 48-capillary DNA Analyzer with GS 500 LIZ or 600 LIZ internal size standards (Applied Biosystems, Carlsbad, California, USA). Alleles were manually scored using the program GeneMapper (GeneMapper v.3.7, Applied BioSystems). I amplified 13 microsatellite loci isolated from other salmonid species including: Atlantic salmon, *Salmo salar* (*SSOSL456*; Slettan *et al.*, 1997), bull trout, *Salvelinus confluentus* (*Sco200, Sco202, Sco215, Sco216, Sco220*; DeHaan & Ardren, 2005), Chinook salmon, *Oncorhynchus tshawytscha* (*OtsG83b, OstG253b*; Williamson *et al.*, 2002), Dolly Varden, *Salvelinus malma* (*Smm-17, Smm-21, Smm-22, Smm-24* ; Crane *et al.*, 2004) and rainbow trout, *Oncorhynchus mykiss* (*OMM1105*; Rexroad *et al.*, 2002) (Appendix B Table. B.3). With the exception of *Smm-21*, all markers were polymorphic (non-diagnostic) for Arctic char and Dolly Varden.

The locus, *Smm-21* was, however, monomorphic and diagnostic in Arctic char; all fish were homozygous for a 110 base pair allele. By contrast, Dolly Varden were polymorphic at *Smm-21* with alleles that ranged in size from 120-132.

3.2.3 Statistical analysis

I used MICRO-CHECKER (van Oosterhout et al. 2004) to check the microsatellite data for errors in scoring such as stuttering and null alleles which can compromise subsequent analyses. Next, I used the program FSTAT ver 2.9.3 (Goudet 2001) and ARLEQUIN ver 3.5 (Excoffier & Lischer 2010) to generate the basic descriptive statistics of sample size (N), number of alleles (N_A), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosity. Tests for departures from Hardy-Weinberg Equilibrium (HWE) for each locus-population combination were performed with GENEPOP ver 4.2 (Raymond & Rousset 1995) using an exact test in which probability values were determined using a Markov chain method (P) controlling for multiple tests.

3.2.4 Population structure

To test for population subdivision for all samples pooled within Lake Aleknagik and Lake Nerka, I ran simulations of K = 1 to K = 10, repeated five times in the Bayesian program STRUCTURE for each sampling year (2012, 2013) separately to account for inter-annual differences and visualized the data using DISTRUCT (Rosenberg 2004). To infer support for the most probable number of subpopulations for each sampling year, ΔK (Evanno *et al.* 2005) was calculated across multiple runs of STRUCTURE using STRUCTURE HARVESTER (Earl & vonHoldt 2012). To assign individuals as Arctic char or Dolly Varden, an *ad hoc* approximation of species identity was used where fish whose Q-value were ≥ 0.95 (i.e. at least 95% of the genome suggestive of Dolly Varden) were classified as Dolly Varden and fish whose Q-values were ≤ 0.05 were classified as Arctic char. Any fish that had Q-values ≥ 0.05 but ≤ 0.95 were noted as possible hybrids.

3.2.5 Levels of hybridization

To determine the level of hybridization and the genetic classification of hybrids present, I used the programs STRUCTURE ver. 2.3.4 (Pritchard *et al.* 2000) and NEWHYBRIDS vers.1.1 Beta 3 (Anderson & Thompson 2002). For both programs, I used genotypic data from all 13 microsatellite loci for each individual sampled and analyzed from Lake Aleknagik and Lake Nerka. I used STRUCTURE to estimate an individual's probability of belonging to a one of two populations (K= 2, i.e., Arctic char and Dolly Varden – see Results) while minimizing departures from HWE and linkage disequilibrium within groups (Evanno *et al.* 2005). The STRUCTURE analyses were conducted using a burnin-period of 50,000 Markov Chain Monte-Carlo (MCMC) iterations proceeded by an additional 450,000 steps, replicated five times to verify consistency across runs (Taylor *et al.* 2008). I then used STRUCTURE to estimate the admixture proportion value (Q) and posterior probability intervals for each fish. I defined Q_{DV} as the proportion of the genome estimated to originate from Dolly Varden. Thus, to establish general cutoff values for the two species I defined pure Dolly Varden as individuals with Q_{DV} values $\geq$ 0.95 and pure Arctic char as individuals with Q_{DV} values $\leq$ 0.05.

Next, to generate a range of Q_{DV}-values that would be more indicative of hybrid individuals, I used the program HYBRIDLAB (Nielsen *et al.* 2006) to create 100 simulated F_1, F_2, and backcrossed hybrids generated from the random selection of alleles from different loci in non-admixed allopatric populations of Arctic char and Dolly Varden. The allopatric populations were then designated as priors or "learning samples" by implementing the using the USEPOPINFO model combined with simulated hybrids and char from Lake Aleknagik and Lake Nerka through five replicated analyses in STRUCTURE (Pritchard *et al.* 2000). I took the average area between the upper and lower limits of possible Q_{DV}-values for the simulated hybrids across the five replicated analyses to create a zone of hybridity. Admixed individuals from the Wood River basin whose Q_{DV}-values fell inside this range were classified as putative hybrids and were used to generate the level of hybridization for each lake.

To account for low sample sizes for allopatric populations, a second zone of hybridity was generated using 50 Arctic char from Lake Aleknagik and Lake Nerka with Q_{DV} values closest to 0 ($Q_{DV} < 0.05$, pure Arctic char) and 50 Dolly Varden with Q_{DV} values closest to 1.0 ($Q_{DV} \geq 0.88$, due to limited samples) using the same methods as above. Both zones of hybridity were then compared to the range generated by Taylor *et al.* (2008) for Arctic char and Dolly Varden in southwestern Alaska.

I next used NEWHYBRIDS separately on Lake Aleknagik and Lake Nerka samples to determine an individual's probability of belonging to one of six genetic classes: pure Arctic char, pure Dolly Varden, F_1 hybrid, F_2 hybrid, backcross with Arctic char, backcross with Dolly Varden. The analysis by NEWHYBRIDS uses allelic differences between hybridizing species in a Bayesian model-based clustering framework with MCMC to estimate the posterior probability of an individual belonging to distinct hybrid classes (Anderson & Thompson 2002). The same allopatric populations used in the STRUCTURE analysis were also run in NEWHYBRIDS amongst the samples as non-admixed reference populations for a minimum of 450,000 MCMC steps.

To independently assess the use of a $K = 2$ in STRUCTURE and two parental species in NEWHYBRIDS (i.e., Arctic char and Dolly Varden), a factorial correspondence analysis (FCA) in GENETIX (Belkhir *et al.* 2001) was conducted on allele frequencies of Lake Aleknagik and Lake Nerka char alone and with allopatric populations of Arctic char from the Canadian Arctic and Dolly Varden from the Egegik fishing district located south of Dillingham and Bristol Bay, Alaska at the outflow of Becharof Lake. Allopatric populations were assigned to species using a combination of morphology and diagnostic microsatellite markers.

3.2.6 Spatial distribution across microhabitats

Genotyped samples of Arctic char, Dolly Varden and hybrids were georeferenced in order to map the spatial distribution of the species across microhabitats.

Global Positioning System (GPS) coordinates of sampling sites were mapped using online resources (http://boulter.com/gps). Variation in spatial distribution was assessed by analyzing sampling sites and sampling years separately. To determine the distribution of age classes between lake and stream sites for each char species in Lake Aleknagik, a size-frequency histogram generated from fork length and frequency of fish present in each sampling site was used to identify different age classes from size-frequency distributions. Species assignment was based upon admixture (Q_{DV})-values and fish that fell into the generated zone of hybridity (see section 3.3.3 for details) were excluded.

3.3 Results

3.3.1 Genetic analysis

A total of 725 Arctic char and Dolly Varden were genotyped at 44 sites across Lake Aleknagik and Lake Nerka. MICRO-CHECKER did not find evidence of alleles failing to resolve owing to scoring error or to large allele dropout in any of the samples. Evidence for a non-amplifying ("null") allele was found in some samples for locus (*Sco216*) although removal of this locus did not significantly alter results. When samples were partitioned into Dolly Varden and Arctic char (see below), all loci were polymorphic except for *Smm-21* in Arctic char, which was monomorphic and diagnostic for each species (Appendix B Table. B.4). Arctic char and Dolly Varden had approximately equal average allelic richness (17.8 vs 17.3) when pooled across the lakes and across sites within Lake Aleknagik. Dolly Varden showed the highest number of alleles for a single locus (39 at locus *OtsG83b* followed by 38 at locus *Sco200*). When examining samples pooled across sites within each lake and for Arctic char and Dolly Varden separately (separation of species being based on STRUCTURE results, seen below), twenty of fifty tests for deviations from Hardy Weinberg Equilibrium (HWE; two samples x 13 loci for Arctic char plus two samples x 12 loci for Dolly Varden) were significant at $P \leq 0.011$. The majority of deviations from HWE originated within Dolly Varden with ten out of the total 20 deviations found in Lake Aleknagik Dolly Varden samples and six from Lake Nerka Dolly Varden.

3.3.2 Population structure

Analysis by STRUCTURE indicated that the most likely number of genetic populations across both lake and stream sites for both lakes in 2012 was K= 2 (Fig. 3.2 A), as evidenced by a mean ΔK of 1,675 vs. 1.1 for K = 3 as the next most likely model. When sampling effort in stream microhabitats was increased in 2013, a K= 3 model was best supported by the ΔK method as evidenced by a mean ΔK likelihood of 14.6 vs. 3.5 for K= 5 as the next most likely model.

For the initial *ad hoc* assignment of individuals to species, 565 fish (78% of samples) had Q_{DV} values ≤ 0.05 and were classified as "pure" Arctic char while another 148 fish (20%) had Q_{DV} values ≥ 0.95 and were classified as "pure" Dolly Varden. All individuals classified as Arctic char by this method were homozygous for the diagnostic Arctic char locus *Smm-21* (allele 110). Likewise, all individuals classified as Dolly Varden by this method were polymorphic at this same locus (ranging from 120-132) and did not possess the diagnostic 110 allele. A total of 12 fish (2%) of samples fell in between the range of $0.05 \geq Q_{DV} \leq 0.95$. Of the 12 fish in this middle range, five samples had the diagnostic alleles indicative of Arctic char and Q_{DV} values ranging from 0.072–0.216 while the other seven samples were polymorphic at the *Smm-21* locus and had Q_{DV} values ranging from 0.493–0.939.

The results from the FCA analysis using known allopatric populations of Arctic char and Dolly Varden and the examination of the diagnostic locus *Smm-21* indicated that the two populations identified both in Lake Aleknagik and Lake Nerka in 2012 were Arctic char and Dolly Varden (Appendix B Fig. B.1). The numerically dominant genetic cluster in Lake Aleknagik was taken to represent Arctic char as those individuals were homozygous at the diagnostic Arctic char locus (*Smm-21*) and grouped with known allopatric populations of Arctic char in the FCA projection (Appendix B Fig. B.2). The second genetic cluster was numerically much smaller and grouped with allopatric Dolly Varden populations in the same FCA projection. Substantial differentiation as measured by pairwise F_{ST} was seen between the two

species in Lake Aleknagik (F_{ST}= 0.172) and in Lake Nerka (F_{ST}= 0.187; $P < 0.001$, Table 3.1). Similarly, for 2013, the ΔK analysis supported a single population of Arctic char, but a two population model for Dolly Varden, with the Yako Creek sample suggesting the existence of two populations of Dolly Varden in that system (Fig. 3.2 B). In all analyses, including when species were run separately, only a single population of Arctic char was ever supported either within or between the two lakes.

3.3.3 Hybridization levels and genetic classification

Analysis of the dataset with STRUCTURE at K= 2 suggested a general absence of hybridization between the two discrete genetic clusters representing Arctic char and Dolly Varden individuals, respectively (Fig. 3.3). The FCA projections further supported the existence of two distinct genetic clusters with few intermediate individuals (Appendix B Fig. B.1-B.2). Simulated hybrids were generated from allopatric populations of Arctic char and Dolly Varden from Lake Aleknagik and Lake Nerka char with admixture values indicative of pure species. These simulated hybrids had Q_{DV} values that were ≤ 0.779 and ≥ 0.445 (using allopatric learning samples, Appendix B Table. B.3) and Q_{DV} ≤ 0.788 and ≥ 0.237, respectively (Lake Aleknagik and Lake Nerka learning samples, Appendix B Table. B.4).

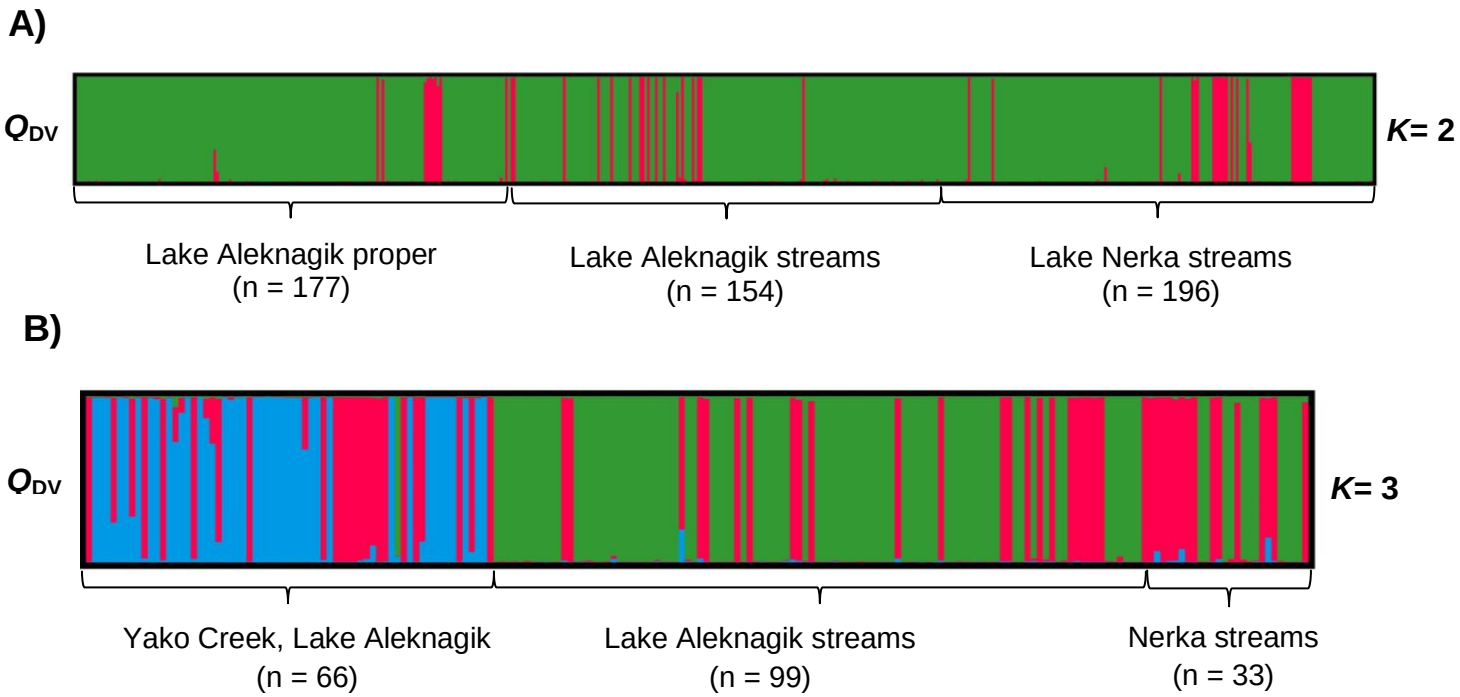

Figure 3.2: Results of STRUCTURE analysis visualized with DISTRUCT for sympatric Alaskan Arctic char (*Salvelinus alpinus*) and Dolly Varden (*S. malma*) assayed at 13 microsatellite DNA loci and sampled from **A)** Lake Aleknagik proper and streams and Lake Nerka stream and river sites in 2012 for *K*= 2; **B)** Yako Creek in Lake Aleknagik with support for two populations of Dolly Varden, Lake Aleknagik stream and Lake Nerka stream sites in 2013 for *K*= 3. Colour codes are: green = Arctic char, red = Dolly Varden, blue = additional population of Dolly Varden from Yako Creek as defined by admixture (*Q*) values.

Table 3.1: Pairwise F_{ST} (θ) estimated by variation across 13 microsatellite DNA loci in sympatric Arctic char (*Salvelinus alpinus*) and Dolly Varden (*S. malma*) from Lake Aleknagik and Lake Nerka, Alaska and allopatric populations of Arctic char and Dolly Varden from the Canadian Arctic and Egegik, Alaska. Arctic char = AC, Dolly Varden = DV. Values accompanied by asterisks are not significantly > 0 (P ≤ 0.0151; after adjustment for multiple simultaneous tests incorporating the false discovery rate procedure of Narum, 2006).

	DV (Aleknagik)	DV (Nerka)	AC (Aleknagik)	AC (Nerka)	AC (Canadian Arctic)
DV (Nerka)	0.009*	-	-	-	-
AC (Aleknagik)	0.172	0.187	-	-	-
AC (Nerka)	0.172	0.187	0.007*	-	-
AC (Canadian Arctic)	0.169	0.179	0.124	0.128	-
DV (Egegik)	0.022	0.0163	0.156	0.149	0.181

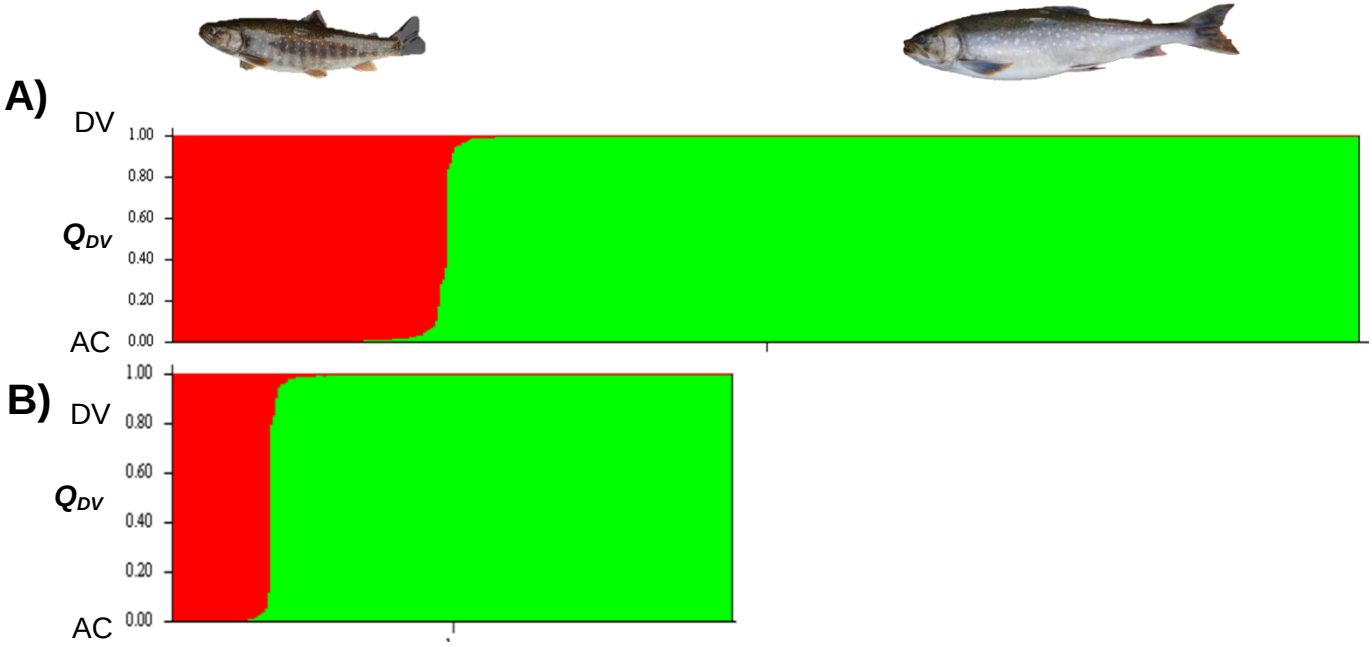

Figure 3.3: Overall genetic classification of sympatric Alaskan Arctic char (*Salvelinus alpinus*) and Dolly Varden (*S. malma*) using 13 microsatellite markers from **A)** Lake Aleknagik (n = 496) and **B)** Lake Nerka (n = 229). Assignment in STRUCTURE was based on the probability of belonging to two *a priori* genetic groups (*K*= 2). Individuals are arranged according to decreasing Q_{DV} -values (i.e., the proportion of red in each line) and both sampling years, summer 2012 and 2013, are shown. Colour codes are: red = *S. malma*, green = *S. alpinus*.

Small sample sizes for allopatric populations (< 25) likely constrained the lower limit of hybridity as the diversity in genotypes was restricted. As the range generated from char in the Wood River Lake system was most comparable to the range generated by Taylor *et al.* (2008) (Q_{DV} between 0.785 and 0.232) for the same system it was selected for use in the subsequent analyses. This range of Q_{DV} values was generated by constraining K= 2 in STRUCTURE, and in doing so, I found that the admixture coefficient for "pure" Arctic char (Q_{DV}) averaged 0.0049 (range 0.128-0.002) while the resultant admixture value for "pure" Dolly Varden (Q_{DV}) averaged 0.99 (range 0.812–0.998). The simulated hybrids had an average admixture value (Q_H) of 0.44 (range 0.237–0.788). Using these boundaries, I established a zone of hybridity expressed relative to the proportion of the Dolly Varden genome, represented by Q_{DV}, and considered fish with Q_{DV} values that fell within this range to be putative hybrids, fish with Q_{DV} values ≥ 0.788 to be Dolly Varden and fish with Q_{DV} values ≤ 0.237 to be Arctic char. The majority of fish fell within the parental ranges of Arctic char and Dolly Varden and only two fish (0.40%) from Lake Aleknagik and one fish (0.45%) from Lake Nerka had admixture values within the zone of hybridity (full summary in Table 3.2). These values did not change when samples were analyzed separately based on shared sampling year and site (e.g. same stream or lake site), and because of small numbers of putative hybrids, any tests of microhabitat differences affecting hybridization prevalence and occurrence were no longer deemed relevant. The three putative hybrids were collected from one lake (Bear Bay, site 5) and one stream site (Happy Creek, site 19) in Lake Aleknagik and one stream site (upper Lynx Creek, site 30) in Lake Nerka during the sampling period of 2012.

Table 3.2: Summary of sampling locations, date, total number of fish genotyped (*N*), and proportion of samples assigned as Arctic char (*Salvelinus alpinus*) (AC), Dolly Varden (*S. malma*) (DV) and putative hybrids (HYB) in the Wood River Lake system, southwest Alaska. Arctic char were defined as having Q_{DV} values of ≤ 0.237, Dolly Varden were defined as having Q_{DV} ≥ 0.788, and hybrids were defined as having Q_{DV} values between 0.237 —0.788.

	Latitude and Longitude	Date	*N*	Total AC	Total DV	Total HYB
Lake Aleknagik	59.343301°N-158.806686°W	July-August 2012	332	303	27	2
		July-August 2013	164	77	87	0
Lake Nerka	59.559679°N - 159.023666°W	June-August 2012	196	173	22	1
		July-August 2013	33	17	16	0
Wood River	59.262936°N - 158.573313°W	October 2012	8	0	8	0

Bayesian assignment of individuals into parental or hybrid (i.e., first- or later generation hybrids or backcrosses) genotypes with NEWHYBRIDS indicated a higher proportion of hybrids than estimated by STRUCTURE for Lake Aleknagik (7% vs. 0.4% by STRUCTURE) but was comparable for Lake Nerka (0% vs. 0.45% by STRUCTURE). A total of 76% of the Lake Aleknagik samples were classified as pure Arctic char, 17% as Dolly Varden, 0% as F_1, 2% as F_2, 0% as backcrosses with Arctic char and 5% as backcrosses with Dolly Varden using NEWHYBRIDS (Fig. 3.4). The fish assigned as backcrosses with Dolly Varden were scattered across stream habitats in Lake Aleknagik with most of the individuals originating from Yako Creek (2013 sites), Happy Creek (both years), Silversalmon Creek and Hansen Creek (2012). The F_2 individuals were found in two stream sites (Happy Creek, site 19 and upper Bear Creek, site 10). Most (6/7) samples of Dolly Varden with spawning colouration collected from the Wood River were assigned to the F_2 hybrid class. With the exception of one fish from Happy Creek, all F_2 individuals possessed the *Smm-21* alleles diagnostic for Dolly Varden. For Lake Nerka, all individuals were assigned to parental classes, with a total of 82.5% of samples classified as pure Arctic char and 17.5% as Dolly Varden.

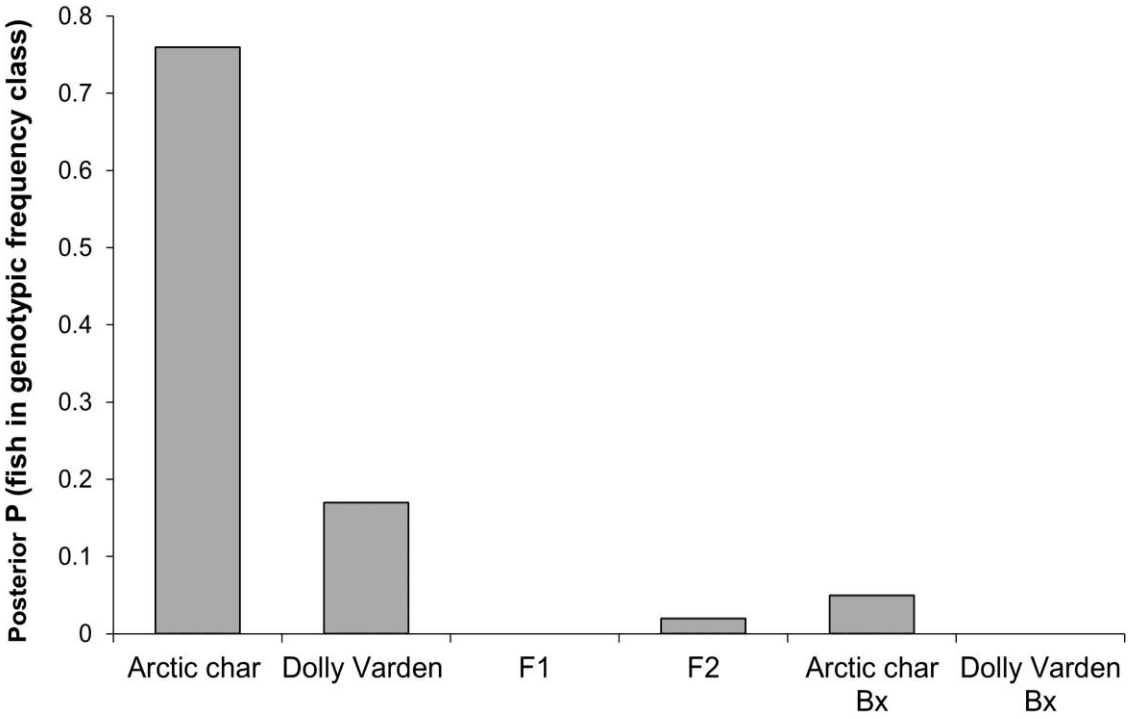

Figure 3.4: Summary of NEWHYBRIDS results for sympatric Arctic char (*Salvelinus alpinus*) and Dolly Varden (*S. malma*) from Lake Aleknagik into six genetic classes. AC = Arctic char, DV = Dolly Varden, Bx = backcross.

3.3.4 Spatial discreteness between species

The frequency and distribution of Arctic char (i.e., $Q_{DV} < 0.05$) in lake and stream habitats was widespread when compared to Dolly Varden. Arctic char were the only char species found in lake habitats at the time of sampling (Fig. 3.5), but could also be found in most streams both in Lake Aleknagik and Lake Nerka (Fig. 3.6–3.9). In streams, Arctic char typically occupied sites near the mouths of streams, but they could also be present in upper stream sites, located several kilometers upstream from the lake. The distribution of Dolly Varden was generally found in the middle to upper reaches of most streams although some streams were exclusively Dolly Varden (e.g., Site 44, Silversalmon Creek). In October, during the spawning period for both species, spawning Arctic char were collected off beaches at the outflow of large creeks on the northernmost region of the lake while mature Dolly Varden were found spawning in the Wood River to the south (C. Schwanke, ADF&G, personal communication). Stream resident Dolly Varden were also observed spawning in early-mid September at the outflow of Lynx Lake, located above Lynx Creek in Lake Nerka (D. Schindler, University of Washington, Seattle, personal communication).

The distribution of the different age classes across stream and lake habitats differed for Arctic char and Dolly Varden in Lake Aleknagik (Fig. 3.10). Young-of-the-year (YOY) to mature ages classes of Dolly Varden were seen in stream habitats, but were not collected during the sampling period from any lake sites. Age 3+ Dolly Varden appeared to reach a maximum size of approximately 270 mm in fork length and displayed vibrant spotting patterns. Young-of-the-year to age 3+ or 4+ Arctic char were also present in the majority of stream microhabitats similar to Dolly Varden, but were also common at lake sites. The numerically dominant char species in the lake, Arctic char, was present at beach sites as YOY and again as 2+ age classes in deeper microhabitats in the lake.

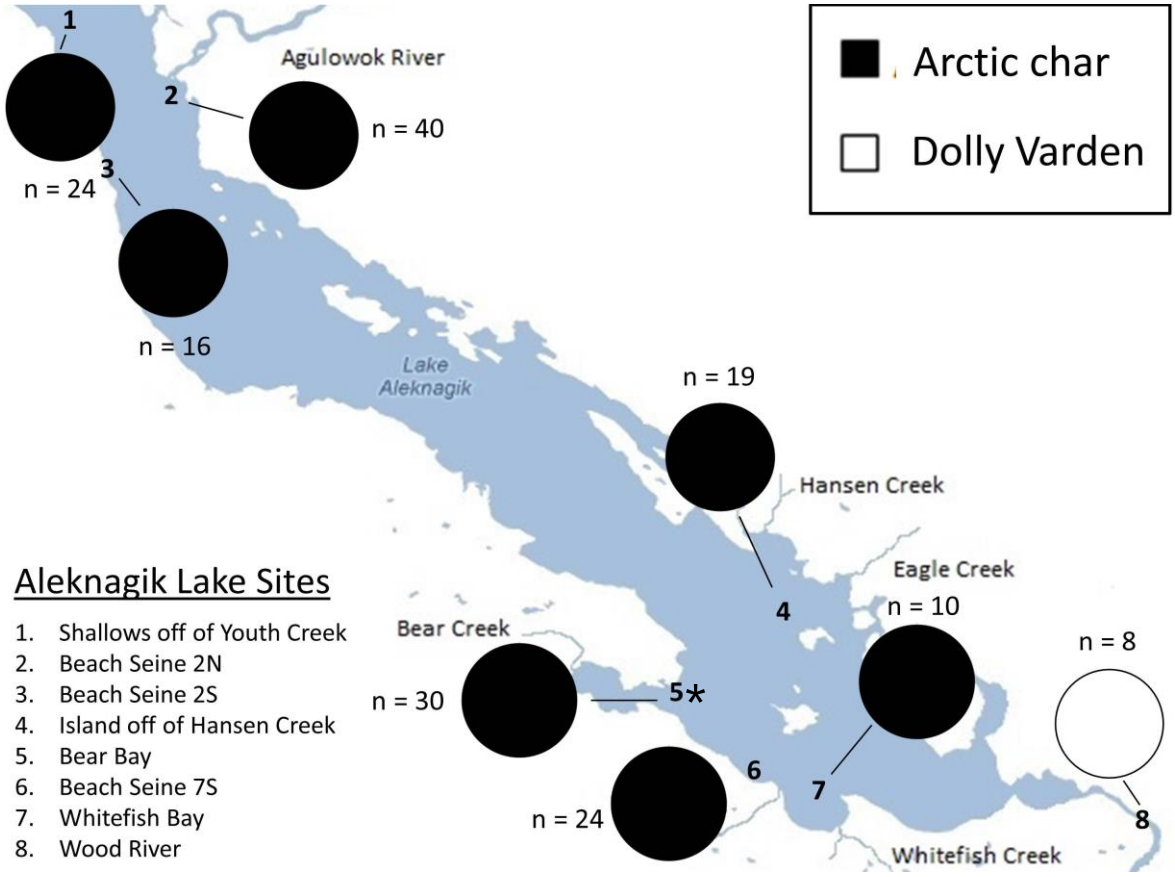

Figure 3.5: Proportion of genotypes defined by admixture (Q_{DV}) values corresponding to Arctic char (*Salvelinus alpinus*) and Dolly Varden (*S. malma*) from within the lake proper and on beaches sites in Lake Aleknagik during summer 2012 (black = *S. alpinus*, white = *S. malma*). Arctic char were defined as having Q_{DV} values of ≤ 0.237, Dolly Varden were defined as having Q_{DV} ≥ 0.788, and hybrids were defined as having Q_{DV} values between 0.237 —0.788. A single putative hybrid was found at the starred site (Bear Bay, site 5).

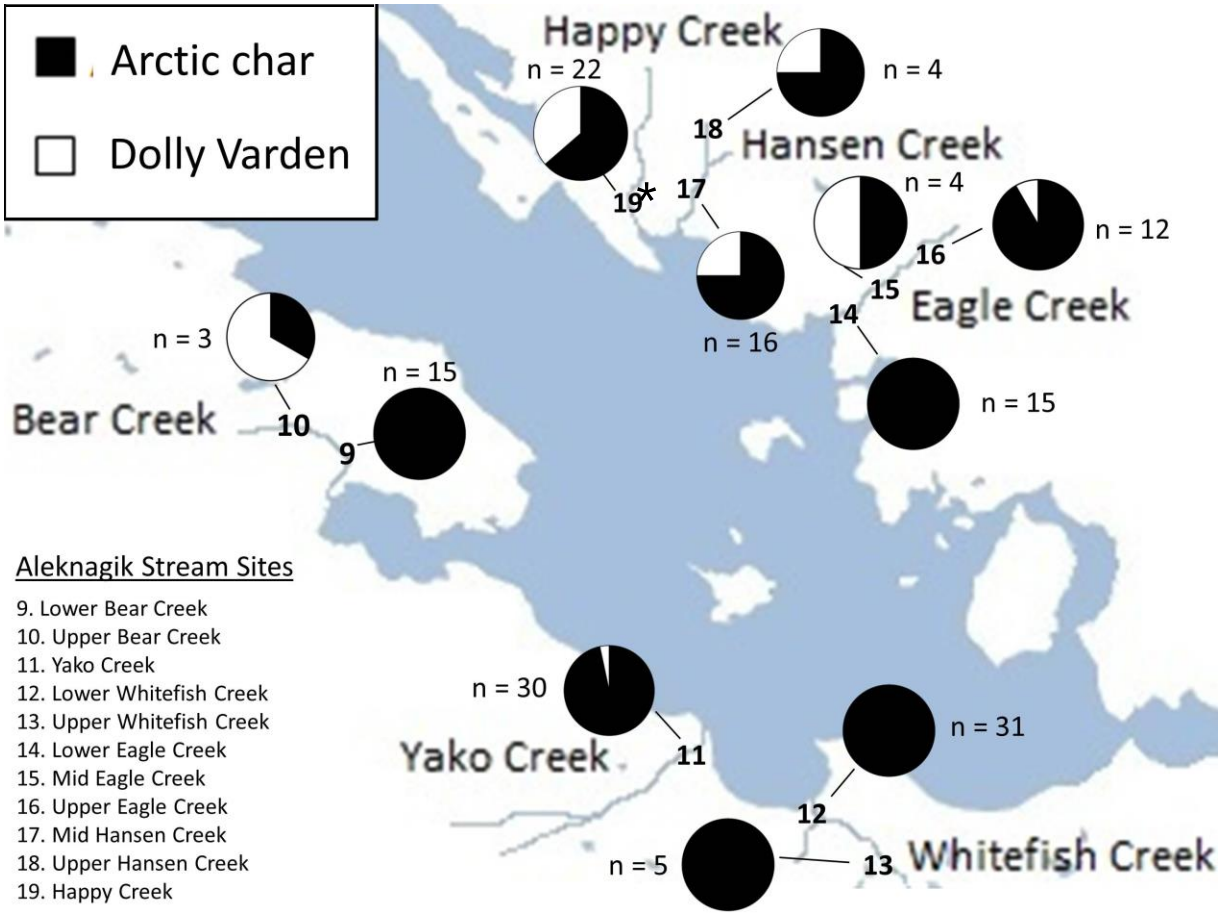

Figure 3.6: Proportion of genotypes defined by admixture (Q_{DV}) values corresponding to Arctic char (*Salvelinus alpinus*) and Dolly Varden (*S. malma*) from stream sites around the southern portion of Lake Aleknagik during summer 2012 (black = *S. alpinus*, white = *S. malma*). Arctic char were defined as having Q_{DV} values of ≤ 0.237, Dolly Varden were defined as having Q_{DV} ≥ 0.788, and hybrids were defined as having Q_{DV} values between 0.237 —0.788. A single putative hybrid was found at the starred site (Happy Creek, site 19).

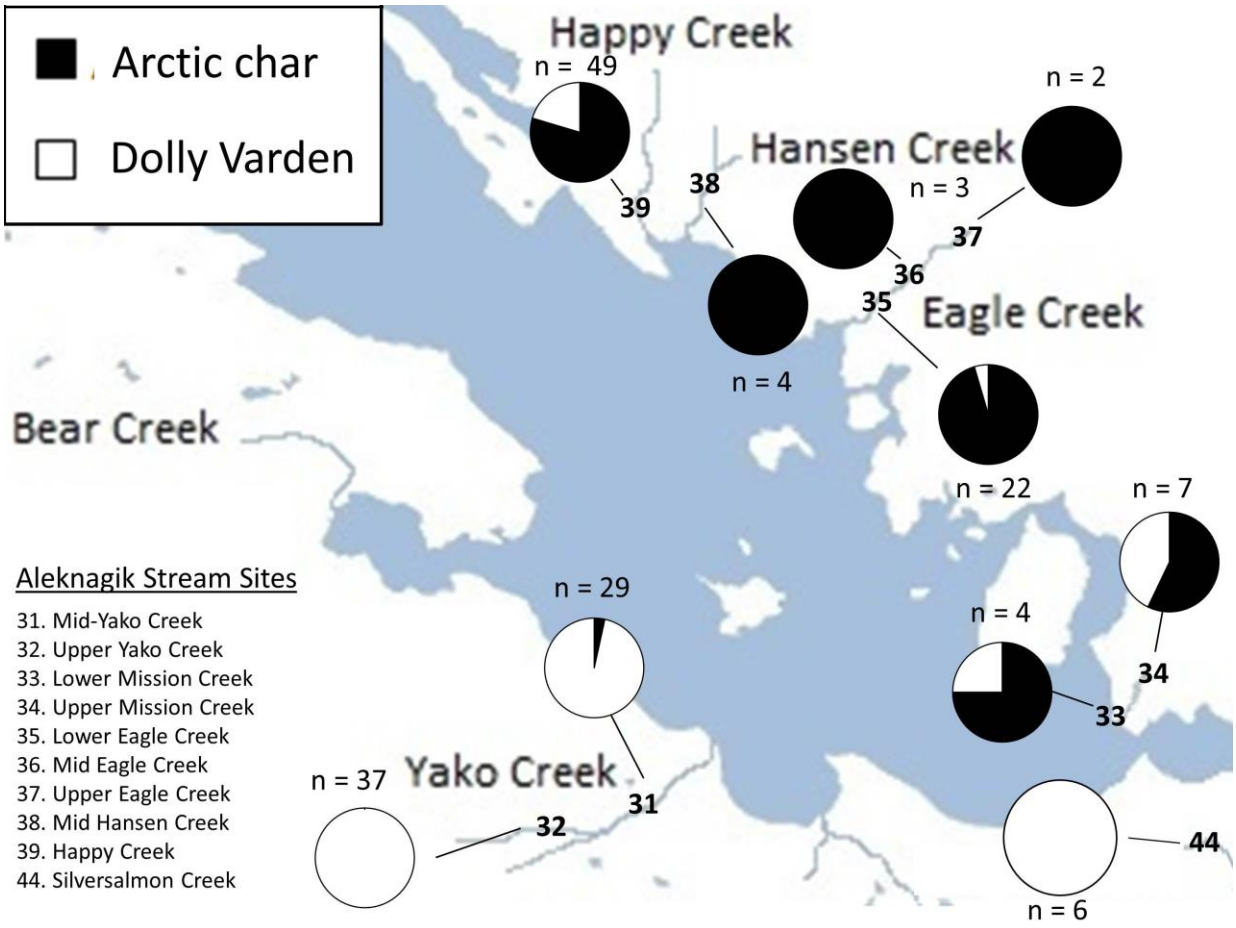

Figure 3.7: Proportion of genotypes defined by admixture (Q_{DV}) values corresponding to Arctic char (*Salvelinus alpinus*) and Dolly Varden (*S. malma*) from stream sites around the southern portion of Lake Aleknagik during summer 2013 (black = *S. alpinus*, white = *S. malma*). Arctic char were defined as having Q_{DV} values of ≤ 0.237, Dolly Varden were defined as having Q_{DV} ≥ 0.788, and hybrids were defined as having Q_{DV} values between 0.237 —0.788.

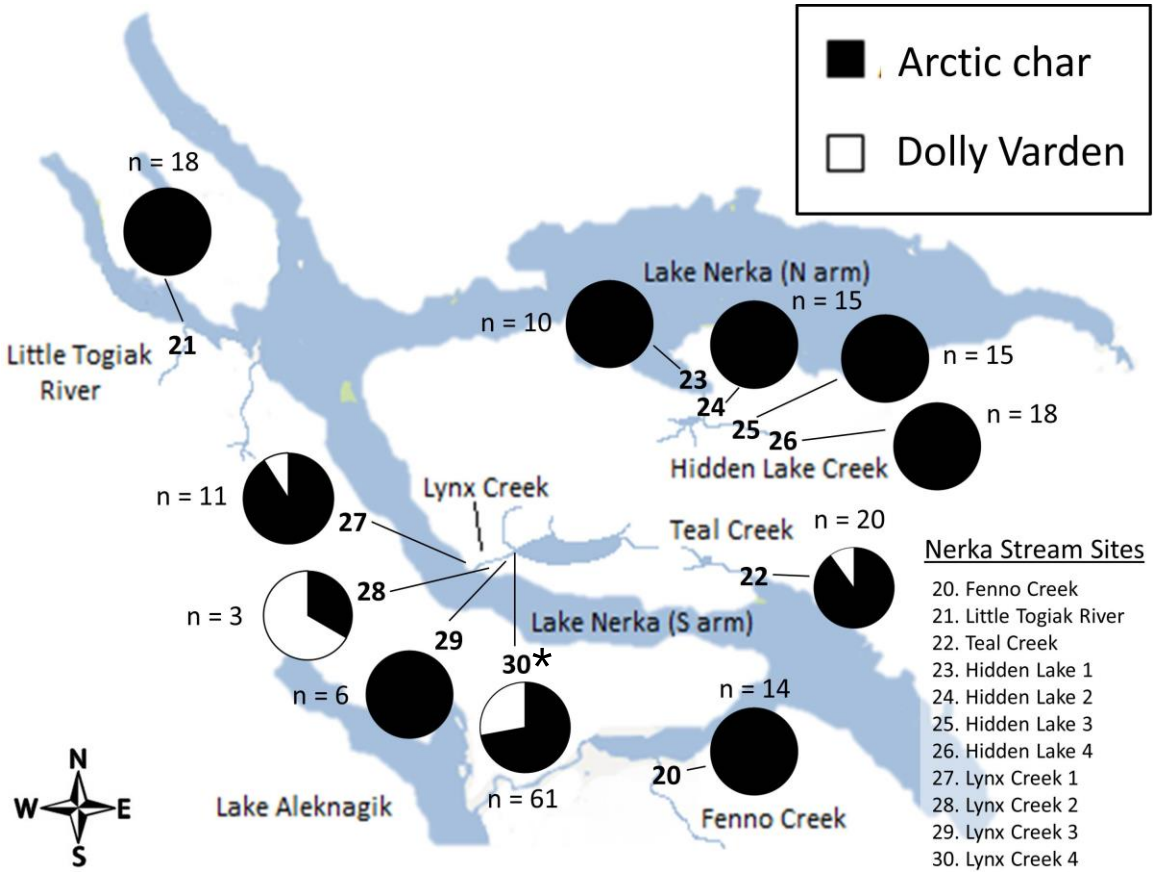

Figure 3.8: Proportion of genotypes defined by admixture (Q_{DV}) values corresponding to Arctic char (*Salvelinus alpinus*) and Dolly Varden (*S. malma*) from stream and river sites around Lake Nerka during summer 2012 (black = *S. alpinus*, white = *S. malma*). Arctic char were defined as having Q_{DV} values of ≤ 0.237, Dolly Varden were defined as having Q_{DV} ≥ 0.788, and hybrids were defined as having Q_{DV} values between 0.237 —0.788. A single putative hybrid was found at the starred site (Lynx Creek 4, site 30).

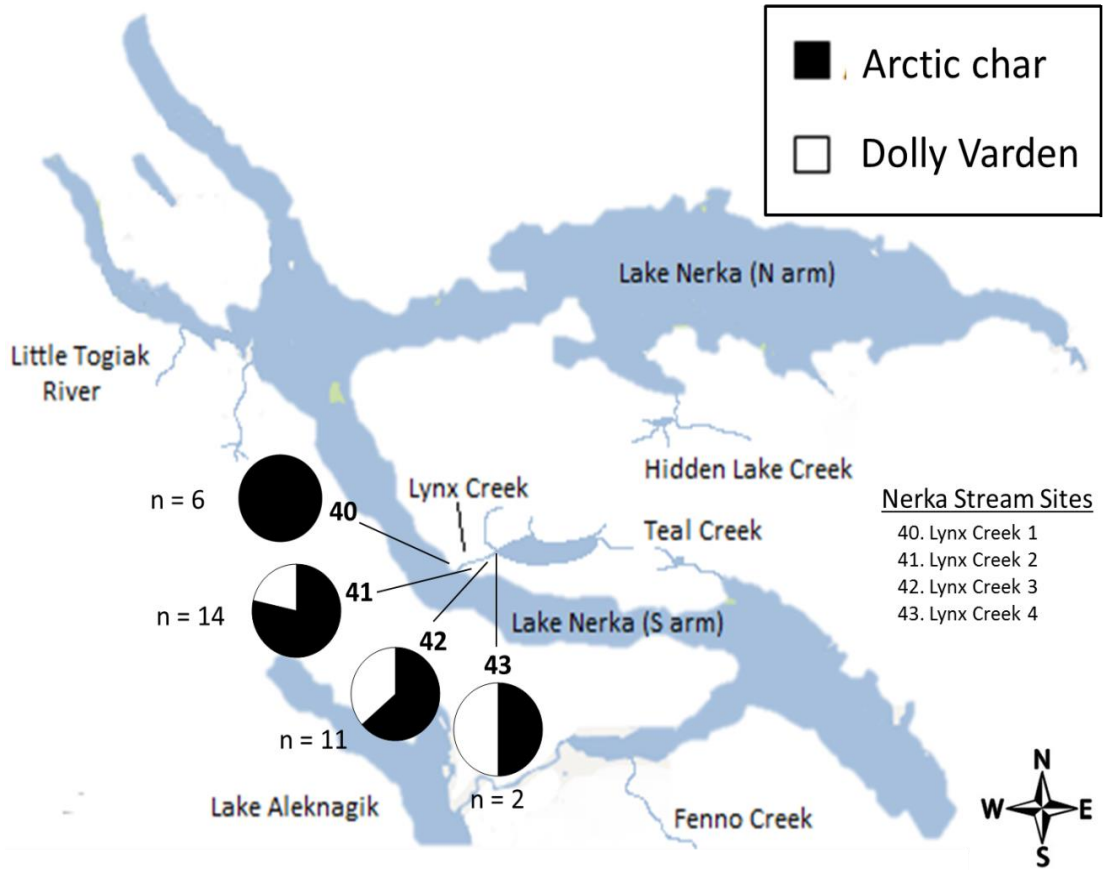

Figure 3.9: Proportion of genotypes defined by admixture (Q_{DV}) values corresponding to Arctic char (*Salvelinus alpinus*) and Dolly Varden (*S. malma*) from stream sites around Lake Nerka during summer 2013 (black = *S. alpinus*, white = *S. malma*). Arctic char were defined as having Q_{DV} values of ≤ 0.237 and Dolly Varden were defined as having Q_{DV} ≥ 0.788 while hybrids, defined as having Q_{DV} values between 0.237 —0.788, were not found.

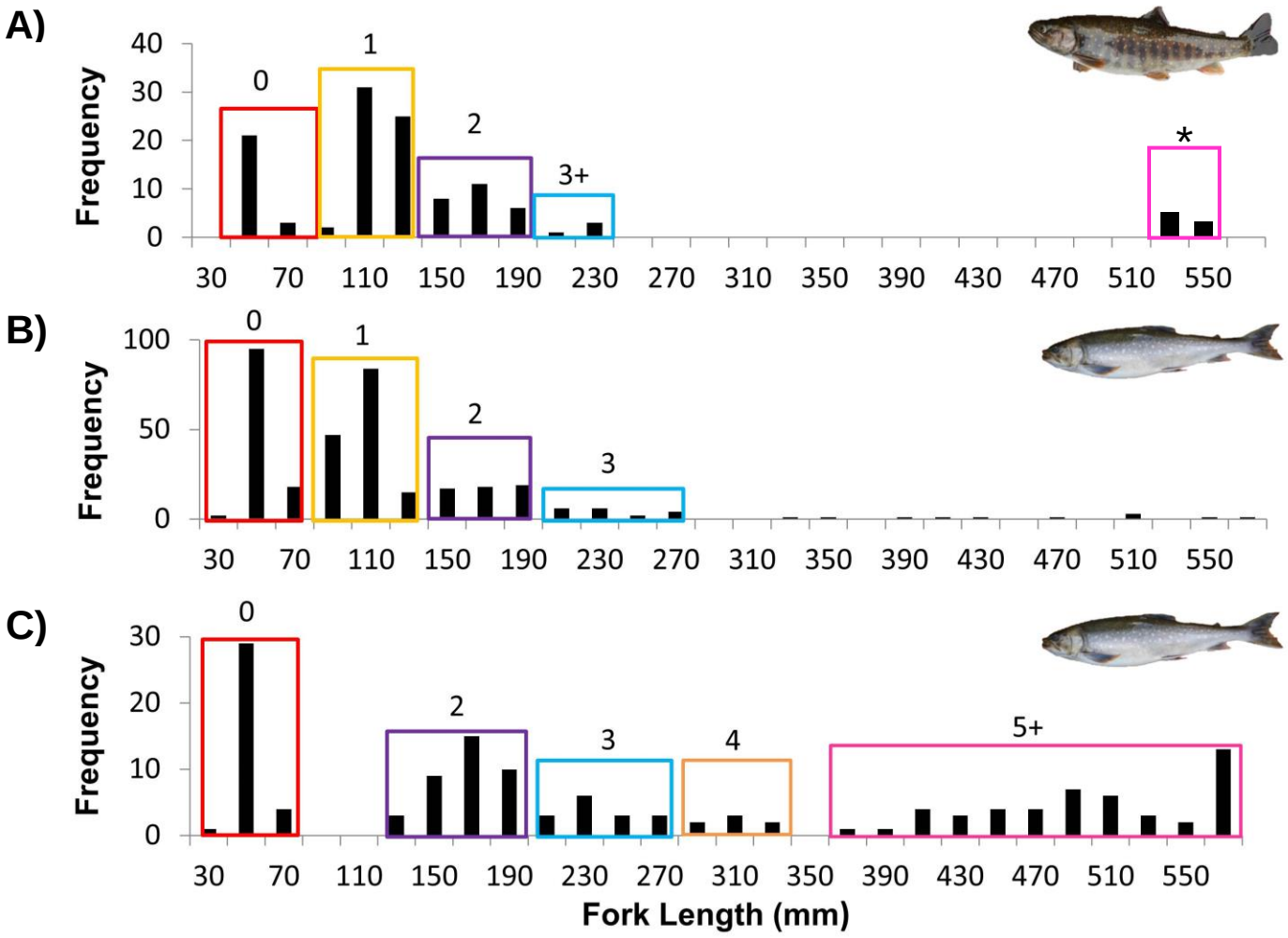

Figure 3.10: Size frequency histogram displaying approximate age classes with estimated age classes outlined by coloured boxes for Lake Aleknagik (n = 494) **A)** Dolly Varden (*Salvelinus malma*) in stream habitats with the exception of starred individuals collected from the Wood River in October **B)** Arctic char (*S. alpinus*) in stream habitats **C)** Arctic char in lake habitats. Age 0 represents young of the year fish and '+' represents all older age classes combined. Dolly Varden were not present in the lake during the July-August sampling period. Arctic char were defined as having Q_{DV} values of ≤ 0.237 and Dolly Varden were defined as having Q_{DV} ≥ 0.788 while hybrids with Q_{DV} values between 0.237 —0.788 were excluded.

3.4 Discussion

Quantification of the degree of natural hybridization between lineages allows us to evaluate the strength of reproductive and genetic barriers between lineages and define species boundaries and can further our understanding of processes that help maintain genetic distinctiveness in the presence of gene flow (Arnold 1992; Jiggins & Mallet 2000; Hagen & Taylor 2001). In many situations, parental species can maintain their genetic integrity in the face of gene flow (Hammar *et al.* 1991); however, the formation of hybrid zones with a variety of evolutionary outcomes (*e.g*. species collapse, reinforcement of reproductive isolation) can also occur (Barton 2001; Latch *et al.* 2011). Arctic char and Dolly Varden hybrids have been identified across their sympatric range from lakes in southwestern Alaska (McPhail 1961; Taylor *et al.* 2008), but the lack of a robust genetic survey across multiple lake and stream microhabitats limited the ability to properly address the hybridization issue between the species. As Arctic char and Dolly Varden are considered sister species and hybridization occurs frequently in char, I initially predicted that hybrids would be more prevalent in sympatric systems and that a hybrid zone between the species may exist in the Wood River basin due to intermediacy in normally discrete morphological counts (McPhail 1961; Taylor *et al.* 2008). In this chapter I have addressed the limitations of the previous studies in two southwestern Alaskan lakes and find evidence supporting much lower levels of hybridization between the two species than previously shown when using similar genetic methods to Taylor *et al.* (2008). Understanding the factors which underlie low levels of natural hybridization may help us to resolve important steps in the divergence of Arctic char and Dolly Varden as models in speciation.

3.4.1 Evidence for distinct gene pools between species

The microsatellite data revealed significant differentiation in pairwise F_{ST} between sympatric Arctic char and Dolly Varden samples from the Wood River Lake system and the degree of differentiation in sympatry was equivalent to, or higher than, divergence seen between either species and the and allopatric populations (i.e., between Dolly Varden in sympatry and allopatric Arctic char and *vice versa*). While Arctic char and Dolly Varden clearly separated into two distinct genetic clusters at *K*= 2, a model supporting two subpopulations of Dolly Varden was the most supported genetic structure in the 2013 samples from Lake Aleknagik (i.e., in Yako Creek). This result suggests that Dolly Varden may not disperse far from their presumably natal streams, but more sampling is required to test this idea rigorously. Spatial population genetic structure has also been observed in stream-resident Dolly Varden populations from tributaries in central Hokkaido, Japan and has been associated with the presence of physical barriers (Koizumi & Maekawa 2004; Koizumi 2011). Yako Creek in particular had high habitat complexity with abundance of fallen woody debris and boulders (Pess 2009) which could act as physical barriers isolating pockets of individuals. This has important implications for hybridization as Dolly Varden may be less likely to move from stream habitats to the spawning grounds of Arctic char on the lake beaches (see below). By contrast, across the two interconnected lakes, Arctic char populations had a higher degree of genetic homogeneity as evidenced by a population cluster of one; this suggests that there is extensive gene flow among all Arctic char from different areas both among stream and lake habitats.

3.4.2 Hybridization levels

My results strongly suggest that contemporary hybridization between Arctic char and Dolly Varden is very infrequent in the Wood River Lake system. First, without using any prior designation of species or providing any information collected from different microhabitats (e.g., same site in a particular stream), analyses in STRUCTURE supported the existence of two highly distinct genetic clusters identified as Arctic char and Dolly Varden and there are at least two known diagnostic microsatellite markers (*Sfo18* is also diagnostic between the species – see Taylor *et al.* 2008). These distinct groups were further supported in the FCA projection as Arctic char and Dolly Varden from the Wood River Lake system clustered closely with their respective allopatric populations and separately from one another. Moreover, the FCA projection identified few, if any, obvious hybrid samples that fell into intermediate regions between the two clusters.

Using comparable methods to Taylor *et al.* (2008), my genetic survey and STRUCTURE analysis indicated that the level of hybridization between Arctic char and Dolly Varden in the Wood River Lake system is minimal (0.4%) and much lower than that reported by Taylor *et al.* (2008, 7%). Subsequent analyses with NEWHYBRIDS supported the presence of later-generation hybrids (up to 7% in Lake Aleknagik); however, the proportion of F_1 and F_2 generation hybrids in the Wood River Lake system was still minimal (2% in Lake Aleknagik and 0% in Lake Nerka). The lower level of hybridization reported in my study could be a result of increased sample size (N = 720 vs.105), additional microsatellite DNA markers used (13 loci vs. nine), a greater range of microhabitats sampled (20 discrete streams and various lake sites vs. six sites total) and more intensive top-to-bottom sub-sampling of streams in my study which should have facilitated a more representative sample of genetic diversity in Wood River Lake system. The difference observed in the level of hybridization between the two studies could also result from the five year gap between studies as temporal fluctuations in environmental conditions across years can influence hybridization levels or the fitness of hybrids (Arnold 1997; Chenuil *et al.* 2000).

Low hybridization levels between the species in the Wood River basin may be comparable to other nearby systems containing sympatric populations of Arctic char and Dolly Varden. For example, in the nearby Iliamna Lake system, located approximately 85 km east of the Kvichak River, Taylor *et al.* (2008) found no evidence of F_1 hybrids between the species, but classified approximately 10 out of 65 individuals (15%) through admixture (*Q*) values as post-F_1 hybrids. Similar to the findings of this study, the high number of putative later-generation hybrids can perhaps be explained by low sampling site diversity and sample size. Only two discrete habitats were sampled from Iliamna Lake, one a series of spring-fed ponds (Pedro Ponds) comprised exclusively of small-bodied Dolly Varden and a beach site from Iliamna Lake proper (Taylor *et al.* 2008). Given that Iliamna Lake is over 2,600 km^2 and only a small proportion of the genetic diversity of char was previously analyzed (Taylor *et al.* 2008), I suggest that a more robust genetic survey across the wide range of microhabitats present within the Iliamna Lake system would produce similar results, i.e. low hybridization levels; to that seen in the Wood River Lake system.

In contrast to other sympatric char systems where hybridization is often a regular occurrence e.g., Arctic char x lake char (Hammar *et al.* 1989), Arctic char x brook trout (Hammar *et al.* 1991), Dolly Varden x bull trout (with 0-25% of individuals presumed to be of hybrid origin, Baxter *et al.* 1997, Redenbach & Taylor 2003), the level of hybridization between Arctic char and Dolly Varden in the Wood River Lake system is unexpectedly low, especially considering their sister species status. While other sympatric char systems provide evidence of spatial overlap of species during breeding (e.g. bull trout spawning in upstream habitats where Dolly Varden predominate, Bustard & Royea 1995, but see Hagen & Taylor 2001), Arctic char and Dolly Varden appear to be longitudinally segregated along streams and presumably spawn in different habitats at slightly different times of the year. Given that no direct observations of hybridization between Arctic char and Dolly Varden currently exist (Reist & Sawatzky 2010), low levels of interspecific competition for spawning sites and pre-mating spatial segregation may be important aspects of pre-mating isolation between the two species; however, further work is needed to formally establish the

basis and degree of pre-mating reproductive isolation between the species in sympatry.

Despite assigning approximately 7% of samples as hybrids in Lake Aleknagik, Taylor *et al.* (2008) found striking evidence for a complete absence of heterozygous individuals at a diagnostic marker (*Sfo18*). Given that species-specific alleles in heterozygous fish have been observed in hybridized stock populations of brook trout and Arctic char (Gross *et al.* 2004), Taylor *et al.* (2008) speculated that the likely cause of this apparent lack of F_1 individuals was rarity in hybridization events because the majority of hybrids had admixture values near the boundaries in the zone of hybridity and no hybrid had a Q_{DV} value of 0.5 which is suggestive of an F_1 hybrid. They could not, however, rule out the possibility that this locus was under selection or closely linked to another locus which may bias their estimates of hybridization. To circumvent these unknowns, I used another putative diagnostic locus (*Smm-21*) which is polymorphic in Dolly Varden, but fixed in Arctic char (May-McNally, S.L. unpublished). The same absence of heterozygotes seen with *Sfo*18 was observed with *Smm-21*; therefore, it is even more unlikely that selection or linkage at diagnostic markers is underestimating current levels of hybridization. Moreover, a random sample from 2012-2013 analyzed at *Sfo18* (n = 75) showed the same discreteness in species-specific alleles and lack of heterozygotes as seen in Taylor *et al*. (2008).

The STRUCTURE analyses did not find evidence of F_1 hybrid individuals at any age class and little evidence for post-F_1 hybrids in the two lakes (~0.4 % across the two lakes). The lack of F_1 hybrids is further supported through analyses in NEWHYBRIDS. In general, hybrid zones between Dolly Varden and bull trout also show a relative paucity of F_1 hybrids relative to other genotypic classes (e.g. Redenbach & Taylor 2003). Conversely, NEWHYBRIDS suggested that post-F_1 hybrids may constitute up to 7% of the Lake Aleknagik population and that backcrossed hybrids are biased towards introgression with Dolly Varden. As NEWHYBRIDS has been shown to be more sensitive at detecting the correct proportion of hybrid classes present in a sample than STRUCTURE it could be detecting second or third generation hybrids that were classified as parental species

when using only STRUCTURE (Vähä & Primmer 2006). As the credible regions and error margins associated with each sample are accounted for differently in each program, many of the potential late-generation hybrids with borderline Q_{DV}-values nearing parental levels could have easily been misclassified by the STRUCTURE analysis, but detected by NEWHYBRIDS. Although the programs are not directly comparable (i.e., a 0.4% level of hybridization in STRUCTURE ≠ 7% level of hybridization in NEWHYBRIDS), the presence of advanced generation hybrids as suggested by my NEWHYBRIDS analyses could perhaps explain the overlap in morphology between species (*e.g.* gill rakers, pyloric caeca) observed by McPhail (1961) and Taylor *et al.* (2008) in Lake Aleknagik.

Taken together, it seems plausible that F_1 hybrids are generated very rarely in this system, but signatures of their influence may be detected through backcrossing, as suggested by the presence of post-F_1 individuals. This situation perhaps best fits the conceptual framework of Arnold's (1997) "Evolutionary Novelty" model which posits that evolutionary stable lineages can arise when environment-dependent and environment-independent selection act concurrently on hybrid genotypes and when hybrids can exhibit equivalent or higher fitness than parental genotypes. The model was derived based on observations that under natural conditions F_1 individuals are rarely formed in ecosystems without well-defined ecotones or disturbed sites that often act to promote sympatry and inter-breeding between parental species that are otherwise restricted and adapted to alternative habitats (Anderson 1948; Moore 1977). In other words, the presence of well-established habitat differences (lakes versus streams) has perhaps promoted the evolution of distinct habitat use and life history in Dolly Varden and Arctic char that limits the opportunities for hybridization especially in the absence of any obvious ecotones or disturbed habitats.

The relative rarity of Dolly Varden in the Wood River Lake system may also contribute to the low observed levels of hybridization, i.e., the level of hybridization is to some extent dependent on the relative density of parental species (see Wirtz 1999). Across the habitats surveyed, Dolly Varden were numerically under-represented in comparison to Arctic char as seen by the ~76% of samples genotyped from Lake Aleknagik that were classified as Arctic char. In Lake Nerka,

Arctic char were also the dominant species in the streams and rivers. The lower abundance of Dolly Varden in this system probably limits interspecific encounters during reproductive periods and thus may have an impact on the dynamics of hybridization and directionality of introgression (Hubbs 1955; Mayr 1963; Burgess *et al.* 2005; Lepais *et al.* 2009). Furthermore, through studies of extrinsic selection on hybrid zones, it has observed that phenotypically and physiologically intermediate hybrid individuals often occupy intermediate habitats across ecotones or clines (Arnold 1997; Fritsche & Kaltz 2000; Culumber *et al.* 2011). Although the few post-F_1 generation hybrids that were observed were scattered throughout the Lake Aleknagik system, they could show highest fitness in currently unknown, and presumably less common, intermediate habitats (e.g., stream outflow sites near the lake). If high densities of hybrid offspring were present in Lake Aleknagik, the carrying capacity of these intermediate habitats would perhaps be exceeded and extrinsic selection could act against hybrid individuals in parental environments. When the density of hybrids is low and intermediate habitat sites are available, perhaps selection against hybrids is weaker and they can better persist throughout their lifecycle. If hybrid survival is high when hybrids are rare, an evolutionary stable situation that promotes low levels of gene flow between two otherwise discrete species may develop and be potentially long-standing (Arnold 1997).

3.4.3 Spatial segregation across lake-stream microhabitats

Arctic char are the predominant and more widely distributed char species across the majority of microhabitats within Lake Aleknagik and Lake Nerka. During the summer sampling period, Arctic char were the only species collected from the lake, with younger (age 0) age classes inhabiting nearshore beach habitats and older individuals collected from a variety of depths. My spatial distribution mapping, however, also supports the presence of Arctic char in some stream habitats. The species is generally abundant from the mouth to middle regions of streams, but was also occasionally present at the top-most reaches of streams located several kilometers from the mouth.

From age class distributions, it appears that Arctic char migrate into stream habitats from the lake at a young life stage, demonstrated by a dramatic absence of age 1 Arctic char from any lake microhabitat and a high abundance of this year class near the lower reaches of streams. Although age 1 Arctic char might have been underrepresented in the gill nets and beach seine collections, it seems more plausible that they move into the small streams to exploit available invertebrate resources while still at an appropriate size for the shallow depths. Once certain size thresholds are met, estimated to be around ages 2+ or 3+ due to the sharp decline in stream habitat abundance at these age classes, Arctic char may migrate back into the lake (or deep rivers) where they remain at least until the time when large numbers of spawning sockeye salmon (*Oncorhynchus nerka*) enter streams. Large Arctic char were observed swimming back into the mouths of larger streams, perhaps as a transient habitat shift, in July-August to capitalize on pulses of sockeye salmon eggs that become available when the salmon begin spawning.

At the time of sampling, Dolly Varden were only found in stream microhabitats and were in highest abundance in the upper reaches of streams, with the exception of a few Dolly Varden dominated streams (e.g., Silversalmon creek). A complete range of age classes, from young-of-the-year (YOY) fish to those exhibiting spotting colours characteristic of maturing fish were also present in stream microhabitats. When plentiful, Dolly Varden could be found feeding near the mouths of streams on sockeye salmon eggs, a behaviour which may relate to the daily upstream-downstream migration seen with juvenile coho salmon (*Oncorhynchus kisutch*) in Lake Aleknagik (Armstrong & Schindler 2013). Small YOY Dolly Varden were also observed in upper reaches of certain streams such as Yako Creek which suggests that adult spawning areas extend well upstream. Anadromous Dolly Varden were excluded from this study due to their absence in the system during summer periods, but are presumed to spawn in the Wood River or near the stream mouths of Lake Aleknagik's creeks such as Yako and Happy creeks (C. Schwanke, ADF&G, personal communication). Thus, it seems possible that both young anadromous and small-bodied non-anadromous Dolly Varden co-exist in similar stream microhabitats until they adopt their alternative life histories.

Arctic char and Dolly Varden overlap spatially in streams, but my results indicate that this sympatry occurs primarily with Dolly Varden and immature Arctic char or in periods when sockeye salmon eggs are plentiful. Previous work in the Karluk River system, located just southeast of my study area, further supports my findings that Dolly Varden prefer stream habitats while Arctic char favour the lake (Delacy & Morton 1943). Consequently, I suggest that well-established niche segregation limits the potential for hybridization events to occur during spawning periods in Dolly Varden and Arctic char and in sympatric char more generally. The spatial distribution of smaller Dolly Varden in upper stream reaches and larger Arctic char in the remaining available niche space mirrors the distribution observed with other sympatric char such as Dolly Varden and bull trout (*Salvelinus confluentus*). In northwestern British Columbia where they occur in sympatry, Dolly Varden are apparently limited to stream habitats while bull trout are adfluvial; they spatially overlap with Dolly Varden in many tributaries as juveniles in their first year or two of life, but migrate to lakes as older juveniles to feed before returning to streams to spawn as adults (Hagen & Taylor 2001). Although bull trout and Dolly Varden do not appear to be adapted to alternative trophic or habitat resources while sympatric in streams (Hagen & Taylor 2001), the extent of such differentiation between Arctic char and Dolly Varden is unknown, but could contribute to resource driven ecological segregation in these fish. Preliminary diet analyses reveal that Arctic char have higher numbers of salmon eggs, a calorie rich food source, in their stomach than do similar sized Dolly Varden feeding in the same microhabitat (May-McNally, S.L., unpublished data) suggesting that Arctic char may be able to outcompete Dolly Varden in certain situations and environments. This finding could help explain the current distributions and the predominance of Arctic char in the Wood River Lake system, but further work (e.g. detailed diet analysis, stable isotopes) is needed to examine the degree of trophic niche overlap and resource partitioning between the species.

Overall, this chapter of my thesis has demonstrated that Dolly Varden and Arctic are highly distinct genetically in the Wood River Lake system. Despite their sympatry, hybridization between the two species is very rare and a high degree of

spatial segregation occurs between them at various life stages suggesting that pre-mating barriers to interbreeding are important in this system.

Chapter 4:

General conclusions

4.1 Summary of findings

My thesis produced two main findings:

1) I provided strong support for three genetically distinct sympatric Arctic char (*Salvelinus alpinus*) morphs ('large', 'medium', and 'small'–bodied char) from a southwestern Alaskan lake showing substantial F_{ST} (up to 0.09) and differences in admixture proportions across forms. Significant associations between genetic admixture proportions and gill raker counts among body-size morphs (r = -0.73, P < 0.001) and growth trajectories further support the idea that resource polymorphism may promote genetic divergence in Holarctic populations of Arctic char.

2) My work also found that the level of natural hybridization between Arctic char and Dolly Varden (*Salvelinus malma*) across multiple microhabitats in two southwestern Alaskan lakes is much lower than estimated from earlier genetic surveys. The percentage of recent hybrid genotypes in the population was found to be less than 0.5% across lakes through STRUCTURE analyses, but up to 7% of the samples may represent late-generation backcrosses as suggested through analyses by NEWHYBRIDS. I also found that Dolly Varden char are concentrated in stream habitats while Arctic char dominated lake habitats and downstream portions of streams. Pre-mating spatial segregation in discrete habitats (lake vs. streams) may contribute to the low levels of contemporary hybridization that I have documented.

4.2 Defining a species

The diversity of Arctic char ecophenotypes and morphs has led to taxonomic uncertainty within the group and has been summarized as the 'char problem' (Nordeng 1983; Klemetsen 2010). Some authors have suggested that many of these forms are distinct species, as seen with the up to 32 taxa recognized in Europe (Kottelat & Freyhof 2007), while others promote the idea of a single species 'complex' which encompasses all the possible variants of Arctic char (McPhail 1961; Reist *et al.* 2013). The morphs of Lower Tazimina Lake showed substantial ecological differences; they feed at different trophic levels and differ in colouration and size at maturity (Woods *et al.* 2013). Genetic differentiation also exists among the morphs despite evidence for some gene flow among them and was correlated with morphological trait differences (e.g., gill rakers) associated with divergence in trophic ecology (May-McNally *et al.* 2014)

Although there is no evidence of behavioural reproductive isolation between large and small forms and there does seem to be some gene flow between them, they do exhibit substantial ecological and genetic divergence (Woods *et al.* 2013; May-McNally *et al.* 2014). The question of when these ecological and genetic differences "cross the line" to become species-level differences is important topic in evolutionary biology. This issue is even more apparent in some other systems where Arctic char morphs show complete reproductive isolation in sympatry (e.g., Westgaard *et al.* 2004). Reist (2013) argued that Arctic char is probably best described as a species 'complex' given that the various forms reflect contemporary-scale differentiation caused by local habitat differences, morphological and diet driven trophic polymorphism and not deeper evolutionary-scale differentiation driven by large-scale geomorphological events such as glaciation. This characterization seems to apply to the Lower Tazimina Lake and Lake Hazen (Nunavut) morphs which showed a lack of complete reproductive isolation, but exhibited morphological differences and trophic polymorphisms. Further, trophic differentiation in Arctic char may be possible in relatively short evolutionary time-frames, as suggested by translocation experiments of lacustrine populations in Maine where Arctic char

shifted from their original food source to a novel diet across only ~ 25 years or about 5-6 generations (Michaud *et al.* 2008). In addition to changes in diet, the Arctic char accumulated significant phenotypic differences suggested to have arisen as a result of this trophic shift (Michaud *et al.* 2008). Rapid phenotypic change accompanying trophic specialization is not unique to char and has also been observed in Galapagos finches (*Geospiza* spp.) which can develop substantial phenotypic variability and divergence in resource use within a few generations depending on food availability (Grant & Grant 2006). Given the estimated recent time of deglaciation in the Lower Lake Tazimina area (~12 000 ─ 15 000 BP) (Stilwell & Kaufman 1996; Ramstad *et al.* 2004) and lack of complete reproductive isolation, the Lower Tazimina Lake morphs are probably best associated with local or contemporary-scale differentiation and as variants within the Arctic char species 'complex'. This argument, however, becomes more complicated in systems with morphs such as the 'dark' and 'pale' sympatric forms of Arctic char in Gander Lake, Newfoundland, and the two morphs in Lake Aigneau, Québec, which represent populations that have diverged to a much deeper level (Reist *et al.* 1995; Gomez-Uchida *et al.* 2008; Power *et al.* 2009; Arbour *et al.* 2011; May-McNally *et al.* 2014).

Understanding the nature of species and their interrelationships is central to evolutionary biology, but is complicated in char and many other taxa by hybridization and introgression even between what are thought to be relatively divergent species. The status of Arctic char and Dolly Varden as distinct biological species has only recently become better supported with genetic data (Taylor *et al.* 2008). My study in the Wood River Lake system of Alaska provided a more robust 'acid test' of the biological species status of Arctic char and Dolly Varden in sympatry. The data presented in Chapter 3, in addition to the work of Taylor *et al.* (2008), supports the substantial genetic distinctiveness of these taxa at microsatellite DNA loci and established that the species only rarely hybridize. The general lack of hybridization between Dolly Varden and Arctic char in the Wood River Lake system is consistent with the observation that the species appear to spawn in different areas of the Aleknagik Lake watershed (Arctic char on beaches, Dolly Varden in rivers and streams) even though they clearly overlap in stream habitats as juveniles (Chapter

3). The occasional gene flow between Dolly Varden and Arctic char is likely facilitated by infrequent species encounters during spawning, perhaps to low occurrences of smaller-bodied 'sneaker' males as seen with sympatric bull trout and Dolly Varden in northwestern British Columbia (Hagen & Taylor 2001). Opportunistic sneaker behaviour can be a common evolutionary stable strategy across many species of salmonids (Hendry & Stearns 2003). The formation of what I inferred to be some post-F_1 individuals also suggests that F_1 hybrids are viable and that no post-zygotic intrinsic genomic incompatibilities exist between Arctic char and Dolly Varden (Jiggins & Mallet 2000). Following strict adherence to the original biological species concept (BSC, Mayr 1942), incomplete reproductive isolation and genomic congruence would suggest that Arctic char and Dolly Varden represent a single taxon. Recent work, however, suggests that natural hybridization can be transient and result in the reinforcement of pre-mating isolating behaviours (Arnold 1997; Gow *et al.* 2006; Hausdorf 2011). Substantial, but not necessarily complete, reproductive isolation between sympatric lineages may signify that the majority of pre- and post-mating barriers are already in place. Given environmental stability, it is expected that reinforcement through hybridization would lead to the finalization of mating barriers (Servedio & Noor 2003; Mallet 2005). It therefore seems most appropriate for taxonomic classification to follow a modified BSC as proposed by Coyne and Orr (2004) and designate evolutionary-scale lineages that border on the edge of complete reproductive isolation, such as Dolly Varden and Arctic char, as valid species (Hausdorf 2011).

4.3 Implications for conservation of char diversity

Climate change, in combination with direct human influence on ecosystems, e.g., habitat destruction, pollution, introduced species, and over-fishing, may result in novel selection pressures on many northern fishes potentially resulting in a loss of species diversity (Maitland 1995; Hoffmann & Sgro 2011; Reist *et al.* 2013). Arctic char and Dolly Varden may be particularly at risk from climate change due to the combination of a highly northern range (especially for Arctic char), long lifespans of

up to 40 years for Arctic char and 15 years for Dolly Varden, and old ages at maturation (Hendry & Stearns 2003; Berg *et al.* 2010). Under most models of climatic change, increased average water temperatures and longer seasonal periods are expected in the coming decades (Minns & Moore 1992; Reist *et al.* 2006; Hoffmann & Sgro 2011). Cold-adapted fishes with low resistance to warm temperatures such as Arctic char may not be capable of tolerating thermal stress in conjunction with increasing anthropogenic perturbation from northern development (Johnson 1980; Baroudy & Elliott 1994; Reist *et al.* 2013). Further, Arctic char populations balancing multiple stressors may be even more susceptible to the spread of parasites and diseases (Reist *et al.* 2006). As Arctic char are a common subsistence species for Aboriginal peoples in many northern regions including Alaska and Dolly Varden can be harvested year-round for subsistence or through commercial fisheries, an increase in parasite and disease load may have a negative impact on fish quality for consumption (Armstrong & Morrow 1980; Johnson 1980; Gordeeva *et al.* 2010).

Climate change may be especially critical in fragile Arctic and sub-Arctic lacustrine systems containing landlocked or sympatric morphotypes of Arctic char. These typically species-poor ecosystems may be especially impacted by the reduction or loss of species incapable of handing or dispersing from the changing thermal environment (Reist *et al.* 2013). Reduction in relative species abundance and increased seasonal length of may impact food web dynamics and composition of other species and prey abundance at different tropic levels (Reist *et al.* 2006, 2013). The Arctic char morphs present in Lower Tazimina Lake consumed discrete diets (Woods *et al.* 2013) and resource competition between the large and small forms is likely to be impacted if conditions do not remain stable. Additionally, Arctic char may be spatially limited to deeper, cooler regions of the lake (predicted to be as deep as >30 m) if average water temperatures continue to rise (Power *et al.* 2012). Currently in Lower Tazimina Lake, the large and small morphs segregate between the limnetic and benthic regions of the lake as predicted through stable isotope differences (Woods *et al.* 2013). If the uppermost regions of the lake (in particular the shallow limnetic zone) increase in temperature the small morph may be

displaced and potentially lost. As these morphs represent a unique component of the genetic diversity of Arctic char present in Alaska, protection from additional human-induced stressors that might exacerbate the effects of climate change and monitoring of these populations in response to increased warming may be essential for their survival. Environmental changes could also result in increased gene flow amongst morphs and change the genetic structure of the complex in Lower Tazimina Lake (Taylor *et al.* 2006; Vonlanthen *et al.* 2012).

For sympatric populations of Arctic char and Dolly Varden in Alaska, climate change may result in alterations to the frequency and therefore the pattern of hybridization (Hoffmann & Sgro 2011). Disturbance of stable climate regimes could cause the balance between the species to shift and may result in changes in ecological selection against F_1 hybrids (Gilman & Behm 2011). Given that disturbed environments are often more favourable for hybridization, the survival and viability of F_1 hybrids may be facilitated through climate change (Seehausen 2006; Seehausen *et al.* 2008). The extreme situation would be the breakdown of pre-mating isolating barriers between the two species, potentially leading to the formation of a hybrid swarm and substantial genetic admixture between the species i.e., 'speciation in reverse' as observed with sympatric pairs of threespine sticklebacks (*Gasterosteus aculeatus*) of Enos Lake on Vancouver Island (Taylor *et al.* 2006) and Lake Victoria cichlids (Seehausen *et al.* 1997, 2008). Although the stickleback and cichlid divergences are probably less than 15,000 years old while Dolly Varden and Arctic char probably diverged from one another in pre-Wisconsinan glaciation times (i.e., ~ 1 million years ago, Grewe *et al.*, 1990), McPhail (1961) proposed that pre-mating isolation is also central to the genetic discreteness of Dolly Varden and Arctic char in sympatry. Some breakdown of spatial mating isolation between Arctic char and Dolly Varden may be possible if warming of once thermally suitable spawning grounds leads to a reduction of available spawning sites in the lake, increasing the odds of interspecific interactions during mating periods if the streams and rivers become more thermally suitable for Arctic char. In addition to spatial shifts in spawning sites, warmer and more sporadic lake and ocean (in terms of anadromous Dolly Varden) temperatures could affect the timing and success of spawning events (Mieszkowska

et al. 2006; Sundby & Nakken 2008). Lake Aleknagik Arctic char and Dolly Varden spawn at slightly different, but nevertheless overlapping, times in the autumn (September-October for Arctic char and October-November for Dolly Varden, C. Schwanke, ADF&G, personal communication) and changes in climate could result in a higher degree of overlap and thus increase the potential for hybridization. To conserve the most char genetic diversity, continual monitoring of the Arctic char and Dolly Varden populations of the Wood River Lake system would be required to assess the degree of impact from climate warming. Reducing additional human induced habitat destruction may also help to mitigate any effects of climate change by limiting additional stressors that may act alone or synergistically to promote a species reversal for sympatric populations (Hubbs 1955; Taylor *et al.* 2006; Vonlanthen *et al.* 2012).

4.4 Concluding thoughts and future directions

My research builds on previous work surrounding sympatric Arctic char morphs and sympatric populations of Arctic char and Dolly Varden from lakes in southwestern Alaska and has firmly established that the two char are strikingly genetically distinct in sympatry. I have also provided better information on the spatial segregation of the two char that may contribute to their high degree of inferred reproductive isolation. Taken together, my work contributes to a better understanding of the evolutionary history of Arctic char and it fits broadly into a continuum of evolutionary scenarios from the origin of resource polymorphism and divergence of sympatric forms to species-level interactions. Further, as Arctic char are an important food and recreational source for many people in the north, understanding the evolutionary processes underlying adaptive divergence may facilitate predictions about how they will respond to habitat loss or climate change; knowledge that can result in more nuanced and detailed conservation strategies.

4.4.1 Genetic basis of divergence

Using the recent advancements in genome-level technology, the Lower Tazimina Lake morphs may represent a system in which to further study genetic changes surrounding the early stages of intraspecific sympatric ecological divergence. For the sympatric Arctic char morphs, it remains unknown whether differences in particular gene families also exist between the large, medium and small forms– knowledge of which could provide important clues about the type of genetic change which may have occurred during the first stages of adaptive divergence. Genetic differences between intraspecific morphs associated with the earliest stages of divergence could be identified given that their genomes have not yet been entirely confounded by further genetic differences associated with complete divergence which can remove so-called genomic "islands of speciation" (Bernatchez *et al.* 2010). The Lower Tazimina Lake morphs therefore represent ideal organisms in which to conduct a transcriptome analysis targeting functional and regulatory genes that differ between the two or potentially three growth forms (Renaut *et al.* 2010). By taking samples of various tissues from juvenile and mature large, medium and small-bodied morphs in Lower Tazimina Lake, the extracts of total RNA could be reverse-transcribed to create cDNA libraries, which in turn, could be used to probe a 16,006 cDNA microarray originally designed for the Atlantic salmon (*Salmo salar*) – a microarray proven to be a successful substitute for other Salmonidae species without a fully sequenced genome such as lake whitefish (e.g., *Coregonus clupeaformis*, Bernatchez *et al.* 2010). Using the information from the microchip array one could test for differences in gene expression (up-regulation or parallel expression) between large, medium and small morphs and contrast age and tissue type. This could identify genes or gene families important in the early stages of divergence and whether they are associated with known ecological differences between alternative Arctic char growth forms.

4.4.2 Basis of reproductive isolation: ecology and life history

By conducting a robust genetic survey of sympatric populations of Arctic char and Dolly Varden to better estimate the degree of admixture, my data clearly support the hypothesis that hybridization between the species is rare (given by the complete lack of F_1 hybrids) in the Wood River Lake system and that they remain highly distinct across various alternative microhabitats. This result supports previous, less extensive work by Taylor *et al.* (2008) and the separate biological species designation of Arctic char and Dolly Varden. These data indicate that there is simply no basis for the contention that the separate species status of Dolly Varden is "questionable" based on the sharing of some mtDNA haplotypes as suggested by Brunner *et al.* (2001).

With the addition of genome-wide genetic assays, a more detailed analysis of the level of hybridization, admixture and population structure would be possible. Although the species show some degree of spatial segregation, fine-scale spatial mapping of individuals through GIS and/or PIT tagging could be used to access the degree of niche and resource overlap. As the movement and diet of older Arctic char (> 300 mm) in Lake Aleknagik has been previously studied (McBride 1980) and it was found that the species largely capitalizes on sockeye salmon smolts, it would be informative to analyze diets across both lake and stream habitats where the two species co-exist and at younger life stages when the gape size of their mouths are too small to consume smolts. Interestingly, a preliminary diet analysis of the species in streams has suggested that Arctic char and Dolly Varden consume different amounts of sockeye salmon eggs (with Arctic char appearing to consume more) which could be related to differences in gape sizes or competitive interactions as seen other studies of char (Baxter *et al.* 1997; Hagen & Taylor 2001). Competitive exclusion may help to explain the current spatial distribution of char in Lake Aleknagik, where Dolly Varden are generally restricted to upstream areas of streams and Arctic char to lakes, and why the prevalence of Dolly Varden is lower than Arctic char in certain stream habitats during the summer months.

My results, in combination with previous work by McPhail (1961), suggest that Arctic char and Dolly Varden of the Wood River Lake system possess alternative life history patterns. In Alaska, Arctic char are presumed to be non-anadromous, lake-resident fish (Johnson 1980; Denton *et al.* 2010), and Dolly Varden are presumed to show both anadromous and non-anadromous life histories in Lake Aleknagik (C. Schwanke, ADF&G, personal communication), but these characterizations have not yet been explicitly tested in Wood River Lake system char using otolith chemistry analyses (Limburg 1995; Kraus & Secor 2004; Lowe *et al.* 2010).

Using genetic methods, my results agree with and extend previous work suggesting that the evolution of alternative sympatric Arctic char ecophenotypes can be associated with, and may indeed promote, reproductive isolation as a component of ecological speciation. Additionally, I found contemporary levels of natural hybridization between sympatric Arctic char and Dolly Varden to be minimal, likely reflecting the evolution of strong pre-mating barriers to reproduction. In conclusion, my thesis highlights the complex evolutionary history and genetic diversity of Arctic char in Alaska - an understudied portion of its range.

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Appendix A Chapter 2

Table A.1: Genetic diversity values for Lower Tazimina Lake morphs and four populations of Arctic char (*Salvelinus alpinus*) from southwestern Alaska: sample codes; *N*, number of samples; *A*, number of alleles per locus; A_R, Allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; *P* (HW), probability of departure from HWE (p≤ 0.0185).

	Sco 220	***Sco 200***	***Smm 22***	***Sco 215***	***Ssos 1456***	***Otsg 253b***	***Smm 24***	***Omm 1105***	***Otsg 83b***	***Smm 17***	***Sco 216***
Lower Tazimina Lake											
N	89	89	89	89	90	90	90	90	90	90	89
A	30	20	20	3	8	16	22	16	22	11	27
A_R	30	20	20	3	7.9	16	21.9	16	22	11	27
Ho	0.966	0.899	0.888	0.438	0.344	0.811	0.856	0.756	0.956	0.633	0.910
H_E	0.947	0.890	0.920	0.493	0.597	0.881	0.925	0.873	0.923	0.746	0.900
F_{IS}	-0.021	-0.01	0.035	0.112	0.424	0.080	0.076	0.135	-0.035	0.152	-0.012
P(HW)	0.242	0.363	0.170	0.248	**0***	**0.0075***	0.042	0.028	0.084	**0***	0.988
Large Morph											
N	31	31	31	31	31	31	31	31	31	31	31
A	20	11	11	2	3	11	11	11	11	6	12
A_R	20	11	11	2	3	11	11	11	11	6	12
Ho	1	0.935	0.903	0.323	0.258	0.742	0.742	0.742	0.935	0.645	0.935
H_E	0.932	0.867	0.881	0.444	0.286	0.855	0.864	0.845	0.866	0.691	0.875
F_{IS}	-0.074	-0.081	-0.026	0.277	0.099	0.134	0.143	0.124	-0.082	0.087	-0.071
P(HW)	0.750	0.425	0.639	0.209	0.584	0.213	0.347	0.389	0.618	0.189	0.708

	Sco 220	*Sco 200*	*Smm 22*	*Sco 215*	*Ssos 1456*	*Otsg 253b*	*Smm 24*	*Omm 1105*	*Otsg 83b*	*Smm 17*	*Sco 216*
Intermediate Morph											
N	18	18	18	18	19	19	19	19	19	19	19
A	14	12	13	2	4	10	15	11	14	7	14
A_R	14	12	13	2	3.95	9.89	14.63	10.84	13.68	6.89	13.58
Ho	0.944	0.944	0.944	0.667	0.526	0.789	0.842	0.842	0.947	0.579	0.895
H_E	0.921	0.903	0.924	0.514	0.585	0.879	0.923	0.896	0.905	0.686	0.863
F_{IS}	-0.027	-0.047	-0.023	-0.308	0.102	0.104	0.09	0.062	-0.049	0.166	-0.034
P(HW)	0.172	0.702	0.637	0.343	0.675	0.193	0.662	0.447	0.865	0.0756	0.532
Small Morph											
N	40	40	40	40	40	40	40	40	40	40	39
A	30	14	19	3	7	11	21	10	21	9	24
A_R	29.7	13.9	18.9	3	6.9	11	20.7	10	20.9	9	24
Ho	0.950	0.850	0.850	0.425	0.325	0.875	0.950	0.725	0.975	0.650	0.897
H_E	0.963	0.879	0.932	0.512	0.350	0.843	0.917	0.834	0.943	0.685	0.930
F_{IS}	0.014	0.034	0.089	0.172	0.071	-0.0380	-0.037	0.133	-0.034	0.144	0.035
P(HW)	0.841	0.314	0.075	0.265	0.164	0.415	0.468	0.253	0.670	**0***	0.459
Caribou Lakes											
N	25	25	25	25	24	25	25	25	25	25	24
A	10	13	12	2	3	9	9	2	9	4	6
A_R	9.96	12.72	11.88	2	3	8.84	8.92	2	9	4	6
Ho	0.920	0.800	0.760	0.400	0.583	0.720	0.760	0.080	0.800	0.480	0.167
H_E	0.87	0.84	0.908	0.372	0.582	0.745	0.83	0.075	0.887	0.552	0.782
F_{IS}	-0.057	0.048	0.163	-0.076	0.039	0.03	0.091	-0.021	0.099	0.127	0.783
P(HW)	0.0981	0.666	0.0706	1	0.706	0.603	0.315	1	0.606	0.385	**0***
Summit Lake											
N	59	59	59	59	59	59	59	59	59	57	59

	Sco 220	***Sco 200***	***Smm 22***	***Sco 215***	***Ssos 1456***	***Otsg 253b***	***Smm 24***	***Omm 1105***	***Otsg 83b***	***Smm 17***	***Sco 216***
A	10	7	14	1	2	6	8	8	6	7	15
A_R	10	7	14	1	2	5.97	7.97	8	5.97	7	14.90
Ho	0.881	0.627	0.898	NA	0.373	0.678	0.644	0.729	0.780	0.351	0.831
H_E	0.875	0.763	0.882	NA	0.472	0.712	0.738	0.799	0.757	0.441	0.864
F_{IS}	-0.008	0.180	-0.019	NA	0.211	0.048	0.128	0.089	-0.031	0.374	0.023
P(HW)	0.0812	0.0219	0.364	—	0.166	0.120	0.093	0.078	0.888	**0***	0.0586
Iliamna Lake											
N	42	42	42	42	42	42	42	42	41	41	41
A	22	27	23	5	5	17	19	7	18	12	23
A_R	21.81	26.83	22.83	4.98	4.98	16.95	18.90	6.98	18	12	23
Ho	0.857	0.905	0.857	0.381	0.548	0.952	1	0.857	0.854	0.732	0.829
H_E	0.940	0.957	0.939	0.434	0.654	0.919	0.917	0.792	0.917	0.780	0.931
F_{IS}	0.090	0.056	0.088	0.124	0.165	-0.037	-0.092	-0.083	0.070	0.063	0.110
P(HW)	**0.011***	0.0300	0.0806	0.018*	0.086	0.290	0.997	0.829	0.570	0.186	0.106
Lake Aleknagik											
N	70	70	70	70	70	70	70	70	70	70	70
A	24	19	23	3	5	18	18	9	17	11	33
A_R	24	19	23	3	5	18	18	9	17	11	33
Ho	0.929	0.886	0.971	0.271	0.557	0.910	0.886	0.800	0.886	0.729	0.929
H_E	0.946	0.928	0.927	0.351	0.651	0.922	0.902	0.721	0.895	0.841	0.962
F_{IS}	0.019	0.046	-0.047	0.227	0.094	0.008	0.018	-0.11	0.010	0.134	0.034
P(HW)	0.420	0.076	0.275	0.109	0.316	0.085	0.143	0.699	0.279	**0.0048***	0.0344

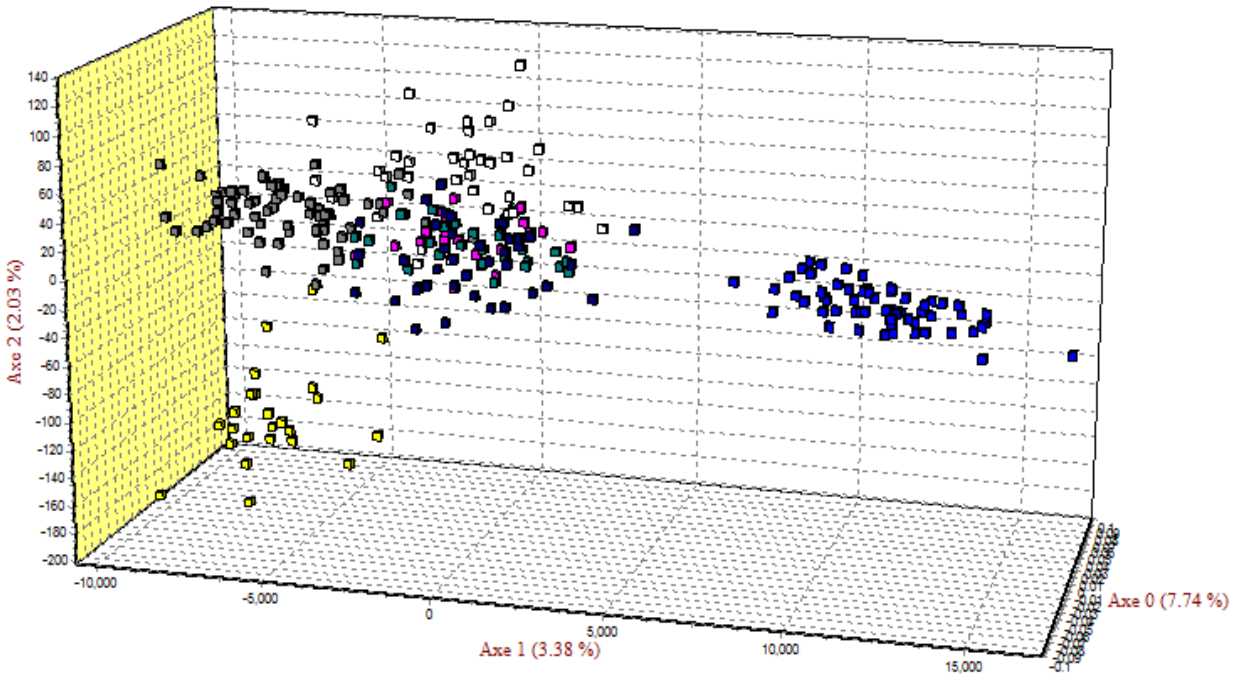

Figure A.1: Factorial correspondence analysis (FCA) of five populations of Arctic char (*Salvelinus alpinus*) from southwestern Alaska. Lower Tazimina Lake morphs have been separated into large, intermediate and small populations according to the assigned genetic groupings based on *Q* value. Populations are denoted as follows: Yellow = Caribou Lakes; Blue = Summit Lake; White = Iliamna Lake; Grey = Lake Aleknagik; Dark Blue = Small morphs; Teal = Intermediate morphs; Pink = Large morphs

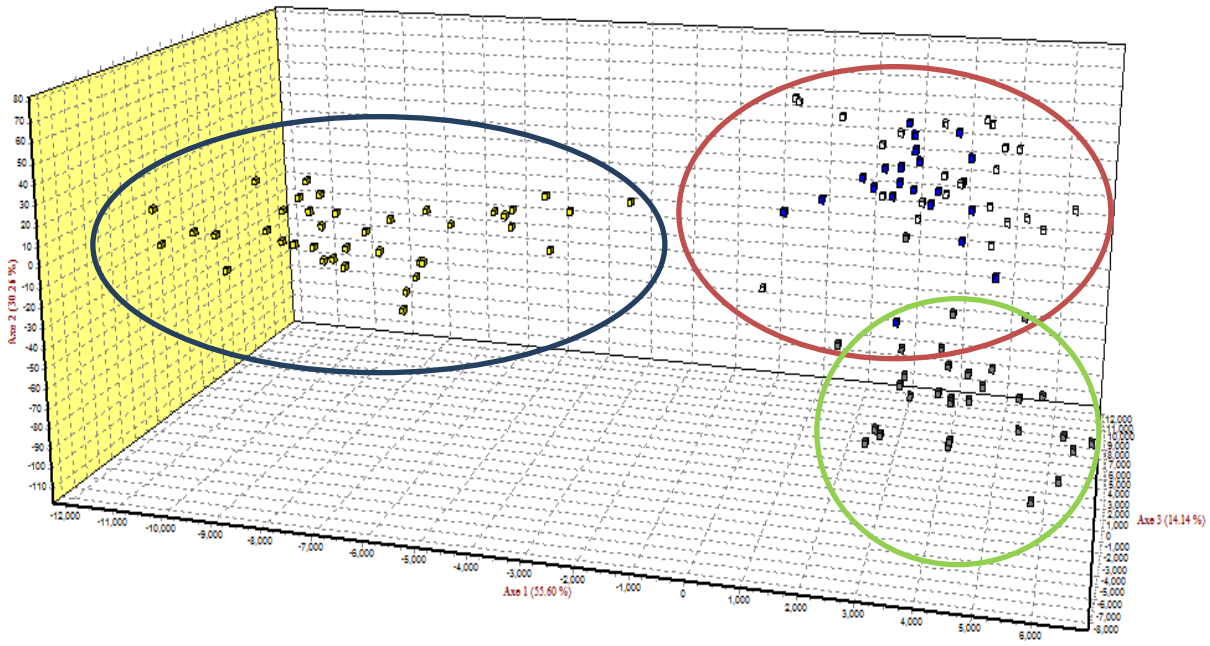

Figure A.2: Factorial correspondence analysis (FCA) of Lower Tazimina Lake, southwestern Alaska, morphs and Iliamna Lake Arctic char (*Salvelinus alpinus*). Populations are denoted by circles as follows: Yellow = Iliamna Lake; Blue and White = Large and Intermediate morphs; Green = Small morphs

Appendix B Chapter 3

Table B.1: Lake Aleknagik and Lake Nerka site names and assigned site ID numbers (July-August, 2012) with corresponding sample size, sampling locale, total number of samples collected, total samples genetically analyzed, and the given proportions of samples successfully genotyped as Arctic char (*Salvelinus alpinus*) (AC), Dolly Varden (*S. malma*) (DV) and hybrid (HYB). Arctic char were defined as having Q_{DV} values of ≤ 0.237, Dolly Varden were defined as having Q_{DV} ≥ 0.788, and hybrids were defined as having Q_{DV} values between 0.237 —0.788.

Site ID	Site Name	Locale	Total *N*	*N* Analyzed	AC/DV/HYB
1	Youth Creek	Aleknagik	24	24	24/0/0
2	Beach Seine 2N	Aleknagik	98	40	40/0/0
3	Beach Seine 2S	Aleknagik	20	16	16/0/0
4	Island off Hansen Creek	Aleknagik	35	18	18/0/0
5	Bear Bay	Aleknagik	37	30	29/0/1
6	Beach Seine 7S	Aleknagik	30	24	24/0/0
7	Whitefish Bay	Aleknagik	31	20	20/0/0
8	Wood River	Wood River	8	8	0/8/0
9	Lower Bear Creek	Aleknagik	57	15	15/0/0
10	Upper Bear Creek	Aleknagik	3	3	1/2/0
11	Lower Whitefish Creek	Aleknagik	73	31	31/0/0
12	Upper Whitefish Creek	Aleknagik	5	5	5/0/0
13	Yako Creek	Aleknagik	51	30	29/1/0
14	Lower Eagle Creek	Aleknagik	35	15	15/0/0
15	Mid Eagle Creek	Aleknagik	4	4	2/2/0
16	Upper Eagle Creek	Aleknagik	12	12	11/1/0
17	Mid Hansen Creek	Aleknagik	30	17	13/4/0
18	Upper Hansen Creek	Aleknagik	6	4	3/1/0
19	Happy Creek	Aleknagik	31	22	14/7/1
20	Fenno Creek	Nerka	14	14	14/0/0
21	Little Togiak River	Nerka	18	18	18/0/0
22	Teal Creek	Nerka	20	20	18/2/0
23	Hidden Lake 1 (lower)	Nerka	54	10	10/0/0
24	Hidden Lake 2 (lower-mid)	Nerka	15	15	15/0/0
25	Hidden Lake 3 (mid-upper)	Nerka	34	15	15/0/0
26	Hidden Lake 4 (upper)	Nerka	20	18	18/0/0
27	Lynx Creek 1 (lower)	Nerka	19	11	10/1/0
28	Lynx Creek 2 (lower-mid)	Nerka	3	3	1/2/0
29	Lynx Creek 3 (mid-upper)	Nerka	6	6	6/0/0
30	Lynx Creek 4(upper)	Nerka	93	61	43/17/1

Table B.2: Lake Aleknagik and Lake Nerka site names and assigned site ID numbers (July-August, 2013) with corresponding sample size, sampling locale, total number of samples collected, total samples genetically analyzed, and the given proportions of samples successfully genotyped as Arctic char (*Salvelinus alpinus*) (AC), Dolly Varden (*S. malma*) (DV) and hybrid (HYB). Arctic char were defined as having Q_{DV} values of ≤ 0.237, Dolly Varden were defined as having Q_{DV} ≥ 0.788, and hybrids were defined as having Q_{DV} values between 0.237 —0.788.

Site ID	Site Name	Locale	Total *N*	*N* Analyzed	AC/DV/HYB
31	Mid Yako Creek	Aleknagik	29	29	1/28/0
32	Upper Yako Creek	Aleknagik	40	37	0/37/0
33	Lower Mission Creek	Aleknagik	4	4	3/1/0
34	Upper Mission Creek	Aleknagik	8	7	4/3/0
35	Lower Eagle Creek	Aleknagik	22	21	20/1/0
36	Mid Eagle Creek	Aleknagik	4	3	3/0/0
37	Upper Eagle Creek	Aleknagik	2	2	2/0/0
38	Mid Hansen Creek	Aleknagik	4	4	4/0/0
39	Happy Creek	Aleknagik	49	49	39/10/0
40	Lynx Creek 1 (lower)	Nerka	6	6	10/1/0
41	Lynx Creek 2 (lower-mid)	Nerka	14	14	1/2/0
42	Lynx Creek 3 (mid-upper)	Nerka	6	11	6/0/0
43	Lynx Creek 4(upper)	Nerka	2	2	1/1/0
44	Silversalmon Creek	Wood River	6	6	0/6/0

Table B.3: Repeat motif, optimal annealing temperature (°C), species of origin, GenBank accession number, characteristics of marker in Arctic char (*Salvelinus alpinus*) and Dolly Varden (*S. malma*) and original reference for the 13 microsatellite markers used in this study.

Locus	Repeat motif	°C	Species of Origin	GenBank No.	Diagnostic/ Polymorphic	Reference
OMM1105	$(AGAC)_{23}$ $(GATA)_{16}$	62	*Oncorhynchus mykiss*	AF352768	Polymorphic	Rexroad *et al.*, 2002
OtsG83b	$(TGTC)_7$-N^a_{51}-$(TATC)_{34}$	52	*Oncorhynchus tshawytscha*	AF393189	Polymorphic	Williamson *et al.*, 2002
OtsG253b	$(GACA)_{10}$ $(GATA)_{14}$	52	*Oncorhynchus tshawytscha*	AF393193	Polymorphic	Williamson *et al.*, 2002
Sco200	$(ATAG)_{15}$	56	*Salvelinus confluentus*	AY88869	Polymorphic	DeHaan & Ardren, 2005
Sco202	$CTAT_{(10)}$	60	*Salvelinus confluentus*	AY88871	Polymorphic	DeHaan & Ardren, 2005
Sco215	$(GAAA)_6(GA)_6(GGGA)_1$ $(GA)_{13}$	60	*Salvelinus confluentus*	AY88884	Polymorphic	DeHaan & Ardren, 2005
Sco216	$(CAGT)_{18}(CAGG)$ $_{10}C_1(AGAT)_7$	60	*Salvelinus confluentus*	AY88885	Polymorphic	DeHaan & Ardren, 2005
Sco220	(ATAG)5$(ATAC)_2$ $(ATAG)_{15}$ $(ATTG)_1(ATAG)_{17}$	60	*Salvelinus confluentus*	AY88889	Polymorphic	DeHaan & Ardren, 2005

Locus	Repeat motif	°C	Species of Origin	GenBank No.	Diagnostic/ Polymorphic	Reference
Smm-21	$(TC)_4TTTC(TC)_{21}$	56	*Salvelinus malma*	AY327128	Diagnostic for AC[b]	Crane *et al.*, 2004
Smm-22	$(TAgA)_{19}$	55	*Salvelinus malma*	AY327129	Polymorphic	Crane *et al.*, 2004
Smm-24	$(TATC)_{33}$	56	*Salvelinus malma*	AY327130	Polymorphic	Crane *et al.*, 2004
SSOSL456	$(AC)_{12}AG(AC)_{10}$	58	*Salmo salar*	Z69645	Polymorphic	Slettan *et al.*, 1997

[a] Any nucleotide

[b] Arctic char

Table B.4: Genetic diversity values at 13 microsatelllite loci for Arctic char (*Salvelinus alpinus*) and Dolly Varden (*S. malma*) from two southwestern Alaskan Lakes with two sampling years (2012-2013) pooled. Sample codes: DV, Dolly Varden; AC, Arctic char; *N*, number of samples; *A*, number of alleles per locus; A_R, Allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient of an individual relative to the subpopulation; *P* (*HWE*), probability of departure from Hardy-Weinburg equilibrium ($p \leq 0.011$).

Locus		DV (Aleknagik)	DV (Nerka)	AC (Aleknagik)	AC (Nerka)
Sco220	*N*	109	36	383	179
	A	32	23	34	29
	A_R	3.7	5.2	33.8	28.8
	H_O	0.771	0.667	0.943	0.922
	H_E	0.939	0.944	0.95	0.937
	F_{IS}	0.18	0.297	0.008	0.016
	P(HWE)	0*	0*	0.0327	0.119
Sco200	*N*	112	35	383	181
	A	38	27	28	23
	A_R	3.7	5.3	27.3	22.7
	H_O	0.9375	0.943	0.898	0.873
	H_E	0.951	0.948	0.921	0.906
	F_{IS}	0.015	0.006	0.025	0.037
	P(HWE)	0*	0.0126*	0.0297	0.108
Smm22	*N*	112	37	383	182
	A	18	19	32	23
	A_R	3.4	5	31.4	22.9
	H_O	0.884	0.919	0.922	0.885
	H_E	0.886	0.924	0.916	0.934
	F_{IS}	0.002	0.06	-0.006	0.053
	P(HWE)	0.0089*	0.305	0.175	0.0607

Locus		DV (Aleknagik)	DV (Nerka)	AC (Aleknagik)	AC (Nerka)
Sco215	*N*	2	3	381	171
	A	3	3	5	6
	A_R	3	3	5	6
	H_O	0.5	0	0.276	0.222
	H_E	0.833	0.8	0.319	0.287
	F_{IS}	0.5	1	0.14	0.225
	P(HWE)	0.333	0.0701	0.0374	0.0013*
Ssosl456	*N*	111	37	381	183
	A	8	4	7	6
	A_R	2.5	2.8	6.9	5.9
	H_O	0.784	0.703	0.617	0.623
	H_E	0.682	0.649	0.653	0.662
	F_{IS}	-0.15	-0.084	0.055	0.059
	P(HWE)	0.0855	0.921	0.0214	0.389
Otsg253b	*N*	112	37	382	183
	A	20	17	23	17
	A_R	3.3	4.7	22.6	17
	H_O	0.893	0.811	0.88	0.847
	H_E	0.861	0.891	0.907	0.901
	F_{IS}	-0.038	0.091	0.031	0.06
	P(HWE)	0*	0.001*	0.102	0*
Smm24	*N*	101	36	348	183
	A	25	21	21	21
	A_R	3.5	5.2	21	20.8
	H_O	0.871	0.944	0.905	0.907
	H_E	0.907	0.942	0.902	0.908

Locus		DV (Aleknagik)	DV (Nerka)	AC (Aleknagik)	AC (Nerka)
	F_{IS}	0.040	-0.0030	-0.0030	-0.0050
	P(HWE)	0*	0.0218	0.658	0.987
Omm1105	*N*	108	37	383	182
	A	18	15	11	12
	A_R	3.3	4.8	10.9	11.9
	H_O	0.694	0.833	0.773	0.747
	H_E	0.861	0.906	0.752	0.803
	F_{IS}	0.194	0.081	-0.027	0.069
	P(HWE)	0*	0.242	0.311	0.369
Otsg83b	*N*	110	37	382	183
	A	39	30	28	25
	A_R	3.7	5.45	27.5	24.6
	H_O	0.927	0.865	0.893	0.869
	H_E	0.951	0.958	0.911	0.917
	F_{IS}	0.026	0.101	0.02	0.052
	P(HWE)	0*	0.0144*	0.113	0.177
Smm17	*N*	99	35	381	183
	A	15	15	14	15
	A_R	3.3	4.5	13.7	14.9
	H_O	0.485	0.6	0.822	0.754
	H_E	0.857	0.88	0.856	0.851
	F_{IS}	0.436	0.321	0.04	0.114
	P(HWE)	0*	0*	0.711	0.0882
Sco216	*N*	102	36	382	183
	A	37	21	38	36
	A_R	3.7	5.26	37.6	35.8
	H_O	0.36	0.417	0.901	0.891

Locus		DV (Aleknagik)	DV (Nerka)	AC (Aleknagik)	AC (Nerka)
	H_E	0.943	0.946	0.958	0.962
	F_{IS}	0.616	0.563	0.06	0.075
	P(HWE)	0.0043*	0*	0.028	0.0074*
Smm21	*N*	108	35	NA	NA
	A	6	4	1	1
	A_R	2.19	2.3	1	1
	H_O	0.574	0.429	NA	NA
	H_E	0.572	0.444	NA	NA
	F_{IS}	-0.003	0.035	NA	NA
	P(HWE)	0.0114*	0.261	NA	NA
Sco202	*N*	107	35	374	180
	A	7	3	17	14
	A_R	1.56	1.7	16.9	14
	H_O	0.28	0.314	0.797	0.806
	H_E	0.275	0.273	0.822	0.838
	F_{IS}	-0.019	-0.154	0.03	0.038
	P(HWE)	0.157	1	0.439	0.0014*

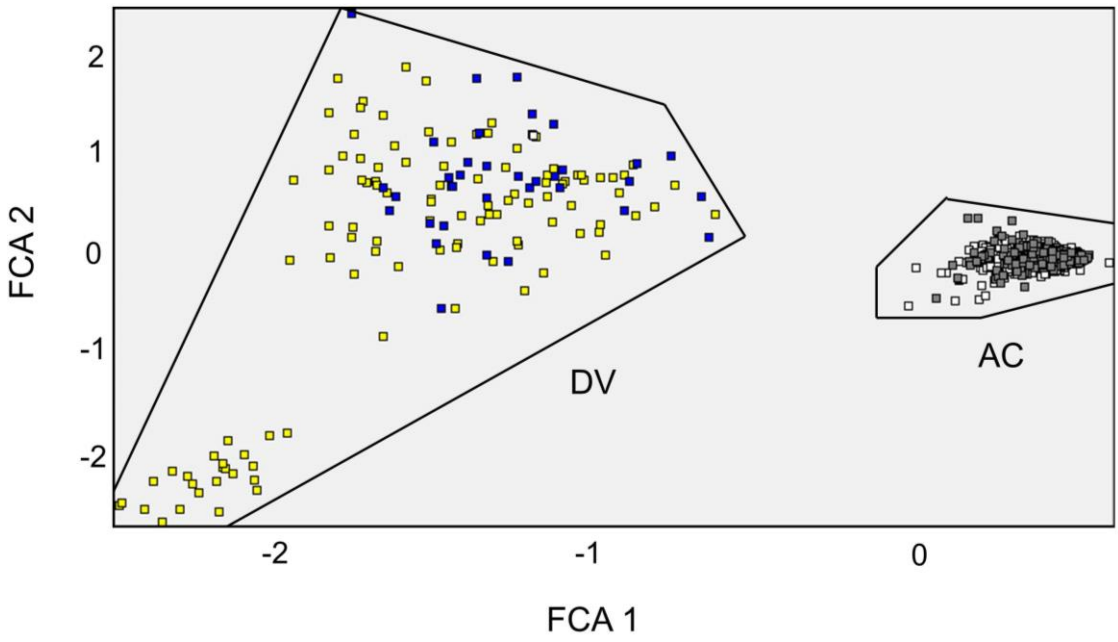

Figure B.1: Factorial correspondence analysis (FCA) based on variation at 13 microsatellite DNA loci in Arctic char (*Salvelinus alpinus*) and Dolly Varden (*S. malma*) from the Wood River Lake system in southwestern Alaska. Dolly Varden are denoted by yellow squares in Lake Aleknagik and blue in Lake Nerka and Arctic char by grey squares in Lake Aleknagik and white in Lake Nerka.

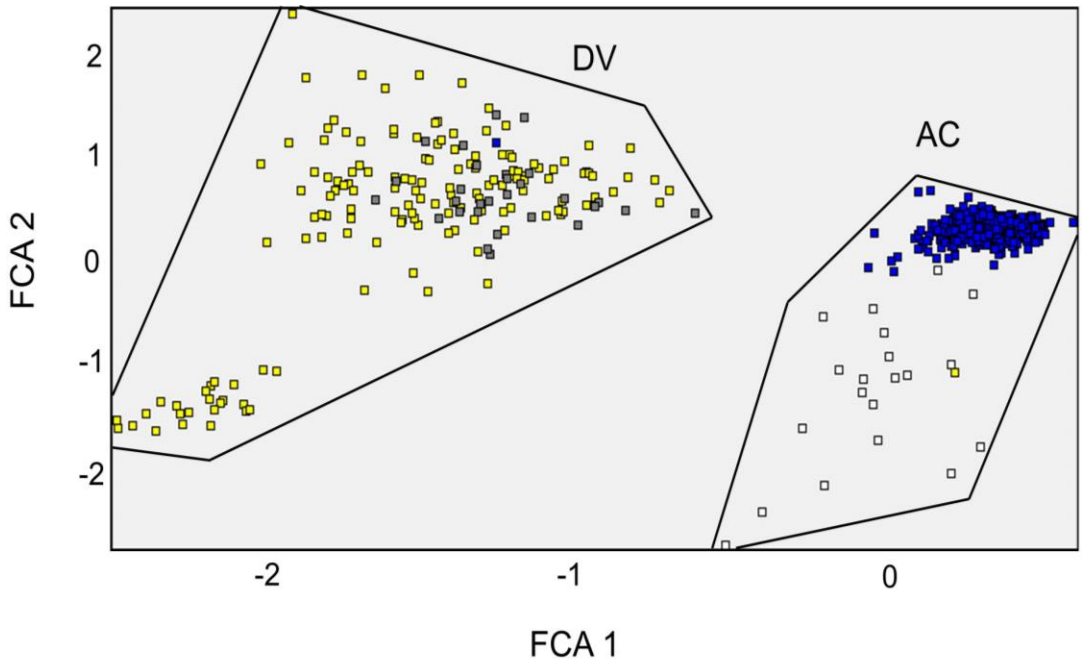

Figure B.2: Factorial correspondence analysis (FCA) based on variation at 13 microsatellite DNA loci in Arctic char (*Salvelinus alpinus*) and Dolly Varden (*S. malma*) from the Wood River Lake system in southwestern Alaska compared to Arctic char from Resolute Bay, Nunavut in the Canadian Arctic and Dolly Varden from the Egegik fishing district on the southwestern coast of Alaska. Allopatric Arctic char are represented by white squares, allopatric Dolly Varden by grey squares and sympatric Arctic char and Dolly Varden from the Wood River Lake System by blue and yellow squares respectively.

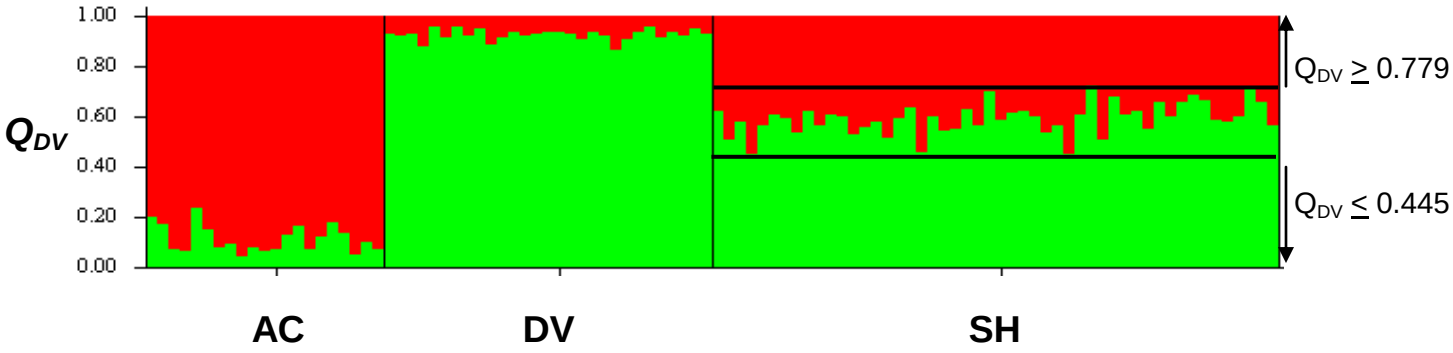

Figure B.3: STRUCTURE plot generated from allopatric Arctic char (AC, n = 21) from the Canadian Arctic, allopatric Dolly Varden (DV, n = 29) from the Egegik fishing district in southern Alaska and associated simulated hybrids (SH, n = 50). Each fish is represented by a vertical bar that denotes admixture fractions (*Q*) for *K* =2 genetic clusters (red and green portions of each bar). Horizontal bars indicate the upper and lower in the SH plot represent the upper and lower boundaries for *Q*-value defined hybrids.

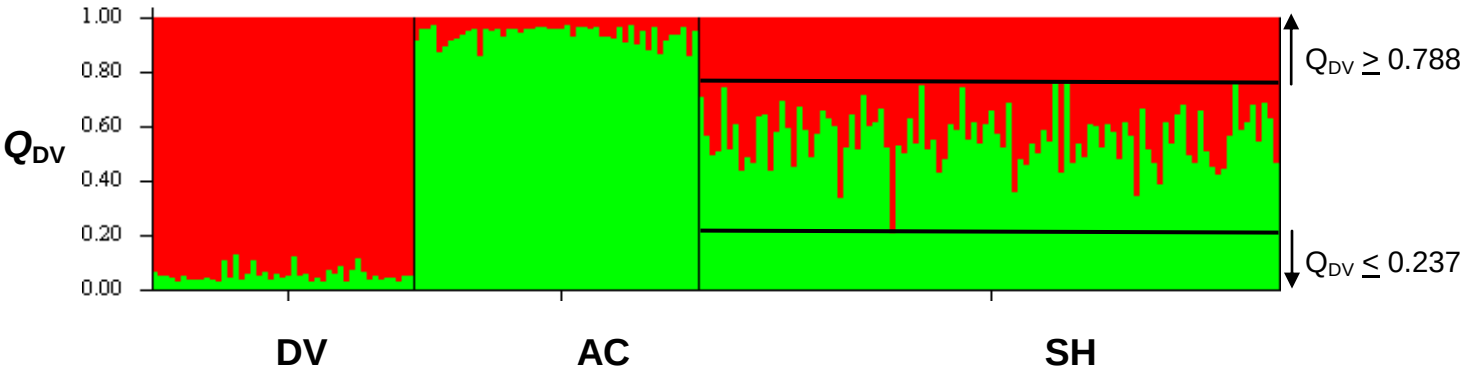

Figure B.4: STRUCTURE plot generated from presumed non-admixed Arctic char (AC, n = 50) and Dolly Varden (DV, n = 50) from the Wood River Lake system in southwestern Alaska and associated simulated hybrids (SH, n = 150). Each fish is represented by a vertical bar that denotes admixture fractions (*Q*) for *K* =2 genetic clusters (red and green portions of each bar). Horizontal bars indicate the upper and lower in the SH plot represent the upper and lower boundaries for Q_{DV}-value defined hybrids.