COLONIZATION AND LOCAL ADAPTATION OF SOCKEYE SALMON (*ONCORHYNCHUS NERKA*) OF LAKE CLARK, ALASKA

by

Kristina Marie Ramstad

B.A. Wellesley College 1993

M.Sc. University of Washington 1998

Presented in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

The University of Montana

2006

Approved by:

_____

Chair, Board of Examiners

_____

Dean, Graduate School

_____

Date

Abstract

Ramstad, Kristina M., Ph.D., May 2006 Organismal Biology and Ecology

Colonization and Local Adaptation of Sockeye Salmon (*Oncorhynchus nerka*) of Lake Clark, Alaska

Chair: Fred W. Allendorf

Adaptive divergence is expected among sockeye salmon (*Oncorhychus nerka*) populations because they spawn in diverse habitats and experience spatially and temporally restricted gene flow. Genetic bottleneck effects are also expected among sockeye salmon populations because they are a highly structured species with excellent colonizing abilities. To test if phenotypic divergence among sockeye salmon populations is due to local adaptation or genetic drift, I compared neutral genetic and heritable phenotypic variation in spawning populations of sockeye salmon throughout the Lake Clark area of Alaska.

I assessed nuclear (microsatellites and allozymes) and mitochondrial DNA variation of 15 sockeye salmon populations. Reduced allelic and haplotypic diversity and strong divergence of Lake Clark populations relative to Six-mile and Iliamna Lake populations suggest a bottleneck associated with the colonization of Lake Clark by sockeye salmon between 100 and 400 generations ago. The Sucker Bay Lake population had an exceptionally severe reduction in allelic diversity at microsatellite loci but not mtDNA, suggesting this population has experienced consistently smaller effective population size than other Lake Clark populations. Geographic distance and spawning habitat apparently do not contribute to neutral genetic divergence among populations. However, temporal isolation based on spawning time and founder effects associated with ongoing glacial retreat contribute to the genetic population structure of Lake Clark sockeye salmon.

Significant phenotypic divergence among spawning populations was associated with spawning habitat differences, but not neutral genetic divergence among populations. For example, female body color was lighter and egg color darker in glacial than non-glacial habitats due possibly to reduced selection for red spawning color in glacial habitats and an apparent trade-off between body and egg color in females. Mean P_{ST} (phenotypic divergence among populations) exceeded neutral F_{ST} for most phenotypic traits indicating that phenotypic differences among populations could not be explained by genetic drift alone. Plasticity is an unlikely source of phenotypic differences because Lake Clark sockeye salmon spend nearly all their lives in a common environment. These data suggest that Lake Clark sockeye salmon populations are adapted to spawning in beach, tributary, and glacial habitats and provide the first evidence of a glacial spawning phenotype among sockeye salmon.

Acknowledgements

It is not possible to provide due thanks here to the many people that have contributed to my dissertation. They span the globe from Montana to New Zealand and include members of my dissertation committee, numerous field and lab technicians, and many people that shared their knowledge, expertise, and resources with me. I could not have completed this work without their support.

I thank Fred Allendorf, my major advisor, for all that he has taught me and for his encouragement, support, and guidance. Thanks also to the other members of my dissertation committee, Lisa Eby, Doug Emlen, Scott Mills, Andy Sheldon, and Carol Woody, for their guidance of this research. I have learned a tremendous amount from each of you that will continue to guide my work.

For Chapters 2, 3, and 4, I owe thanks to all who collected samples in the field including Jake Allgeier, Rita Corcoran, Patricia Hernandez, Craig Kasemodel, Kerry Kilcoyne, Penny Knuckles, Duncan Oswald, Todd Rinaldi, Dan Rugerillo, Heather Rugerillo, Carl Saunders, Martin Stafford, Dalit Ucitel, and Johnny Zutz. Thanks also to those who collected data in the lab including Kevin Sage, Ghazal Ataian, Flo Gardipee, and Elizabeth Benolkin. Thanks to Jim Seeb, Tom Quinn, Ian Stewart, and Tim Brabets for sharing samples and data with me, Damon Holzer for his GIS expertise, Andy Gray, Dimitri Churikov, and Chris Kondzela for helpful advice on mtDNA analysis, Chris Foote, Gordon Luikart, Jennifer Neilsen, Jeff Olsen, Joel Reynolds, Ellie Steinberg, Fred Utter, and John Wenburg and for helpful discussions and reviews of draft manuscripts. Many thanks to Vanetta Burton and Joe Ball of the Montana Co-op Research Unit for administering this project and the National Park Service for critical field support. I am indebted to Dan Young for the varied roles he played, including fireside songleader, in making this research both fun and successful.

Many thanks to the Māori elders that generously gave their time and knowledge for Chapter 5 of this dissertation. This includes Mildred and Dale Connor, George Martin, Ropata Taylor, Barney Thomas, Bunna Riwaka, Glenice Paine, Jim Hintze Elkington, Ross Kawharu, Bill Webber, Priscilla Paul, Ben Hippolite, Jim Elkington, Ru and Moana Rene, and Whetu McGregor. Thanks also to Cathleen Carew and Hillary McGregor for help in coordinating and transcribing interviews, Pete Gaze and Mike Aviss for expert tuatara input, Penny Kukuk and Rich Clow for advice in working with TEK, Richard Romijn for digging up historical tuatara documents, Jenny Neale and Rich Arnold for survey design advice, and Gavin Laurie, Matt Bestram, and Dan Whaley for assistance with the technical audio and video aspects of this project. This project would not have been possible without the dedicated efforts of Diana Beech, Glenice Paine, and Anaru Paul. I deeply enjoyed working with them on this project and appreciate all their efforts.

Funding for Chapters 2, 3, and 4 was provided by the Office of Subsistence Management of the U.S. Fish and Wildlife Service, the National Park Service, and the Alaska Science Center of the U.S. Geological Survey. Chapter 5 was funded by the National Science Foundation Training Within Environmental Biology Program (T-WEB) of the University of Montana, the School of Biological Sciences and University Research Office of Victoria University of Wellington, New Zealand, and the Allan Wilson Centre for Molecular Ecology and Evolution, New Zealand.

Many thanks to all at the Montana Conservation Genetics Lab as well as Charlie Daugherty, Nicky Nelson, and Charlie's Angels for their patience, support, and good humor.

Much love and thanks to Sheryl, Bob, Rachel, and Bruce for their unwavering support and encouragement and to Max, Henry, and Louisa for providing perspective and inspiration.

Table of Contents

List of Tables

List of Figures

Chapter 1 - Introduction

Populations are products of opposing evolutionary forces. Gene flow promotes homogeneity among populations while genetic drift and selection promote divergence among populations. When reproductive isolation is coupled with significantly different selective regimes, adaptive variation among populations, or local adaptation, may result and accrue over time (Endler 1984; Stearns 1992; see review by Taylor 1991). Phenotypic divergence among populations may indicate adaptation but could also be attributed to phenotypic plasticity (the range of expression of the genotype in interaction with the environment; Stearns 1992) or genetic drift (Adkison 1995; Hartl and Clark 1997). Thus, attributing local differentiation to local adaptation is difficult.

Organisms exploiting different habitats may be locally adapted and display heritable phenotypic variation both in the absence of gene flow (Taylor and Bentzen 1993; Johnson and Black 2000) and in the presence of gene flow (Wood and Foote 1996; Foote et al. 1999) among habitat types. This adaptive divergence among conspecific populations is sometimes a precursor to speciation (Robinson and Schluter 2000). Northern freshwater fishes may be particularly prone to rapid and sympatric phenotypic diversification. Many of these species exhibit dramatic phenotypic variation that is trophically associated, heritable, and persists in the presence of high gene flow.

Reproductive isolation and spawning habitat variation promote divergence of genotypes and phenotypes among spawning populations of salmonids (Ricker 1972; Beacham and Murray 1987; Taylor 1991; Blair et al. 1993). Phenotypic differences are often attributed to local adaptation as habitat characteristics frequently correlate with heritable morphological traits, behaviors, and life histories. For example, sockeye salmon (*Oncorhynchus nerka*) body depth is positively correlated with spawning site water depth and negatively correlated with predation (Bishop 1991; Wetzel 1993; Woody 1998) and run timing is correlated with stream thermal regime (Brannon 1987; Woody 1998). The role of genetic drift in promoting phenotypic

differentiation among local spawning populations of salmonids has been less explored (Adkison 1995).

My dissertation research examines the relative importance of colonization (genetic drift due to founder effects) and local adaptation (selection) in shaping spawning populations of sockeye salmon in Lake Clark, Alaska. Phenotypic plasticity is an unlikely source of phenotypic differences among populations of Lake Clark sockeye salmon because fish rear as juveniles in a common nursery lake (Schlenger 1996), collectively outmigrate to sea as smolt (Orrell 1963; Woolington et al. 1990), spend their adult lives in a common oceanic environment (French et al. 1976; Burgner 1980), and return synchronously to Lake Clark to spawn (Burgner 1980; Jensen and Mathisen 1987). Thus, there is little opportunity for spawning populations to experience different environments prior to spawning.

Colonization is the invasion of species into newly created or disturbed habitats (Mayr 1965). The retreat of the Cordilleran ice sheet approximately 13,500 to 11,000 years ago carved and filled in lake basins and formed many freshwater habitats of coastal Alaska (Hamilton 1994). Salmonids survived the Wisconsin glaciation in refugia and ultimately colonized these freshwater systems (McPhail and Lindsey 1970; Lindsey and McPhail 1986; McPhail and Lindsey 1986; Wood et al. 1994). Sockeye salmon are excellent colonizers of newly created habitats because their homing fidelity exists in a dynamic balance with a tendency to stray (Quinn 1984; 1985) and they can quickly establish spawning populations with few individuals (Milner 1987; Milner and Bailey 1989; Milner et al. 2000). Thus, sockeye salmon are vulnerable to founder effects, and genetic drift is expected to influence the genetic population structure of sockeye salmon.

Lakes are the focal points of homing and spawning of sockeye salmon and much of the genetic variation within sockeye salmon is found among nursery lakes (Wood et al. 1994; Wood 1995; Seeb et al. 2000; Withler et al. 2000). However, sockeye salmon exhibit complex patterns of genetic divergence on several spatial and temporal scales and data suggest that significant genetic divergence among spawning populations within nursery lakes as well. Divergence among

populations within lakes is typically associated with differences in spawning habitat type (e.g., beach or tributary) or spawning behaviors (e.g., early or late spawning time; Wilmot and Burger 1985; Varnavskaya et al. 1994; Wood 1995). Restricted gene flow among and differential selection within spawning habitat types could result in habitat specific phenotypes of sockeye salmon due to selection.

Genetic and phenotypic analyses are complementary in studies of adaptive divergence (Bernatchez et al. 1999; Bernatchez and Wilson 1998). Neutral genetic polymorphism data can resolve gene flow among and genetic drift within populations but can say nothing of adaptive phenotypic divergence while the reverse is true for ecological and phenotypic comparisons. In this study, I compare patterns of neutral genetic, phenotypic, and spawning habitat variation to assess the relative effects of genetic drift and selection in shaping spawning populationsof Lake Clark sockeye salmon. More specifically, I tested for evidence of adaptive differentiation between fish spawning in beach and tributary habitats and between fish spawning in glacial and non-glacial habitats.

T-WEB Internship

I also conducted a project to record Māori traditional ecological knowledge (TEK) of tuatara (*Sphenodon* spp.) in New Zealand. This project was conducted as part of my Training Within Environmental Biology (T-WEB) fellowship. The T-WEB program is a doctoral conservation biology program with the goal of training creative, skilled scientists that are flexible thinkers and able to work collaboratively with scientists and decision makers both inside and outside academia. Each T-WEB fellow designs and carries out an internship that provides substantial experience outside their normal research activities in a non-academic setting and promotes critical analysis of the relationship between basic science and environmental decision making. For my internship, I designed and coordinated a collaborative study between university ecologists (University of Montana, USA and Victoria University of Wellington, New Zealand) and Māori

iwi (similar to tribes) resource managers (Te Atiawa Iwi and Ngati Koata Iwi, New Zealand) and spent 6 months in New Zealand recording Māori TEK of tuatara.

1.1 Research objectives and findings

Ecological genetics of sockeye salmon

The role of founder effects in shaping the genetic population structure of Lake Clark sockeye salmon is examined in Chapter 2. I measured variation at 11 microsatellite loci and compared populations throughout Lake Clark, Six-mile Lake, and Lake Iliamna to address three primary questions.

(1) What is the pattern of genetic divergence among spawning populations of Lake Clark, Six-Mile Lake, and Lake Iliamna sockeye salmon?

(2) Is there evidence of genetic bottleneck effects among spawning populations of sockeye salmon within Lake Clark relative to Six-mile Lake and Lake Iliamna?

(3) What physical and biological factors best explain the patterns of genetic divergence among and genetic variation within these spawning populations of sockeye salmon?

Six-mile Lake and Lake Clark populations have historically been grouped together for management purposes and are geographically proximate. However, microsatellite analysis suggests that Six-mile Lake populations are genetically similar to Lake Iliamna populations and divergent from Lake Clark populations. Reduced allelic diversity and strong divergence of Lake Clark populations relative to Six-mile Lake and Lake Iliamna populations suggest a bottleneck associated with the colonization of Lake Clark by sockeye salmon. The Sucker Bay Lake population of Lake Clark exhibited the strongest bottleneck signal observed in this study. Geographic distance and spawning habitat differences apparently do not contribute to isolation

and divergence among spawning populations. However, temporal isolation based on spawning time and founder effects associated with ongoing glacial retreat and colonization of new spawning habitats contribute to the genetic population structure of Lake Clark sockeye salmon. This chapter was published in the journal *Molecular Ecology* (13:277-290) in 2004.

In Chapter 3, I use two additional types of genetic markers (allozymes and mtDNA) to further examine patterns of genetic bottleneck effects and population structure in Lake Clark sockeye salmon. Comparing bottleneck signals in mitochondrial DNA (mtDNA) and nDNA (e.g., microsatellites and allozymes) can provide information on the duration and timing of bottleneck events (Wilson et al. 1985; Birky 1991). The following three questions are addressed in Chapter 3.

(1) Do allozyme and mtDNA data suggest the same pattern of founder effects as microsatellites in Lake Clark sockeye salmon?

(2) Is the reduced genetic variation of Sucker Bay Lake sockeye salmon due to a recent, acute bottleneck effect or consistently low effective population size?

(3) Does the Six-mile Lake population share similar allozyme allele and mtDNA haplotype frequencies with Lake Iliamna or Lake Clark populations?

Allozyme and mtDNA results were congruent with the microsatellite data in suggesting a common founder event in Lake Clark sockeye salmon and confirmed the divergence of Lake Clark populations from neighboring Six-mile Lake and Lake Iliamna populations. The Sucker Bay Lake population had an exceptionally severe reduction in allelic diversity at microsatellite loci but not at mitochondrial DNA. This suggests that the reduced microsatellite variation in Sucker Bay Lake fish is due to consistently smaller effective population size than other Lake Clark spawning populations, rather than a more acute or additional bottleneck since founding. This chapter is currently in press as an article in the American Fisheries Society Proceedings entitled Sockeye Salmon Evolution, Ecology, and Management.

The objective of chapter 4 was to test for evidence of local adaptation of sockeye salmon to different spawning habitats of Lake Clark, Alaska. In this chapter, I describe phenotypic differences among fish spawning in each of four habitat types (glacial beaches, non-glacial beaches, glacial tributaries, non-glacial tributaries) and compare patterns of phenotypic divergence among spawning populations to neutral genetic divergence among populations and differences in spawning habitat. The following specific questions were addressed.

(1) What is the pattern of phenotypic variation among spawning populations of Lake Clark sockeye salmon?

(2) Is phenotypic divergence among populations correlated with differences in spawning habitat?

(3) Is phenotypic divergence among spawning populations correlated with neutral genetic divergence?

I found significant phenotypic divergence among populations that is associated with spawning habitat differences, but not with neutral genetic divergence. For example, female body color is lighter and egg color darker in glacial than non-glacial habitats due possibly to reduced selection for red spawning color in glacial habitats and an apparent trade-off between body and egg color in females. In addition, divergence among spawning populations in most phenotypic traits exceeds neutral genetic divergence indicating that phenotypic differences among populations cannot be explained by genetic drift alone. Thus, phenotypic divergence among spawning populations of sockeye salmon is likely the product of local adaptation.

T-WEB Internship

The objectives of my T-WEB internship were three fold. First, I aimed to learn more about tuatara biology and ecology. Second, I hoped to document the cultural significance of tuatara to Māori. Finally, I hoped to learn something of the nature of TEK itself through comparing this

case study to others in the literature. All study objectives were met as this study provided the first evidence of seven former sites of tuatara occupation, suggested five additional sites that tuatara may presently occupy, suggested novel hypotheses for scientific testing, and contained tuatara cultural roles that have not been previously reported. The study also resulted in a lasting digital archive of Māori TEK for each participating iwi, illustrated that TEK can persist as species decline, and suggested that TEK can serve as a valuable source of ecological information of rare species.

1.2 Synthesis and significance

Ecological genetics of sockeye salmon

Phenotypic divergence among spawning populations of Lake Clark sockeye salmon is likely the product of local adaptation. Phenotypic divergence among populations is not correlated with neutral genetic divergence but is correlated with differences in spawning habitats suggesting that selection is the primary force in driving phenotypic divergence among spawning populations. The data provide additional evidence of beach and tributary ecotypes already reported in the literature, as well as the first evidence of a glacial ecotype of sockeye salmon. Local adaptation to glacial spawning habitats suggests that sockeye salmon are excellent colonizers partly because they can adapt quickly to highly unstable, geologically young habitats.

This work provides an empirical test of the role of founder effects in shaping the population structure of a colonizing species. The correlation between differences in genetic variation and genetic divergence among spawning populations in three types of genetic markers suggests that founder effects have deeply affected the genetic population structure of Lake Clark sockeye salmon. Genetic divergence and geographic distance are not correlated among populations further suggesting these populations are not at migration-drift equilibrium and patterns of neutral genetic population divergence are to due to historical events. This study also

demonstrates the importance of using a variety of genetic marker types to test for bottleneck effects. The combination of microsatellite and mtDNA data suggests that isolation and low effective population size (N_e) have promoted further loss of genetic variation in the Sucker Bay Lake population since sharing in a common founder event with other Lake Clark populations.

Commonly founded populations have provided some of the best evidence of rapid adaptive divergence in wild salmon because phenotypic divergence due to selection must have arisen since populations became established (Quinn et al. 1996; Kinnison et al. 1998; Hendry et al. 2000b; Quinn et al. 2000). This study concurs with others in suggesting that local adaptation can occur relatively quickly (100 to 400 generations in Lake Clark sockeye salmon) and be present among populations that exhibit little neutral genetic divergence (Hendry et al. 2000b; Stockwell et al. 2003).

T-WEB Internship

We are currently experiencing a species and cultural extinction crisis with profound losses of species (Singh 2002), indigenous languages (Cox 1997; Maffi 1998; Stork 1999), and TEK. This study suggests that if we wish to conserve TEK and cultural diversity, we must conserve species that figure prominently in traditions and maintain links between traditional cultures and species. Including diverse perspectives will promote better species conservation by improving our understanding of species and increasing public support for management and restoration.

Chapter 2 - Founding events influence genetic population structure of Lake Clark sockeye salmon

2.1 Abstract

Bottlenecks can have lasting effects on genetic population structure that obscure patterns of contemporary gene flow and drift. Sockeye salmon are vulnerable to bottleneck effects because they are a highly structured species with excellent colonizing abilities and often occupy geologically young habitats. We describe genetic divergence among and genetic variation within spawning populations of sockeye salmon throughout the Lake Clark area of Alaska. Fin tissue was collected from sockeye salmon representing 15 spawning populations of Lake Clark, Six-mile Lake, and Lake Iliamna. Allele frequencies differed significantly at 11 microsatellite loci in 96 of 105 pair-wise population comparisons. Pair-wise estimates of F_{ST} ranged from zero to 0.089. Six-mile Lake and Lake Clark populations have historically been grouped together for management purposes and are geographically proximate. However, Six-mile Lake populations are genetically similar to Lake Iliamna populations and divergent from Lake Clark populations. Reduced allelic diversity and strong divergence of Lake Clark populations relative to Six-mile Lake and Lake Iliamna populations suggest a bottleneck associated with the colonization of Lake Clark by sockeye salmon. Geographic distance and spawning habitat differences apparently do not contribute to isolation and divergence among populations. However, temporal isolation based on spawning time and founder effects associated with ongoing glacial retreat and colonization of new spawning habitats contribute to the genetic population structure of Lake Clark sockeye salmon. Non-equilibrium conditions and the strong influence of genetic drift caution against using estimates of divergence to estimate gene flow among populations of Lake Clark sockeye salmon.

2.2 Introduction

Populations are products of opposing evolutionary forces. Gene flow promotes homogeneity among populations, genetic drift promotes divergence, and selection may act in either direction. The relative effects of these forces shape the genetic population structure (pattern of genetic variation among and within populations) of a species. Understanding genetic population structure is critical for effective management as it provides a basis for defining management units, can identify populations of unusual genetic composition, and may identify populations at risk of extinction due to low genetic diversity (Avise 1994). Population structure is positively associated with genetic diversity and resilience to disturbance such that large, highly structured populations have high genetic diversity and probability of persistence (Giesel 1974; Altukhov 1981). In contrast, small, panmictic populations are vulnerable to inbreeding, demographic stochasticity, genetic drift and thus, reduced evolutionary potential, and increased probability of extinction (Luikart et al. 1998; Soulé and Mills 1998).

A genetic bottleneck effect occurs when a population experiences a severe reduction in effective population size (Avise 1994). During a bottleneck event, genetic drift (random changes in allele frequencies due to imperfect sampling of alleles between generations) reduces genetic variation within and increases genetic divergence among populations. Founder effects are bottleneck effects that are associated with the founding of a new population. Thus, genetic drift may affect genetic population structure through founding events. Populations that have been in stable environments and connected by dispersal over long periods of time will reach a genetic equilibrium where the loss of alleles due to drift is balanced by the introduction of new alleles through migration (Wright 1951).

Assessing the relative effects of gene flow and genetic drift in shaping contemporary genetic population structure is difficult. If populations are in equilibrium, genetic population structure reflects recent processes and the amount of gene flow among populations can be approximated with the equation: $F_{ST} \sim 1/4Nm+1$ (Wright 1931; Mills and Allendorf 1996). When

populations are not at equilibrium, genetic population structure reflects historical processes and estimates of current gene flow based on F_{ST} are biased and potentially misleading (Hutchison and Templeton 1999). Most natural populations are not in genetic equilibrium (McCauley 1993) due to disrupted dispersal among populations and bottleneck effects (Hutchison and Templeton 1999; Kinnison et al. 2002). Populations found at the periphery of a species range (Lesica and Allendorf 1995; Costello et al. 2003) and in formerly glaciated regions (Congdon et al. 2000; Hewitt 2000; Turgeon and Bernatchez 2001; Castric and Bernatchez 2003) are particularly prone to non-equilibrium conditions due to recent range expansions and founder effects. When the rate of approach to equilibrium is slow compared to disturbance regimes, for example fire (England et al. 2002) and tectonic activity (Jollivet et al. 1999), populations may never achieve equilibrium.

Specific natal homing promotes reproductive isolation and genetic structuring among populations of sockeye salmon (*Oncorhynchus nerka*; Ricker 1972; Quinn 1985; Quinn and Dittman 1990). Lakes are focal points of homing and genetic divergence is typically greater among populations spawning in different lakes than among spawning populations within lakes (Wood 1995; Seeb et al. 2000; Withler et al. 2000). However, there is often significant genetic divergence among spawning populations within lakes due to restricted gene flow among fish spawning in beach and tributary habitats (ecological isolation), geographically distant habitats (spatial isolation), or differing in their time of return or spawning (temporal isolation; Ricker 1972; Wilmot and Burger 1985; Varnavskaya et al. 1994a; 1994b; Wood 1995; Ramstad et al. 2003; Woody et al. 2000). Sockeye salmon are excellent colonizers of newly created habitats because their homing fidelity exists in a dynamic balance with a tendency to stray (Quinn 1984; 1985) and they can quickly establish spawning populations with few individuals (Milner 1987; Milner and Bailey 1989; Milner et al. 2000). Thus, sockeye salmon are vulnerable to founder effects, and genetic drift is expected to influence the genetic population structure of sockeye salmon.

We tested the relative importance of ecological, temporal, and spatial isolation (restricted gene flow) and founder effects (genetic drift) on the genetic population structure of sockeye salmon of Lake Clark, Alaska. Populations spawn in a variety of habitat types (beach, inlet tributary, outlet tributary), across a broad range of spawning times (mid August to early November), and in varying geographic proximities to one another (3 to greater than 300 km) throughout this system. In addition, there is the potential for founder effects because Lake Clark is geologically young, having been created by glacial retreat approximately 12 to 15 thousand years ago (Stilwell and Kaufman 1996). Spawning habitats within Lake Clark vary in time since deglaciation, suggesting they similarly vary in time since first colonization. For example, sockeye salmon spawn in an area of the Upper Tlikakila River that was deglaciated approximately one to two hundred years ago (unpublished data, Dr. Patricia Heiser, University of Alaska, Anchorage). Lake Clark sockeye salmon that spawn in younger habitats may have experienced recent founder effects.

This study provides an empirical test of the role of founder effects in shaping the genetic population structure of a colonizing species. We address three primary questions: (1) What is the pattern of genetic divergence among spawning populations of Lake Clark, Six-Mile Lake, and Lake Iliamna sockeye salmon? (2) Is there evidence of genetic bottleneck effects among spawning populations of sockeye salmon within Lake Clark relative to Six-mile Lake and Lake Iliamna? (and 3) What physical and biological factors best explain the patterns of genetic divergence among and genetic variation within these spawning populations of sockeye salmon.

2.3 Methods

2.3.1 Sample collection

Fin tissue from eleven Lake Clark, two Six-mile Lake, and two Lake Iliamna spawning populations was collected (Figure 1, Table 2). Samples (~100) from three Lake Clark

populations (Currant Creek, Priest Rock Creek, Kijik River) and both Lake Iliamna populations (Lower Talarik Creek, Fuel Dump Island) were collected in a single year. Samples (~50) from all other populations were collected in each of two years to test for inter-annual variation in allele frequencies within populations. Post spawning and spawning fish were captured on their spawning grounds by seine and tangle net. A fin clip (approximately 5mm2) was collected and stored in 100% EtOH from each fish sampled.

We attempted to obtain a sample size of 100 for all populations, which provides a 95% probability of detecting an allele at a frequency 0.015 or greater. We generally met this goal with the exception of Priest Rock Creek (N = 65) where a lower sample size reduces the ability to detect rare alleles. However, the number of fish sampled represents a significant fraction of fish present at this site (~150).

2.3.2 Microsatellite genotyping

Total DNA was extracted using the Puregene® DNA Isolation Tissue Kit (Gentra Systems, Minneapolis, Minnesota). Concentration of DNA was measured with a DyNA Quant 200 Fluorometer (Hoefer, San Francisco, California) after rehydration in Tris-EDTA. Working stocks for each sample were diluted with deionized water to concentrations of 50ng/µl.

Fish were genotyped at 11 microsatellite loci (Table 1). Primers were directly labelled with infrared fluorophore IRD700 and IRD800 (LI-COR, Lincoln, Nebraska). The DNA was amplified in 10µl polymerase chain reactions (PCR; 200 µmol each dNTP, 4 pmol each primer, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% each of gelatin, NP-40 and Triton-x 100, and 0.5 units of DNA polymerase (Promega and/or Perkin-Elmer) in a series of five PCRs (Table 1). Profiles for PCR were: 94°C for 2 min followed by 35-40 cycles of 15 sec to 1 min at 94°C, 15 sec to 1 min at annealing temperature (Table 1) and 30 sec to 1 min at 72°C. Blank reactions (all constituents present but template DNA) were included in each PCR to detect sample contamination.

DNA was electrophoresed on a 6% denaturing polyacrylamide gel and PCR products were scored relative to a known size standard on a LI-COR DNA Analyzer Global Edition IR2 and a LI-COR DNA Sequencer Long Reader 4200 using V4.03 Gene ImagIR software (Scanalytics, Inc., Fairfax, Virgina). An individual fish with known allele sizes was included on every gel and a second gel reader proofed allele sizes to insure accuracy and consistency of scoring across gels. Individuals representing 10% of genotyped fish were reamplified and scored a second time. Comparison of initial and repeated scores revealed a genotyping error rate of less than 2%.

2.3.3 Statistical analysis

Genetic population structure

Departures from Hardy-Weinberg proportions (Guo and Thompson 1992) and heterogeneity of allele frequencies were tested using GENEPOP version 3.2 (Raymond and Rousset 1995). Tests of significance were combined over all loci using Fisher's combined probability test (Sokal and Rohlf 1981). The proportion of genetic variation due to population subdivision was estimated as F_{ST} and computed in FSTAT, version 1.2 (Goudet 1995) according to Weir and Cockerham (1984). Principal component analysis was performed using the covariance matrix of allele frequencies in MINITAB, version 11 (State College, PA) after omitting the largest allele at each locus to allow for the non-independence of allele frequencies within a locus (Johnson 1998). Sequential Bonferroni adjustments were made for all multiple comparisons (Rice 1989).

Bottleneck effects

Recently bottlenecked populations may exhibit gametic disequilibrium (non-random association of alleles at different loci; Waples 1991; 2002), reduced allelic diversity, loss of rare alleles (Allendorf 1986), a mode shift in allele frequency distributions (Luikart *et al.* 1998), increased heterozygosity relative to that expected at mutation-drift equilibrium (Maruyama and Fuerst

1985; Cornuet and Luikart 1996; Luikart and Cornuet 1998), and a reduced value for the statistic *M* (Garza and Williamson 2001). Therefore we tested for the presence of bottleneck effects with a suite of measures.

Gametic disequilibrium was assessed for all pair-wise locus comparisons within each population using a Fisher exact test in GENEPOP version 3.2 (Raymond and Rousset 1995). Number of alleles observed in a population is highly dependent on sample size. Therefore, allelic diversity was assessed as allelic richness, which is a measure of the number of alleles per population corrected for sample size (El Mousadik and Petit 1996). Allelic richness was calculated and compared among major population groups in FSTAT version 1.2 (Goudet 1995). Mode shifts in allele frequencies within populations were assessed graphically (Luikart *et al.* 1998). Heterozygosity excess relative to a non-bottlenecked population in mutation-drift equilibrium having the same number of alleles was tested in BOTTLENECK, version 1.2.02 (Cornuet and Luikart 1996). We assume an infinite alleles model of mutation (IAM) for this analysis though microsatellites are expected to conform more closely to a stepwise mutation model (Shriver *et al.* 1993). In the present context, the use of the IAM is equal to the assumption that the sampled Iliamna populations are not bottlenecked because their observed heterozygosity closely matches that expected at mutation-drift equilibrium under the IAM (Cornuet and Luikart 1996). Significance of heterozygosity excess over all loci was assessed with a Wilcoxon sign-rank test (Cornuet and Luikart 1996; Luikart and Cornuet 1998).

The statistic *M* was calculated according to Garza and Williamson (2001). Three of the eleven loci surveyed were not included in this analysis (*Oki-1*, *One105*, *Ots107*) because their allelic distributions did not meet the test requirement of having rare alleles or empty allelic states within the range of common alleles (>0.1 frequency). Significance was assessed by comparison of mean *M* across loci, for each population with both a critical value (M_C) and the more conservative rule that $M<0.68$ suggests a bottleneck effect (Garza and Williamson 2001). Because *M* is simply the ratio of the number (*k*) to the size range (*r*) of microsatellite alleles, its

value does not rely on any mutation model or population size assumptions. However, the critical value M_C was calculated with the assumption of large pre-bottleneck population size (Ne = 5,000), a constant microsatellite mutation rate ($\mu = 5x\ 10^{-4}$/locus/generation), and a two-phase mutation model where 90% of mutations are a single step and the average size of all other mutations is 3.5 repeats. Assuming an N_e to N ratio of 0.2 (Allendorf *et al.* 1997; Reiman and Allendorf 2001) and a generation interval of 4 years, the pre-bottleneck effective population size of 5,000 translates to 6,250 spawning fish observed per year. While this value seems high, it results in a conservative test because the value of M_C is negatively related to the pre-bottleneck N_e and plateaus above an N_e of 5,000. Thus, a large pre-bottleneck N_e of 5,000 suggests fewer bottleneck effects than a lower N_e and larger values of N_e would not change the qualitative results of this analysis. Both the mean M and mean proportion of rare alleles (frequency <0.1) were compared among major population groups with a one tailed, nonparametric Mann-Whitney test (Zar 1984). Comparisons of allelic richness between Sucker Bay Lake (treating each of two sampling years as a population) and major population groups were made in FSTAT version 1.2 (Goudet 1995).

Factors shaping genetic population structure

Correlation between pair-wise genetic population divergence (F_{ST}) and spawning habitat type, geographic distance, difference in spawning time, and difference in allelic richness among populations was assessed by simple and partial Mantel tests in FSTAT version 1.2 (Goudet 1995) according to Manly (1991). Four predictor variable matrices were defined 1) spawning habitat type (HAB), 2) geographic distance (GEODIST), 3) spawning time (SPTIME), and 4) allelic richness (ALLRICH). The spawning habitat matrix coded for populations spawning in similar (0) and different (1) habitat types (beach or tributary). Geographic distance is the minimum distance (km) through water among all sampling sites and was assessed in ArcInfo. Spawning time was estimated as the mean date of peak spawning across survey years and was based on the presence

of gravid females during sampling. Difference in allelic richness between populations serves as a measure of genetic bottleneck effects. All variables but habitat type were log transformed after values of zero were reassigned the smallest pair-wise value detected in all population comparisons (spawning time=1 day, N=4 comparisons; F_{ST} = 0.0001, N=8 comparisons). Tests were conducted among all surveyed populations (Lake Clark, Six-mile Lake, and Lake Iliamna) and populations within Lake Clark.

A pattern of isolation by distance (IBD, a positive correlation between genetic divergence and geographic distance) is expected among populations in migration-drift equilibrium but not among non-equilibrium populations (Slatkin 1993). In the absence of IBD, a consistently low F_{ST} among populations suggests that historic gene flow has been the primary factor in shaping contemporary genetic population structure, while highly variable F_{ST} values suggests that drift historically overwhelmed gene flow (Hutchison and Templeton 1999). Thus, the relationship between pair-wise population F_{ST} and geographic distance (variables untransformed) was evaluated for IBD and the relative historical effects of gene flow and genetic drift.

Differences in the number of alleles and heterozygosity can be a source of bias in estimating F_{ST} (McDonald 1994; Hedrick 1999). Therefore, we used $F2_{ST}$ to test for a correlation between genetic divergence and genetic diversity. $F2_{ST}$ treats all loci as diallelic by using the frequency of the overall most common allele and pooling all others (McDonald 1994; Allendorf and Seeb 2000).

2.4 Results

All loci were polymorphic in all samples. The total number of alleles per locus ranged from 5 to 21, and the mean number of alleles per population and locus ranged from 3.0 to 10.9 (Table 1). Mean expected heterozygosity ranged from 0.452 to 0.518, and mean number of alleles ranged from 4.3 to 7.1 (Table 2) per population over all loci. There was no evidence of deviation from

Hardy-Weinberg proportions at any locus, in any sample. No significant inter-annual difference in allele frequencies (P>0.05) existed so samples collected from the same population in different years were pooled for further analysis. Significant gametic disequilibrium (P<0.05) was detected in only 4 of 825 pairwise comparisons among eleven loci after sequential Bonferroni correction within populations. There was no consistent tendency toward gametic disequilibrium between any loci or within any population.

2.4.1 Genetic divergence among all populations

There were significant differences in allele frequencies in 96 of 105 pair-wise population comparisons (Table 3). Estimates of F_{ST} ranged from 0 to 0.089 and were greatest between Lake Clark and Lake Iliamna populations. Six-mile Lake and Lake Iliamna populations are genetically similar though Six-mile Lake and Lake Clark populations have historically been grouped for management purposes and are geographically proximate. The Sucker Bay Lake population is highly divergent from all other populations surveyed. Hereafter we refer to two major population groups: the Six-mile Lake group (populations of Six-mile Lake and Lake Iliamna) and the Lake Clark group (all populations within Lake Clark but Sucker Bay Lake).

Principal component analysis supports this pattern (Figure 2). The first principal component explains 57% of the total genetic variation and differentiates among the Lake Clark group, Sucker Bay Lake, and the Six-mile Lake group. Loadings suggest this component is primarily influenced by allele frequency differences at four loci (*Omy325*: alleles 152, 156; *One*μ*18*: 181,171; *One*μ*21*: 140; *Omy77*: 105, 109). The second principal component explains 16% of the total genetic variation, further differentiates the Sucker Bay Lake population, and differentiates between populations of Six-mile Lake and Lake Iliamna. The second principal component is influenced primarily by allele frequency differences at four loci (μ*Sat60*: 118, 130; *One*μ*21*: 130,140; *One*μ*13*: 168; *Omy77*: 105). Lake Clark group populations had a pair-wise F_{ST} of 0.060 (95% CI 0.021 – 0.111) with Sucker Bay Lake and 0.054 (95% CI 0.023 – 0.086) with

the Six-mile Lake group. Between Sucker Bay Lake and populations of the Six-mile Lake group, F_{ST} was 0.049 (95% CI 0.018 – 0.082).

2.4.2 Genetic divergence within the Lake Clark group

There was significant genetic structuring within the Lake Clark group though many populations are genetically very similar (Table 3, Figure 3). There was no difference in allele frequencies between the two Kijik Lake populations sampled (Little Kijik River, Kijik Lake South Beach) and many of the greatest pair-wise F_{ST} values within the Lake Clark group were between Kijik Lake and other populations (range from 0.008 to 0.024, Table 3). Priest Rock Creek differed in allele frequencies from all other populations sampled (Table 3). This pattern of divergence within Lake Clark is supported by principal component analysis (Figure 3). The first principal component explains 44% of the genetic variation within Lake Clark and separates the Kijik Lake populations from all others. Component loadings suggests differences in allele frequencies at seven loci (*Oki1-1*: alleles 110, 114; *Oki1-2*:148, 156; *Omy325*:152; *μSat60*:118,130; *Oneμ21*: 140; Oneμ18: 185; *Oneμ13*: 168). The second principal component explains 19% of the genetic variation and differentiates the Priest Rock Creek population. Allele frequencies differences at seven loci are also indicated by second component loadings (*Oki1-2*: 148,156; *Omy325*: 152; *Oneμ21*, 130,140; *Oneμ18*: 181; *Oneμ13*: 160, *One105*: 132,136; *Omy77*: 105,109).

2.4.3 Genetic diversity and bottleneck effects

The data suggest a bottleneck among Lake Clark fish relative to fish of the Six-mile Lake group. We found a total of 92 alleles in the Lake Clark group fish (959 sampled) and 105 alleles in fish of the Six-mile Lake group (383 sampled), despite our sample sizes greatly favoring finding more alleles in the Lake Clark populations. Mean allelic richness (number of alleles corrected for sample size) of the Lake Clark group (5.0) is significantly lower than that of the Six-mile Lake group (6.0; $P<0.01$). There was no mode shift in allele frequency distribution in any population surveyed or population group (Figure 4). However, the mean proportion of rare alleles is

significantly lower in the Lake Clark (0.58) than Six-mile Lake (0.64) group ($U'_{10,4}$=37, P=0.01) and eight of ten Lake Clark group populations have a lower proportion of rare alleles than all four populations of the Six-mile Lake group. Similarly, eight of ten populations in the Lake Clark group have an excess of heterozygosity relative to that expected at mutation-drift equilibrium (Figure 5). This effect was not statistically significant within populations (P from 0.12 to 0.68) but the probability of obtaining this pattern across populations (eight of ten exhibiting heterozygosity excess) is low (P=0.04) assuming a population is equally likely to exhibit a heterozygosity excess or deficit. The statistic M suggests bottleneck effects (P<0.05 and M<0.68) in six populations within Lake Clark and one Six-mile Lake group population (Table 2). The smallest values were found in the Upper Tlikakila (M=0.595) and Little Lake Clark Beach (M=0.637) populations of Lake Clark. Currant Creek, Kijik Lake South Beach, and Priest Rock Creek also showed significant bottleneck effects (M=0.640 to 0.678). Mean M among Lake Clark group populations (0.680) was reduced relative to populations in the Six-mile Lake group (0.742; $U'_{10,4}$=33, P=0.05).

The most severe bottleneck effect found was in Sucker Bay Lake. The Sucker Bay Lake sample had less than half the number of alleles found in the Six-mile Lake group (48 versus 105). Mean allelic richness in Sucker Bay Lake fish (4.2) was lower than that of both the Lake Clark (5.0, P=0.084) and Six-mile Lake (6.0, P<0.001) groups. The Sucker Bay Lake population did not exhibit a mode shift in allele frequency distribution but had 37% fewer rare alleles than populations of the Six-mile Lake group (Figure 4). Heterozygosity excess among Sucker Bay Lake fish was greater than all other populations surveyed (Figure 5). This effect was present in eight of eleven surveyed loci and over all loci (0.502) where it was far in excess of that expected if the population were in mutation-drift equilibrium (0.389; P<0.005). The Sucker Bay Lake population has the second lowest M of all populations surveyed (M=0.615, P<0.05; Table 2).

2.4.4 Factors promoting population structure

The data do not support isolation by spawning habitat type (beach versus tributary). There is no tendency for populations spawning in a given habitat type to be genetically more similar to one another than to populations spawning in a different habitat type (Table 4). This result held across all surveyed populations (Lake Clark, Six-mile Lake, Lake Iliamna, P=0.97) and among Lake Clark populations both when Sucker Bay Lake is included (P=0.72) and when it is excluded (Lake Clark group, P=0.85).

Geographic distance and genetic divergence are significantly correlated among all surveyed populations (P<0.01) but not among Lake Clark populations (P=0.45; Table 4; Figure 6). Partial Mantel tests reveal that the effect of geographic distance on genetic divergence among all populations is insignificant. Geographic distance is correlated with both spawning time (P<0.01) and allelic richness (P<0.001) among all populations. After removal of spawning time effects (r=0.505, P<0.001), the addition of geographic distance was not significant (r=0.124, P=0.21). Similarly, geographic distance was not significantly correlated with genetic divergence (r=0.072, P=0.47) after removal of the effect of allelic richness (r=0.515, P<0.001). The proportion of variation in F_{ST} explained by a model including spawning time and allelic richness is not increased with the addition of geographic distance (R^2 = 0.41 with and without GEODIST). Thus, geographic distance does not explain the variation in F_{ST} among all populations itself but is correlated with variables that are significantly correlated with genetic divergence (SPTIME and ALLRICH).

Among all populations surveyed, both spawning time and allelic richness are significantly correlated with F_{ST} (SPTIME and ALLRICH: P<0.001) and with each other (P<0.01). Each of the two variables explains a significant amount of the variation in pair-wise F_{ST} after the effects of the other variable are removed (Table 4) and the best model includes only these two variables (R^2 = 0.41). Spawning time and allelic richness have similar magnitude of effect as evidenced by their nearly equal correlation coefficients both before (SPTIME: r=0.505;

ALLRICH: r=0.515) and after the removal of the effects of the other variable (SPTIME corrected for ALLRICH: r=0.375; ALLRICH corrected for SPTIME: r=0.389; Table 4).

Spawning time and allelic richness explain a significant amount of the variation in genetic divergence among Lake Clark populations as well. F_{ST} is significantly correlated with spawning time (P<0.01) and marginally correlated with allelic richness (P=0.08) while the two factors (SPTIME, ALLRICH) are independent of each other (P=0.62). The correlation coefficient of spawning time is consistently higher than that of allelic richness (Table 4). Further, the smaller reduction in the correlation coefficient of spawning time (before correction for ALLRICH: r=0.409; after: r=0.393) post removal of the effect of allelic richness suggests it is more closely correlated with F_{ST} than allelic richness (before correction for SPTIME: r=0.247; after: r=0.218; Table 4). However, the amount of variation in F_{ST} among Lake Clark populations explained by spawning time (R^2=0.17) is increased with the addition of allelic richness to the model (R^2=0.22) and there is a strong correlation between the allelic richness of a population and its mean divergence from all other populations as measured by $F2_{ST}$ ($F_{1,9}$=10.43; P=0.01; Figure 7). Thus, allelic richness is also highly associated with genetic population structure of Lake Clark sockeye salmon.

Much of the correlation among spawning time, allelic richness, and F_{ST} among Lake Clark sockeye salmon can be attributed to Sucker Bay Lake. Neither spawning time (r=0.172, P=0.24) nor allelic richness (r=-0.087, P=0.58) explains a significant amount of variation in pair-wise population F_{ST} within Lake Clark when Sucker Bay Lake is excluded (Lake Clark group). The trend for a negative correlation between allelic richness and mean $F2_{ST}$ per population is still evident but not statistically significant when Sucker Bay Lake is excluded ($F_{1,8}$=3.31, P=0.11; Figure 7).

The lack of IBD suggests non-equilibrium conditions among Lake Clark, Six-mile Lake, and Lake Iliamna populations of sockeye salmon (Table 4, Figure 6). F_{ST} is highly variable both among all populations surveyed (0 to 0.089) and among Lake Clark populations (0 to 0.077)

suggesting that historical drift has been a powerful force in shaping the genetic population structure of these populations. Much of the variation in F_{ST} among Lake Clark populations is due to Sucker Bay Lake and F_{ST} among Lake Clark populations is consistently low (0 to 0.024) when this population is excluded. This pattern suggests that gene flow has historically dominated drift among most Lake Clark populations but that drift has driven the divergence between Lake Clark populations and those of Sucker Bay Lake, Six-mile Lake and Lake Iliamna.

2.5 Discussion

2.5.1 Reproductive isolation and genetic population structure

Six-mile Lake populations (Lake Clark Outlet, Tazimina River) are more similar genetically to Lake Iliamna than Lake Clark populations. The genetic similarity among fish spawning in Six-mile Lake and Lake Iliamna is surprising because Six-mile Lake is closer to Lake Clark than to Lake Iliamna and has therefore historically been grouped with Lake Clark for management purposes. In addition, the Newhalen River (~39 km long) between Six-mile Lake and Lake Iliamna is a barrier to fish migration at high water velocities (Poe and Mathisen 1981; 1982). However, no current or recent barrier to fish migration between Lake Clark and Six-mile Lake is known. Satellite imagery documents the presence of a major outwash fan from the Tazimina Valley (Personal communication, Dr. Patricia Heiser, University of Alaska, Anchorage) that could have blocked fish entry to Lake Clark in the past. A barrier of this kind could have caused either isolation between sockeye salmon already present in Lake Clark and Six-mile Lake or delayed colonization of Lake Clark by sockeye salmon.

Reproductive isolation and genetic divergence among population of Lake Clark, Six-mile Lake, and Lake Iliamna sockeye salmon are likely not driven by differences in spawning habitat type or geographic distance. Differences in spawning time, however, are highly correlated with genetic divergence among populations. There is little difference in peak spawning time among

populations of Sucker Bay Lake (~ August 30), Six-mile Lake (August 25 to September 13), and Lake Iliamna (August 20 to 30). However, mean peak spawning time in populations of the Lake Clark group (September 18 to October 11) varies from other populations by one (Kijik system) to four weeks (Hatchet Point Beach, Priest Rock Creek, Little Lake Clark Beach, Lower Tlikakila River). Therefore, temporal isolation contributes to the genetic divergence between the Lake Clark group and other populations but cannot explain the high level of genetic divergence between Sucker Bay Lake and the Six-mile Lake group.

There is significant genetic population structure within the Lake Clark group (all populations spawning within Lake Clark but Sucker Bay Lake) though many populations are genetically similar. Fish spawning in Kijik Lake (South Beach and the outlet Little Kijik River) do not differ from one another in allele frequencies but are significantly differentiated from all other populations of the Lake Clark group. Priest Rock Creek fish are also highly differentiated from all other Lake Clark populations. Within the Lake Clark group, pair-wise population genetic divergence is not correlated with differences in spawning habitat, geographic distance between populations, or differences in spawning time. Thus, the pattern of genetic population structuring among sockeye salmon of the Lake Clark group is not one of simple ecological, geographic, or temporal isolation.

2.5.2 Bottleneck effects and genetic population structure

Evidence for genetic bottleneck effects

The data suggest a bottleneck effect in Lake Clark sockeye salmon relative to Six-mile Lake and Lake Iliamna. The prevalence of a reduced proportion of rare alleles (9 of 11 populations), heterozygosity excess (9 of 11 populations, statistically insignificant except in Sucker Bay Lake), and reduced M (7 of 11 populations) among populations within Lake Clark relative to the Six-mile Lake group suggest a bottleneck event that reduced the genetic diversity of Lake Clark sockeye salmon over all. The most extreme bottleneck signal was found in the Sucker Bay Lake

population of Lake Clark. The strong genetic divergence among most Lake Clark populations, the Sucker Bay Lake population, and populations spawning in Six-mile Lake and Lake Iliamna also suggest significant bottleneck effects.

Genetic drift changes allele frequencies and decreases genetic variation within populations. Therefore, if bottleneck effects have influenced the genetic population structure of Lake Clark sockeye salmon, we would expect a negative correlation between genetic divergence among and genetic variation within populations. We found a negative correlation between mean genetic divergence ($F2_{ST}$) and mean allelic richness among Lake Clark populations. Further, there is a significant, positive correlation between pair-wise F_{ST} and difference in mean allelic richness among both all populations surveyed and Lake Clark populations suggesting that difference in allelic diversity is couple with degree of genetic divergence between populations. Taken together, these results suggests that genetic population structure among sockeye salmon of Lake Clark, Six-mile Lake, and Lake Iliamna has been significantly influenced by genetic drift that is likely due to bottleneck effects.

Are the bottleneck effects also founder effects?

It is important to identify populations that have undergone recent and acute bottlenecks (within the past 100 generations, <20 Ne) because they may be affected by problems due to small population size (demographic stochasticity, inbreeding, fixation of deleterious alleles, reduced evolutionary potential, increased probability of extinction) and may respond to mitigative management (Cornuet and Luikart 1996; Luikart et al. 1998). Gametic disequilibrium, allele frequency distribution mode shifts, and heterozygosity excess are highly transient effects and only expected in populations that have experienced recent and acute genetic bottlenecks (Waples 1991; Cornuet and Luikart 1996; Luikart et al. 1998; Luikart and Cornuet 1998). In contrast, reduced allelic richness and possibly reduced M will persist much longer and may be present in

populations that experienced bottlenecks in the more distant past (age>100 generations; Garza and Williamson 2001; Spong and Hellborg 2002).

Bottleneck effects within Lake Clark could be due to several factors including recent declines in the number of sockeye salmon returning to the Kvichak system, an historic and cyclical pattern of low returns (Regnart 1998; Faire 2000), a velocity barrier at the Newhalen River (Poe and Mathisen 1981; 1982), reduction in spawning habitat of established populations, and colonization of newly created habitats by a few individuals (founder effects). Recent reductions in numbers of spawning fish are an unlikely cause of these bottlenecks because typically 5 to 10 generations must pass before these measures are effective (Luikart *et al.* 1998; Storz *et al.* 2002) and recent declines have only occurred since 1999 (Faire 2000), a single generation of sockeye salmon. Cyclic reductions in the numbers of spawners and the sporadic presence of a velocity barrier to fish passage at the Newhalen River are also unlikely causes of these bottlenecks because these events are typically short lived and are not known to have persisted for even a single generation of sockeye salmon (4 to 5 years). It is similarly unlikely that reductions in spawning habitat caused these bottlenecks because known habitat loss and disturbance within the system is not as widespread as the bottleneck signals and there is only weak evidence of bottleneck effects in populations known to be affected by recent habitat loss (e.g., Priest Rock Creek). The presence of both significantly reduced allelic richness and *M* in most Lake Clark populations suggests the putative bottleneck occurred within the last 100 to 400 sockeye salmon generations (~400 to 1400 years for Lake Clark sockeye salmon) depending on the demographic recovery of the population (Garza and Williamson 2001). The prevalence of reduced allelic richness (the longest lasting effect) but lack of consistent significant effects for the most transient bottleneck measures (gametic disequilibrium, allele frequency distribution mode shifts, heterozygosity excess) suggests older bottleneck effects, perhaps associated with the colonization of Lake Clark after the last ice age.

The lack of isolation by distance and the high variability of F_{ST} over geographic distance also support a strong, historical effect of genetic drift among these populations. For all Lake Clark populations but Sucker Bay Lake, historical gene flow predominates suggesting that the genetic similarity found among most Lake Clark sockeye salmon is due to a common founding event. This historical pattern may persist today because high contemporary gene flow resists its erosion by drift. For example, high gene flow among most Lake Clark populations but restricted gene flow due to differences in spawning time between these and populations of Sucker Bay Lake, Six-mile Lake, and Lake Iliamna would help maintain this pattern. This historical pattern may also persist because insufficient time has elapse since colonization to reach equilibrium. The rate of approach to migration-drift equilibrium depends on *Ne* or the inverse of migration rate ($1/m$), whichever is greater (Slatkin 1994). Following a founder event, reaching equilibrium can take tens to hundreds of generations (Crow and Aoki 1984; Waples 1998, Kinnison *et al.* 2002).

Why does the bottleneck signal intensity vary among Lake Clark sockeye salmon populations?
The differences in magnitude of bottleneck signals may reflect differences in state of recovery of genetic variation or age of the bottlenecks. Once genetic variation is reduced in a population, it can only be regained by mutation or immigration. The number of migrants exchanged among populations, and the difference in allele frequencies between immigrants and the population receiving them will have a strong effect on the rate of recovery of populations (Cornuet and Luikart 1996; Waples 2002). Populations within Lake Clark that have weaker bottleneck signals may receive greater numbers of genetically diverse immigrants and/or may have been established longer allowing more time for mutation to restore genetic diversity. Conversely, Lake Clark populations exhibiting strong bottleneck signals (e.g. Sucker Bay Lake) may be highly isolated or recently founded. A founder effect associated with Lake Clark need not apply to all populations within the basin equally. Time since colonization likely varies among Lake Clark populations due to the slow retreat of glaciers and creation of spawning habitat at the northeast end of the

system. Thus, populations at the northeast end of Lake Clark that exhibit strong bottleneck signals (Upper Tlikakila River, Little Lake Clark Beach) may have been more recently founded than other Lake Clark sockeye salmon populations. For example, receding glaciers uncovered the Upper Tlikakila River within the last 200 years (unpublished data, Patricia Heiser, University of Alaska, Anchorage). The most transient of bottleneck signals may not be found in these populations because some recovery has already occurred or because the signals were never generated. The latter might be expected with a bottleneck among individuals that have already experienced a previous bottleneck from the colonization of Lake Clark.

2.5.3 Implications and conclusions

Genetic population structure is often interpreted with the assumption of equilibrium without consideration for bottleneck or founder events. Here we provide an empirical example of the influence of founder effects, both historical and ongoing, on contemporary genetic population structure of Lake Clark sockeye salmon. Non-equilibrium conditions and strong historical drift among these populations has implications both for management of these fish and our understanding of the genetic and phenotypic variation among sockeye salmon populations.

The detection of a common bottleneck effect among Lake Clark sockeye salmon will affect the interpretation of studies of selection and phenotypic variation among populations of Lake Clark sockeye salmon (Gould and Lewontin 1979, Adkison 1995). For example, phenotypic divergence between Six-mile Lake and Lake Clark sockeye salmon may be due to colonization by phenotypically different founders rather than divergent selection since colonization. In contrast, phenotypic divergence among most Lake Clark populations would require divergent selection since their common founding.

The divergence between sockeye salmon in Lake Clark and those in Six-mile Lake and Lake Iliamna will allow fishery managers to identify Lake Clark fish in the Bristol Bay mixed stock fishery. For this application, it does not matter why populations differ, only that they differ

enough to allow assignment of individuals to their lake of origin with confidence. The lack of migration-drift equilibrium among these populations suggests that observed genetic divergence does not reflect current levels of gene flow but rather reflects ancestral association of the founders. Estimates of gene flow among most Lake Clark populations based on F_{ST} will be overestimates because much of the genetic similarity among populations is due to common ancestry. For example, where F_{ST} is low (e.g. 0.01), we would estimate high gene flow (Nm~25 migrants per population, per generation) when in fact the populations may exchange few individuals and have large effective population sizes that maintain similar allele frequencies since a common founding event. In contrast, the higher divergence observed among some populations (e.g. F_{ST} =0.8) will underestimate contemporary gene flow (Nm~3 migrants per population, per generation) between populations that are exchanging individuals since a recent founding by colonizers with different allele frequencies.

The magnitude of genetic differentiation among spawning populations of Lake Clark sockeye salmon is larger than that typically found among populations within the same lake (Wood 1995). The reduced allelic diversity and strong divergence of most Lake Clark populations relative to populations of Six-mile Lake and Lake Iliamna suggest a bottleneck associated with the colonization of Lake Clark by sockeye salmon. The greatest bottleneck effect detected and the most genetically distinct population was Sucker Bay Lake in Lake Clark. There is also significant genetic divergence between populations of Lake Clark and Six-mile Lake, the latter being more similar to fish of Lake Iliamna. Isolation by distance and differences in spawning habitat type apparently do not contribute to isolation and divergence among major population groups. However, temporal isolation based on spawning time and reduced allelic diversity due to bottleneck effects are closely correlated with the pattern of genetic population structure of Lake Clark sockeye salmon. The correlation between differences in genetic variation (allelic richness) and genetic divergence between populations suggests that founder effects have deeply affected the genetic population structure of Lake Clark sockeye salmon. The lack of

correlation between genetic divergence and geographic distance among populations further suggests these populations are not at migration-drift equilibrium and patterns of population divergence are to due to historical events. Non-equilibrium conditions and the strong influence of genetic drift caution against using estimates of genetic divergence to estimate gene flow among populations of Lake Clark sockeye salmon.

Table 2-1 Site description, mean peak spawning date, sample size, heterozygosity, mean number of alleles and allelic diversity (number of alleles corrected for sample size), and mean *M* of Lake Clark, Six-mile Lake, and Lake Iliamna sockeye salmon populations. Spawning habitat type is coded as beach (B) and tributary (T). Allelic richness is standardized to the lowest sample size (N=65).

	Site		Lake	Habitat type	Peak spawning date	N	H_E	Number alleles	Allelic richness	*M*
1	FDI	Fuel Dump Island	Iliamna	B	23-Aug	87	0.48	7.1	6.5	0.78
2	TCI	Talarik Creek	Iliamna	IT	28-Aug	97	0.50	6.5	6.0	0.66
3	TAZ	Tazimina River	Six-mile	IT	25-Aug	99	0.51	6.5	5.8	0.76
4	OUT	Lake Clark Outlet	Six-mile	OT	13-Sep	100	0.52	6.3	5.7	0.77
5	SBL	Sucker Bay Lake	Clark	B	30-Aug	100	0.50	4.3	4.2	0.62
6	CHI	Chi Point	Clark	B	24-Sep	99	0.48	6.0	5.4	0.76
7	KR	Kijik River	Clark	IT	20-Sep	99	0.48	5.3	4.9	0.69
8	LKR	Little Kijik River	Clark	OT	18-Sep	98	0.46	5.0	4.6	0.72
9	KLSB	Kijik Lake South Beach	Clark	B	25-Sep	100	0.45	5.0	4.6	0.65
10	PRC	Priest Rock Creek	Clark	Pond	11-Oct	65	0.48	4.9	4.9	0.68
11	CC	Currant Creek	Clark	IT	25-Sep	100	0.48	5.5	5.0	0.64
12	HPB	Hatchet Point Beach	Clark	B	12-Oct	99	0.50	5.9	5.3	0.73
13	LLCB	Little Lake Clark Beach	Clark	B	7-Oct	100	0.47	5.6	5.1	0.64
14	LTLK	Lower Tlikakila	Clark	IT	7-Oct	100	0.46	5.5	4.9	0.70
15	UTLK	Upper Tlikakila	Clark	IT	25-Sep	100	0.48	5.2	4.8	0.59

Table 2-2 Microsatellite loci analyzed including multiplex annealing temperatures and allelic variation per locus and population.

Locus	Annealing temperature (°C)	Allelic size range	Total no. alleles per locus	Mean no. alleles per population	Reference
Oki1-1	56	106-122	5	3.1	Smith *et al.* 1998
Oki1-2	56	140-164	7	3.0	Smith *et al.* 1998
Omy325	56	118-174	21	10.9	O'Connell *et al.* 1997
uSat60	58	112-136	12	5.6	Estoup *et al.* 1993
Oneμ21	58	110-152	16	10.1	Scribner *et al.* 1996
Ots3	48	74-100	12	4.0	Banks *et al.* 1999
Oneμ18	52	163-189	12	6.1	Scribner *et al.* 1996
Oneμ13	52	154-174	11	5.9	Scribner *et al.* 1996
One105	52	124-144	7	4.5	Olsen *et al.* 2000
Ots107	48	90-130	9	4.1	Nelson and Beacham 1999
Omy77	48	85-121	14	5.4	Morris *et al.* 1996

Table 2-3 Pair-wise F_{ST} (below diagonal) and number of loci with significant allele frequency differences after sequential Bonferroni correction (above diagonal) between sockeye salmon of Lake Clark, Six-mile Lake, and Lake Iliamna. NS = comparisons without significant allele frequencies differences over all loci (P>0.05 Fisher's combined probability test). Refer to Table 2 for population numbers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	FDI	TCI	TAZ	OUT	SBL	CHI	KR	LKR	KLSB	PRC	CC	HPB	LLCB	LTLK	UTLK
FDI	-	4	7	7	11	8	7	7	8	6	10	6	7	10	8
TCl	0.016	-	8	7	11	8	11	8	7	7	9	9	9	10	10
TAZ	0.043	0.023	-	NS	9	7	9	10	10	8	8	9	10	10	9
OUT	0.026	0.018	0.003	-	9	7	10	8	10	8	7	9	10	10	9
SBL	0.054	0.045	0.065	0.061	-	8	8	9	9	7	8	7	9	8	7
CHI	0.060	0.062	0.052	0.051	0.063	-	NS	2	2	1	0	NS	0	0	NS
KR	0.052	0.055	0.052	0.049	0.044	0	-	4	3	1	1	0	9	10	0
LKR	0.065	0.071	0.069	0.066	0.077	0.014	0.017	-	NS	2	2	3	4	4	4
KLSB	0.067	0.071	0.071	0.069	0.064	0.012	0.011	0.001	-	3	1	2	4	1	2
PRC	0.062	0.057	0.058	0.058	0.050	0.004	0.005	0.012	0.012	-	2	2	2	2	2
CC	0.067	0.065	0.056	0.057	0.057	0	0	0.011	0.008	0.002	-	1	2	NS	1
HPB	0.055	0.060	0.053	0.048	0.060	0.001	0.004	0.013	0.010	0.009	0.004	-	NS	0	0
LLCB	0.070	0.069	0.059	0.061	0.060	0.003	0.005	0.024	0.017	0.013	0.005	0.002	-	0	NS
LTLK	0.089	0.085	0.069	0.074	0.069	0.003	0.006	0.018	0.013	0.007	0	0.006	0.004	-	NS
UTLK	0.076	0.077	0.065	0.066	0.067	0	0.003	0.018	0.013	0.007	0	0.001	0	0	-

Table 2-4 Results of Mantel tests between genetic divergence (pair-wise F_{ST}) and spawning habitat (HAB), geographic distance (GEODIST), difference in spawning time (SPTIME), and difference in mean allelic richness (ALLRICH) among Lake Clark, Six-mile Lake, and Lake Iliamna sockeye salmon. Controlled variable in partial Mantel tests in parentheses, * indicates significance at P<0.05.

	All populations		Lake Clark populations	
Test	*r*	*P*	*r*	*P*
HAB	-0.003	0.97	0.049	0.72
GEODIST	0.263*	<0.01	-0.106	0.45
SPTIME	0.505*	<0.001	0.409*	<0.01
ALLRICH	0.515*	<0.001	0.247	0.08
SPTIME (ALLRICH)	0.375*	<0.001	0.393*	<0.01
ALLRICH (SPTIME)	0.389*	<0.001	0.218	0.11

Figure 2-1 Map of Lake Clark, Six-mile Lake, and Lake Iliamna with sample sites shown. Refer to Table 2 for population numbers.

Figure 2-2 Principal component analysis of allele frequencies at 11 microsatellite loci. Points represent populations from Lake Clark (black), Six-mile Lake (gray), or Lake Iliamna (white). Percentages in parentheses indicate amount of variation explained by each principal component. Refer to Table 2 for population numbers.

Figure 2-3 Principal component analysis of Lake Clark sockeye salmon (excluding Sucker Bay Lake) allele frequencies at 11 microsatellite loci. Highly divergent populations of Kijik Lake and Priest Rock Creek are identified. Percentages in parentheses indicate amount of variation explained by each principal component. Refer to Table 2 for population numbers.

Figure 2-4 Allele frequency distributions of Six-mile Lake, Lake Clark, and Sucker Bay Lake sockeye salmon. The far left bar of each plot indicates the proportion of rare alleles (frequency less than 0.1).

Figure 2-5 Relationship between mean expected heterozygosity (H_E) observed and expected at mutation-drift equilibrium under the Infinite Alleles Model of mutation (IAM) for Lake Clark, Six-mile Lake, and Lake Iliamna sockeye salmon populations. Recently bottlenecked populations have greater heterozygosity (H_E) than expected at migration-drift equilibrium due to the loss of rare alleles. Non-bottleneck populations have an H_E that is equal to or less than that expected under IAM (on or below equality line). Points represent populations from Lake Clark (black), Six-mile Lake (gray), or Lake Iliamna (white). Refer to Table 2 for population numbers.

Figure 2-6 Plot of F_{ST} against geographial distance among Lake Clark (▲), Six-mile Lake (□), and Lake Iliamna sockeye salmon populations (□).

Figure 2-7 Plot of mean $F2_{ST}$ between and mean allelic richness within spawning populations of Lake Clark sockeye salmon (all Lake Clark populations: P=0.01; excluding Sucker Bay Lake: P=0.11).

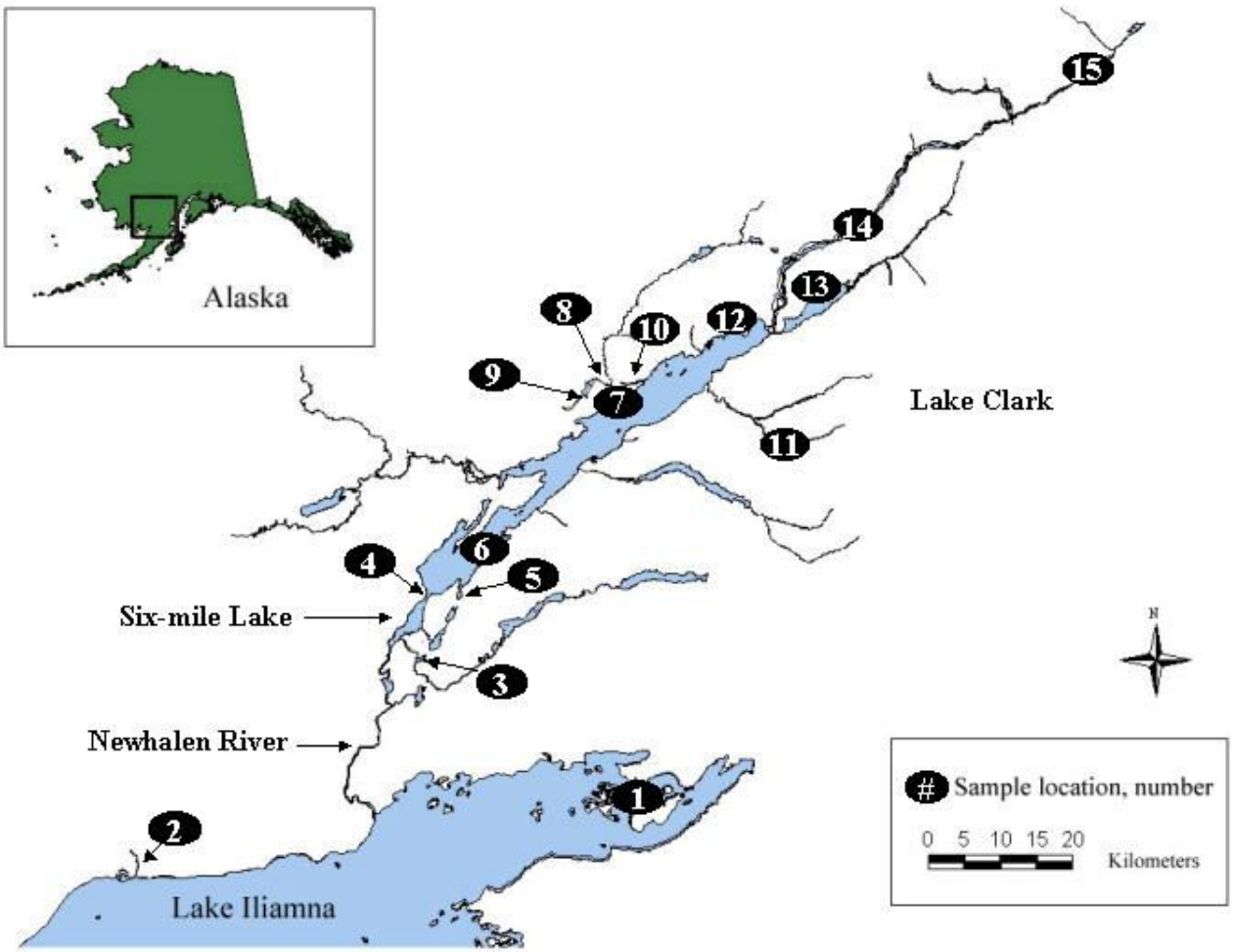
Alaska
Lake Clark
Six-mile Lake
Newhalen River
Lake Iliamna
Sample location, number
0 5 10 15 20
Kilometers
N
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

Figure 2-1

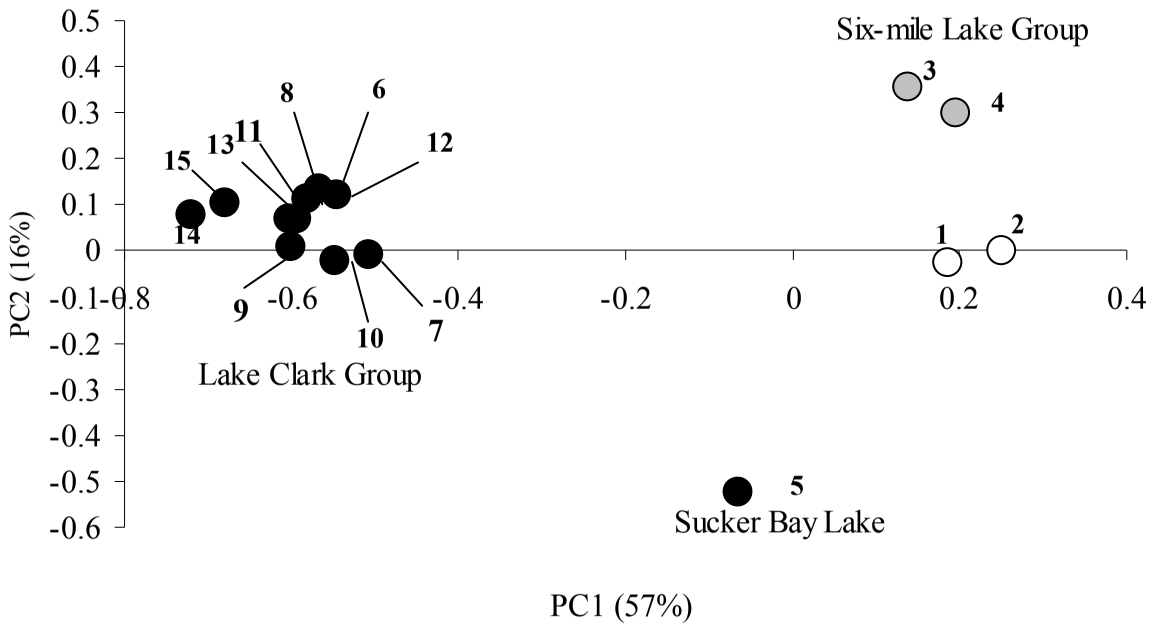
Six-mile Lake Group
3
4
1
2
8
6
12
11
13
15
14
9
10
7
Lake Clark Group
5
Sucker Bay Lake
PC2 (16%)
PC1 (57%)
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4

Figure 2-2

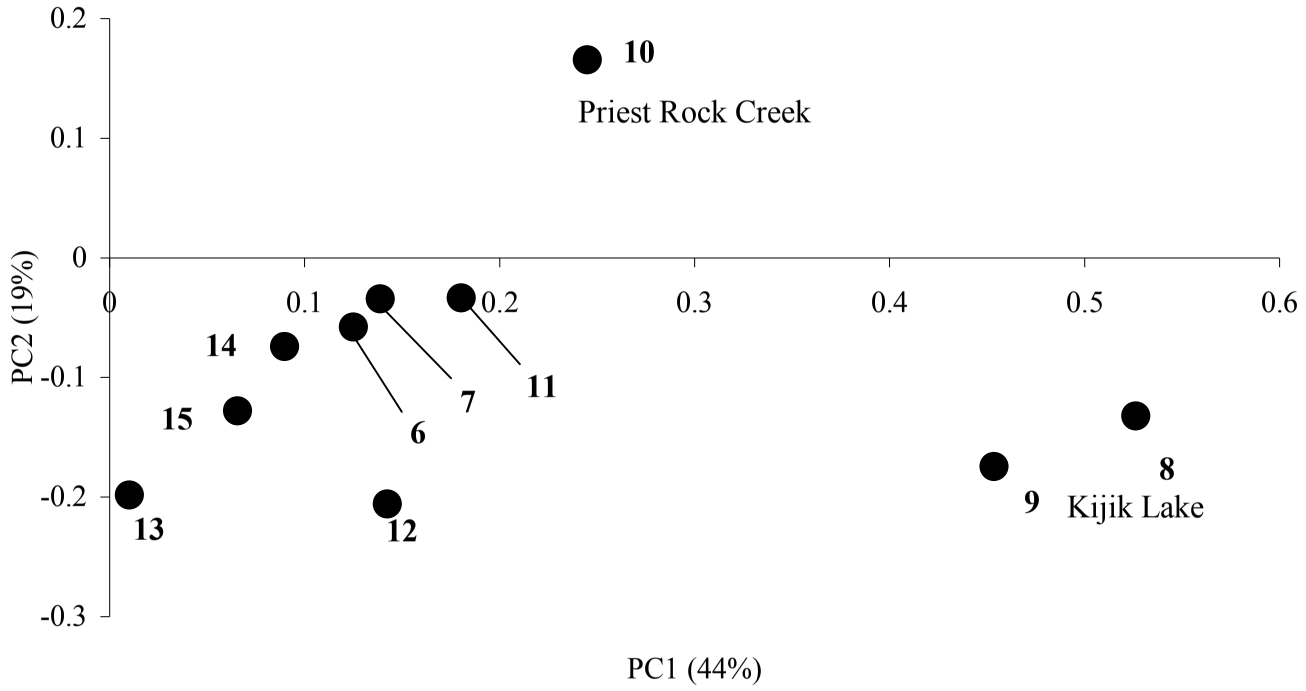
0.2
0.1
0
-0.1
-0.2
-0.3
0
0.1
0.2
0.3
0.4
0.5
0.6
PC2 (19%)
PC1 (44%)
10
Priest Rock Creek
14
15
13
6
7
11
12
8
9
Kijik Lake

Figure 2-3

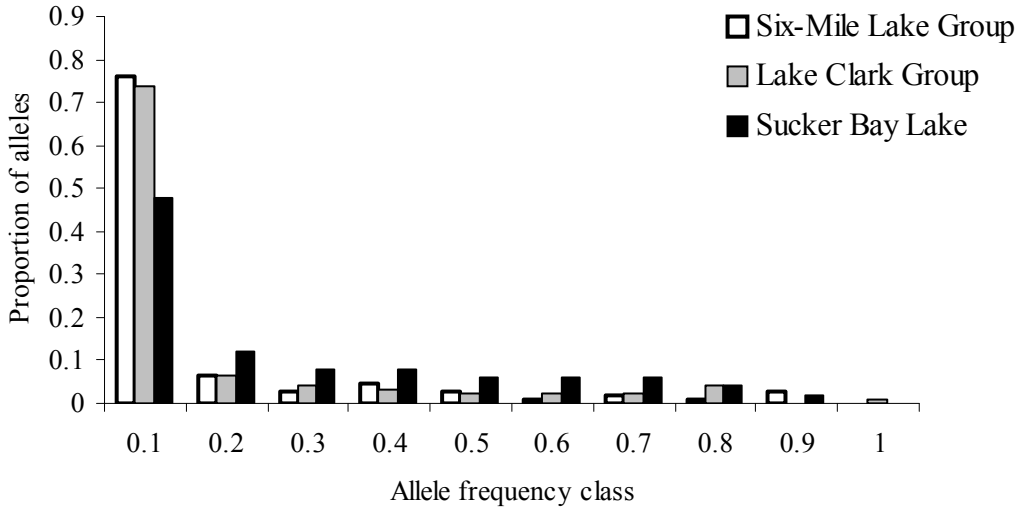
Six-Mile Lake Group
Lake Clark Group
Sucker Bay Lake
Proportion of alleles
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Allele frequency class

Figure 2-4

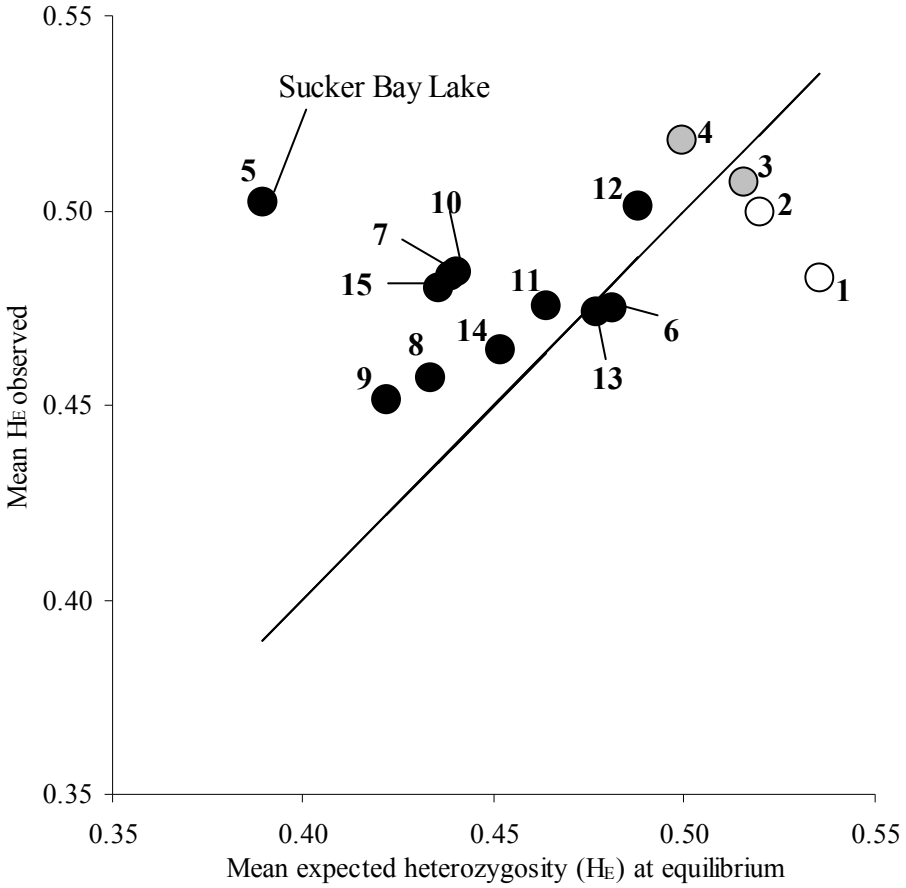
0.55
0.50
0.45
0.40
0.35
Mean HE observed
Sucker Bay Lake
5
4
3
2
1
12
10
7
15
11
6
14
8
9
13
0.35
0.40
0.45
0.50
0.55
Mean expected heterozygosity (HE) at equilibrium

Figure 2-5

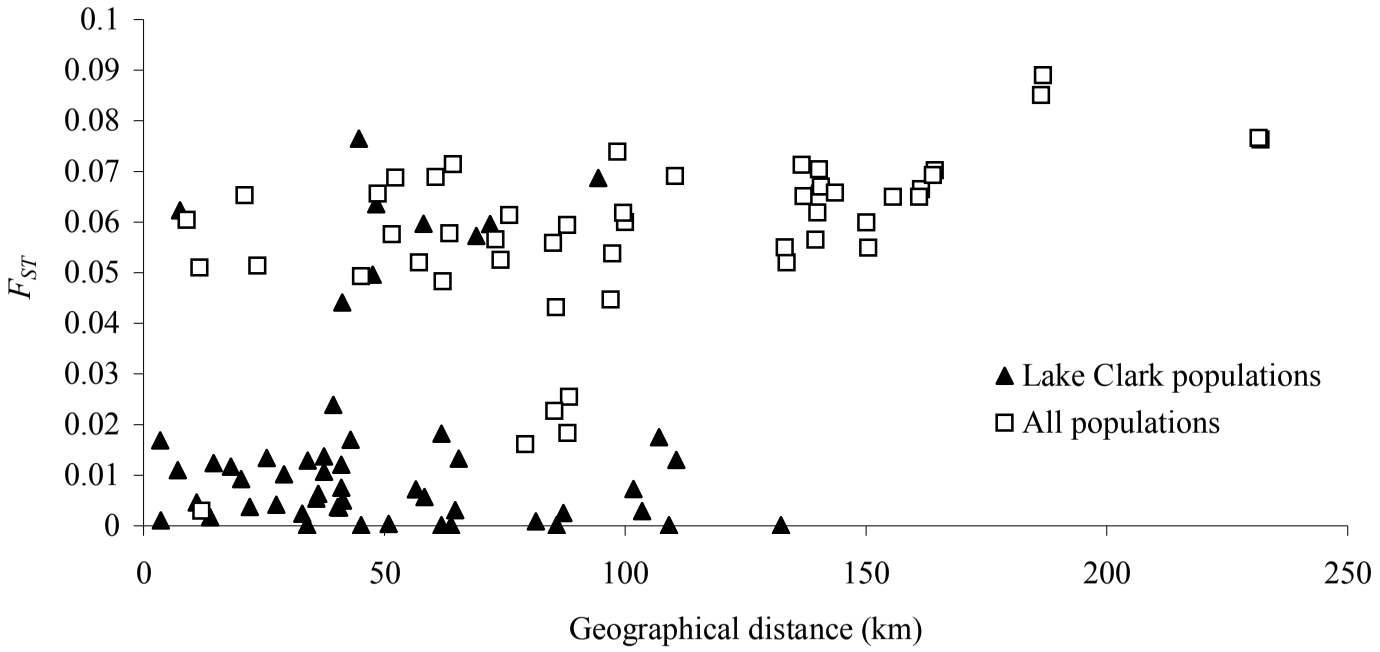
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
F_{ST}
0
50
100
150
200
250
Geographical distance (km)
Lake Clark populations
All populations

Figure 2-6

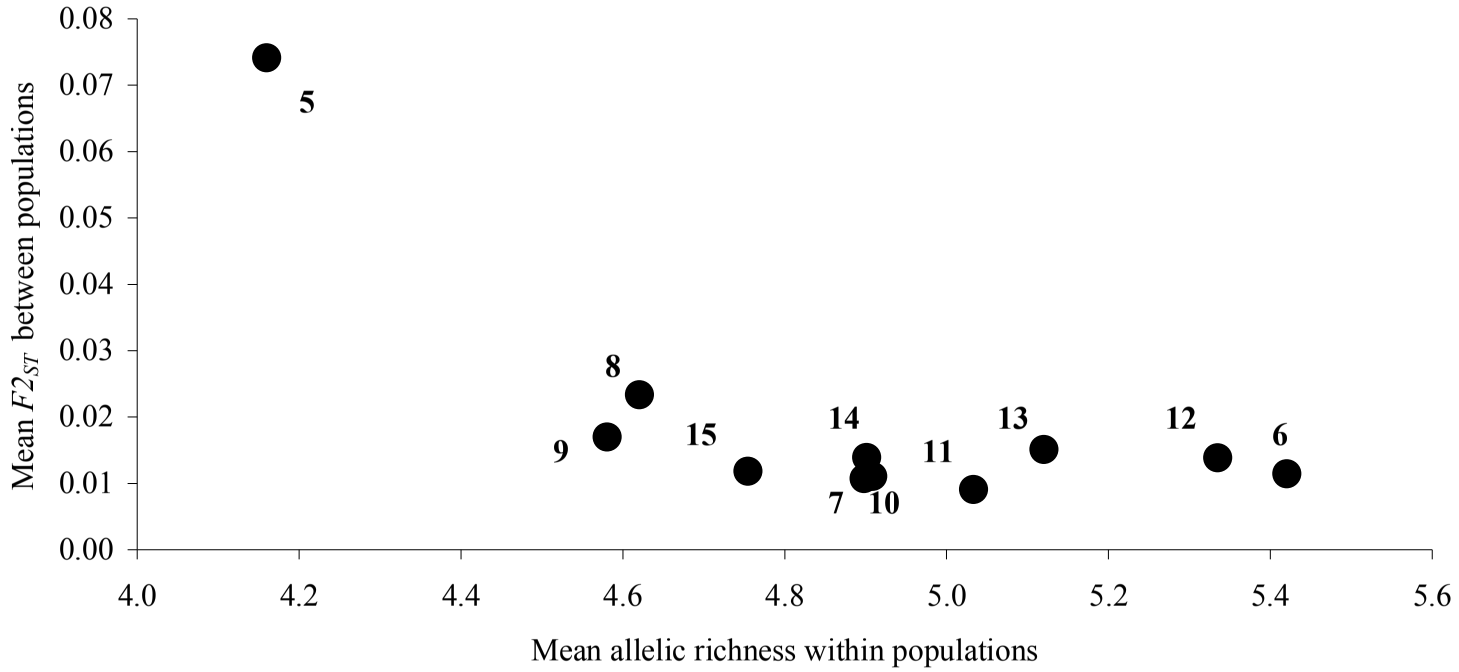
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Mean $F2_{ST}$ between populations
4.0
4.2
4.4
4.6
4.8
5.0
5.2
5.4
5.6
Mean allelic richness within populations
5
8
9
15
14
11
13
12
6
7
10

Figure 2-7

Chapter 3 – Concordance of nuclear and mitochondrial DNA markers in detecting a founder event in Lake Clark sockeye salmon

3.1 Abstract

Genetic bottleneck effects can reduce genetic variation, persistence probability, and evolutionary potential of populations. Previous microsatellite analysis suggested a bottleneck associated with a common founding of sockeye salmon populations of Lake Clark, Alaska. The common founding event resulted in reduced allelic diversity and strong divergence of Lake Clark sockeye salmon relative to neighboring Six-mile Lake and Lake Iliamna populations. Here we use two additional genetic marker types (allozymes and mtDNA) to examine these patterns further. Allozyme and mtDNA results were congruent with the microsatellite data in suggesting a common founder event in Lake Clark sockeye salmon and confirmed the divergence of Lake Clark populations from neighboring Six-mile Lake and Lake Iliamna populations. The use of multiple marker types provided better understanding of the bottleneck in Lake Clark. For example, the Sucker Bay Lake population had an exceptionally severe reduction in allelic diversity at microsatellite loci but not at mtDNA. This suggests that the reduced microsatellite variation in Sucker Bay Lake fish is due to consistently smaller effective population size than other Lake Clark populations, rather than a more acute or additional bottleneck since founding. Caution is urged in using reduced heterozygosity as a measure of genetic bottleneck effects because stochastic variance among loci resulted in an overall increase in allozyme heterozygosity within bottlenecked Lake Clark populations. However, heterozygosity excess, which assesses heterozygosity relative to allelic variation, detected genetic bottleneck effects in both allozyme and microsatellite loci.

3.2 Introduction

A genetic bottleneck occurs when a population experiences a temporary severe reduction in effective population size (N_e; Avise 1994). Reduced N_e promotes loss of genetic variation due to genetic drift (random changes in allele frequencies as a result of imperfect sampling of alleles between generations) and an increase in genetic divergence between bottlenecked populations and those at mutation-drift equilibrium (non-bottlenecked). Founder effects are genetic bottleneck effects that are associated with the founding of a new population.

It is important to identify populations that have experienced acute bottleneck effects (e.g., $\leq$20 N_e) because their low genetic variation may result in reduced evolutionary potential and probability of persistence (Luikart et al. 1998; Soulé and Mills 1998; Briskie and Mackintosh 2004). Similarly, it is important to differentiate between populations that experienced ancient bottlenecks and those that had more recent bottlenecks (e.g., within the past 100 generations), because the latter may also be experiencing problems due to small population size (demographic stochasticity, inbreeding, fixation of deleterious alleles) and may respond to mitigative management (Cornuet and Luikart 1996; Luikart et al. 1998). The two scenarios are not mutually exclusive. For example, populations that experienced bottleneck events in the distant past may continue to lose genetic variation over time due to low N_e and lack of genetically diverse immigrants (Crow and Aoki 1984; Slatkin 1994).

A suite of statistical tests is available to identify genetic signals of bottlenecked populations. These include tests for reduced allelic diversity in general, loss of rare alleles in particular (Allendorf 1986; Luikart et al. 1998), and heterozygosity excess (Maruyama and Fuerst 1985; Cornuet and Luikart 1996; Luikart and Cornuet 1998). Multiple genetic markers, including microsatellites, allozymes, and mitochondrial DNA (mtDNA), may be similarly tested to effectively detect genetic bottleneck effects.

Microsatellites are sensitive to recent bottleneck effects because they have large numbers of alleles as a result of high mutation rates (Weber and Wong 1993; Ellegren 2000). Rare alleles

(frequency <0.01) tend to be abundant at microsatellite loci, and a reduction in their proportion relative to higher frequency alleles is a sensitive indicator of genetic bottleneck effects.

Allozymes generally have fewer alleles than microsatellites because of their lower mutation rates (Nei 1987). This lack of allelic diversity suggests allozyme loci will be less sensitive to bottleneck effects. However, rare alleles are expected to be more abundant than alleles of intermediate frequency at neutral loci regardless of the mutation rate or model (Nei et al. 1976) and natural populations typically exhibit an abundance of rare allozyme alleles (Chakraborty et al. 1980; Luikart et al. 1998). In a survey of natural populations, allozyme loci successfully identified bottlenecked populations but with a lower success rate than microsatellites (Luikart et al. 1998).

Mitochondrial DNA generally has one quarter the effective population size of nuclear DNA (nDNA; e.g., microsatellite and allozyme loci) because is it haploid and maternally inherited (Nei and Tajima 1981; Birky et al. 1983). Thus, genetic drift is amplified at mtDNA relative to nDNA resulting in greater loss of mtDNA variation during a bottleneck, greater divergence between bottlenecked and non-bottlenecked populations, and slower recovery of genetic variation following a bottleneck than nDNA. Patterns of mtDNA variation also contain information about sex-biased dispersal and immigration because mtDNA is maternally inherited. For example, many studies have interpreted strong mtDNA divergence and little or no nDNA divergence among populations as evidence of male mediated gene flow and female philopatry (Palumbi and Baker 1994; Pope et al. 2000; Waits et al. 2000; Tiedemann et al. 2004).

Microsatellites, allozymes, and mtDNA, therefore, all contain information about the bottleneck history and genetic structure of populations. However, the nature of this information differs among the three marker types and comparing bottleneck signals in mtDNA and nDNA can provide information on the duration and timing of bottleneck events (Wilson et al. 1985; Birky 1991). For example, a population that experienced an acute, brief bottleneck in the distant past may presently exhibit little or no reduction in nDNA variation but significantly reduced mtDNA

variation. Similarly, a population that experienced a prolonged bottleneck effect will exhibit a significant reduction in both nDNA and mtDNA variation because of the continued loss of nDNA variation at the rate of $1/2N_e$ per generation.

In this study, we use all three marker types to provide a comprehensive picture of genetic bottleneck effects and population structure of sockeye salmon (*Oncorhynchus nerka*) populations of Lake Clark, Alaska (Figure 1). Lake Clark sockeye salmon comprise one to 75% of the annual return to the Kvichak River watershed, the largest sockeye salmon spawning and rearing system in the world (Rogers et al. 1999; Woody 2004). The Kvichak system was historically the largest contributor of sockeye salmon to the Bristol Bay fishery, valued at up to US$350 million (Fair 2003; Link et al. 2003). However, Kvichak River sockeye salmon are now listed as a "stock of management concern" by the Alaska Department of Fish and Game due to a chronic inability to meet minimum escapement goals over the last 10 years (Westing et al. 2005). Lake Clark sockeye salmon also support subsistence and recreational fisheries and provide critical marine derived nutrients to the Lake Clark ecosystem, the centerpiece of Lake Clark National Park and Preserve (Kline et al. 1993; Willson and Halupka 1995).

A previous study (Ramstad et al. 2004) based on microsatellite analysis reported a founder event in Lake Clark sockeye salmon. Spawning populations of Lake Clark sockeye salmon exhibited reduced genetic variation and strong genetic divergence from populations of neighboring Six-mile Lake and Lake Iliamna (Figure 1). The Sucker Bay Lake population of Lake Clark exhibited the most extreme bottleneck signal with lower allelic richness (A: 4.2) and proportion of rare alleles (0.45) than all other populations surveyed (A = 4.6 to 6.5; proportion of rare alleles = 0.53 to 0.70), and a significant excess of heterozygosity relative to that expected at mutation-drift equilibrium. In addition, populations in Six-mile Lake were more similar in diversity and frequency of microsatellite alleles to Lake Iliamna than they were to Lake Clark populations. This result is surprising because Six-mile Lake is geographically proximate to Lake

Clark and separated from Lake Iliamna by the Newhalen River (~39 km in length) which is a barrier to salmon migration at high water velocities (Poe and Mathisen 1982).

Given the economic, ecologic, and cultural importance of Lake Clark sockeye salmon, it is important to verify the reduced genetic variation detected by microsatellite analysis. Testing this pattern with additional markers may also provide insight into the nature and timing of bottleneck events in Lake Clark sockeye salmon and determine if mitigative management is warranted to conserve recently bottlenecked populations.

In this study, we use two additional genetic markers (allozymes and mtDNA) to further examine patterns of genetic bottleneck effects and population structure in Lake Clark sockeye salmon. We address three specific questions. (1) Do allozyme and mtDNA data suggest the same pattern of founder effects as microsatellites in Lake Clark sockeye salmon? (2) Is the reduced genetic variation of Sucker Bay Lake sockeye salmon due to a recent, acute bottleneck effect or consistently low effective population size? And (3) does the Six-mile Lake population share similar allozyme allele and mtDNA haplotype frequencies with Lake Iliamna or Lake Clark populations?

3.3 Methods

3.3.1 Microsatellites

The microsatellite data used here are a subset of those reported in Ramstad et al. (2004). We drew on these data to provide direct comparisons among marker types in samples of the same populations and, where possible, individual fish. Samples from the eight populations included in this study (Figure 1, Table 1) are identical to those in Ramstad et al. (2004). Data from all three marker types (microsatellites, allozymes, mtDNA) were collected from the same individuals for the Lower Talarik Creek (TCI), Fuel Dump Island (FDI – also known as Flat Island), Kijik River (KR), and Upper Tlikakila River (UTLK) populations. Both microsatellite and mtDNA data were

collected from the same individuals in the Kijik Lake South Beach (KLSB) and Sucker Bay Lake (SBL) populations while mtDNA and allozyme data were collected from the same fish in the Tazimina River (TAZ) population.

3.3.2 Allozymes

Heart, muscle, liver, and eye tissues were collected from approximately 100 adult sockeye salmon captured by seine and tangle net from seven Lake Clark, Six-mile Lake, and Lake Iliamna spawning sites in 2000 and 2001 (Figure 1, Table 1). Sucker Bay Lake fish were not sacrificed for tissue samples out of concern for the extremely small size of this population (<500 fish observed per sampling year) and an inability to insure sampling of only post-spawning fish. Samples were immediately placed on dry ice and kept frozen (-80°C) until used in allozyme analysis. The tissue and gel protocols were those of Seeb et al. (2000); allele and locus nomenclature followed the American Fisheries Society standard (Shaklee et al. 1990).

Six of the 41 allozyme loci surveyed are isoloci (*mAH-1,2*; *sMDH-A1,2*; *sMDH-B1,2*; *TPI-1,2; G3PDH-1,2*; *GPI-B1,2*) which are duplicate loci that share alleles with identical electrophoretic mobility (Allendorf and Thorgaard 1984). Individuals possess four alleles at each isolocus and their genotypes cannot be accurately resolved because it is impossible to assign observed variation to a particular locus of the pair without extensive experimental matings (Waples 1988). In this study, every individual had at least two copies of the most frequent allele at each isolocus. Therefore, we treated isoloci as single, disomic loci that contained all of the variation observed within each individual, at each isolocus.

Twenty-two monomorphic allozyme loci were excluded from further analysis (*sAAT-1,2*; *ADA-1*; *mAH-4*; *CK-A1*; *CK-A2*; *FDH*; *FH*; *PEPA*; *G3PDH-1,2*; *G3PDH-3*; *G3PDH-4*; *mIDHP-1*; *mIDHP-2*; *sIDHP-1*; *LDH-A1*; *LDH-A2*; *LDH-B1*; *LDH-C*; *PEPB-1*; *PEPLT*; *PGDH*; *sSOD-1*). Of the nineteen loci included in subsequent analyses, ten had a common allele frequency of at least 0.99 in all populations surveyed (*sAAT-3*; *sAH*; *GPI-B1,2*; *GPI-A*; *sIDHP-2*; *sMDH-A1,2*;

sMDH-B1,2; *PEPD-1*; *TPI-3*; *TPI-4*; Table 2). The common allele at each locus was **100* except for *mAAT-1* and *TPI-1,2* where the common allele was **-100* and *PGM-1* where a null allele was most common. Frequencies of the null allele at *PGM-1* were estimated by treating the absence of product as homozygous for the null allele and assuming Hardy-Weinberg proportions (Allendorf and Seeb 2000).

3.3.3 Mitochondrial DNA

Dorsal fin tissue clips (approximately 5mm2) were collected from fish from eight spawning populations (Figure 1, Table 1) and immediately stored in 100% ethanol. Total genomic DNA was extracted from fin clips according to Spruell et al. (1999).

Restriction fragment length polymorphism (RFLP) analysis was performed on 23 to 30 fish per population using the ND1/ND2 region of mtDNA. This region was selected because it is variable and representative of genetic variation patterns found in other regions of sockeye salmon mtDNA (Churikov et al. 2001; Doiron et al. 2002). The ND1/ND2 region (~2600bp) was amplified in 35 µl polymerase chain reactions (PCR) using primers given by Carney et al. (1997) and Gharrett et al. (2001). The reaction mixture contained 10mM Tris-HCl (pH 9.0), 50mM KCl, 0.2mM each dNTP, 2.0mM $MgCl_2$, 0.2µM each primer, and 1.75 units of Taq DNA polymerase (Fisher Scientific, Fair Lawn, NJ). Amplification was carried out in an MJ Research PTC-200 Peltier thermal cycler with the following PCR profile: 95°C for 2 min followed by 30 cycles of denaturing at 95°C for 1 min, annealing at 50°C for 1 min, and elongation at 72°C for 3 min; amplification was completed with a final extension step of 72°C for 3 min.

Amplified product was digested with each of four restriction endonucleases (BstN I, BstU I, Ban II, Sau96 I; New England BioLabs, Beverly, MA) in separate, 20µl reactions. Reactions consisted of a 5µl sub sample of the PCR product, 2µl of 10x restriction buffer, and 3 units of restriction enzyme. Digests with BstN I also included 0.2µl of 100X bovine serum albumin (BSA). Digest reactions were incubated at either 60°C (BstN I, BstU I) or 37°C (Ban II,

Sau96 I) for three hrs, the latter was inactivated by exposure to 80°C for twenty minutes. Digest products were electrophoresed on 1.5% agarose gels prepared with SeaKem® LE agarose and ethidium bromide in 0.5x TBE buffer (pH~8.0; 0.045M Tris, 0.045M Boric acid, and 0.001M EDTA). Gels were scanned on Hitachi FMBIO® 100 and Hitachi FMBIO® II and band sizes scored relative to a 100bp DNA ladder size standard (Invitrogen Life Technologies, Carlsbad, CA) using Hitachi FMBIO® Analysis software V8.0. Blank reactions (all constituents present but template DNA) and uncut PCR product were included in each gel to detect sample contamination.

A letter code was assigned to each distinct banding pattern for each enzyme reaction and these were used in combination (order: BstN I, BstU I, Ban II, Sau96 I) to describe composite haplotypes as in Churikov et al. (2001; Table 3). Fragments less than 140bp in length could not be resolved with the methods described above but were diagnostic of only a single banding pattern (C) of a single enzyme (Sau96 I) previously described by Churikov et al. (2001). This pattern was identified by the presence of a larger, easily viewed fragment. Thus, we expect that only novel haplotypes distinguished by bands <140bp would go undetected with this method.

3.3.4 Statistical analysis

Gametic disequilibrium was assessed for all pair-wise locus comparisons within each population using a Fisher exact test in GENEPOP version 3.3 (Raymond and Rousset 1995a). Gametic disequilibrium among loci of different marker types was assessed for only a subset of the populations sampled because data for each marker type was not always collected on the same fish from each population. Therefore gametic disequilibrium was assessed between microsatellite and allozyme loci in four populations (all but TAZ, SBL, Little Kijik River - LKR, and KLSB), between microsatellite loci and mtDNA in six populations (all but TAZ and LKR) and between allozyme loci and mtDNA in five populations (all but SBL, LKR and KLSB). Departures from Hardy-Weinberg proportions (Guo and Thompson 1992) and heterogeneity in population allele

frequencies (Raymond and Rousset 1995b) were tested and pair-wise F_{ST} (Wright 1951; Weir and Cockerham 1984) and R_{ST} (Slatkin 1995; Rousset 1996) calculated in GENEPOP version 3.3 (Raymond and Rousset 1995a). Tests of significance were combined over all loci using Fisher's combined probability test (Sokal and Rohlf 1981). Heterogeneity in haplotype frequencies was tested and pair-wise mtDNA F_{ST} was calculated in ARLEQUIN version 2.000 (Schneider et al. 2000). Principle component analysis (PCA) for allozyme and microsatellite allele and mtDNA haplotype frequencies was performed using the covariance matrix in MINITAB, version 13 (State College, PA). One allele at each allozyme and microsatellite locus and the rarest mtDNA haplotype was omitted from these analyses to allow for the non-independence of allele/haplotype frequencies within loci (Johnson 1998). Sequential Bonferroni adjustments were made for all multiple comparisons (Rice 1989).

A suite of tests was used to detect genetic bottleneck effects. Mean heterozygosity (H_E: heterozygosity expected under Hardy-Weinberg proportions given observed allele frequencies) for all marker types was calculated as Nei's gene diversity (Nei 1987) in FSTAT version 1.2 (Goudet 1995). Because the number of alleles detected in a population is highly dependent on sample size, allelic richness (A: mean number of alleles or haplotypes per population and locus corrected for sample size) was computed and compared among population groups according to El Mousadik and Petit (1996) in FSTAT. Mode shift in allele frequency distribution within populations (lower proportion of rare alleles relative to those with a frequency of 0.2 to 0.3) was assessed graphically for nuclear markers (Luikart et al. 1998). Difference in proportion of polymorphic allozyme loci, mean proportions of rare alleles, and H_E within marker types and among lakes was tested with a one tailed Mann-Whitney U test (H_a: lower values among Lake Clark than Lake Iliamna and Six-mile Lake populations) in MINITAB, version 13 (State College, PA).

Bottlenecked populations exhibit greater H_E than expected if the population was at mutation-drift equilibrium (H_{Eq}: heterozygosity expected given Hardy-Weinberg proportions, the

observed number of alleles, and a constant population size). This effect can be due to either a greater reduction in allelic diversity than heterozygosity (allelic deficit) or a random increase in H_E due to drift (Luikart et al. 1998; Tarr et al. 1998). Heterozygosity excess relative to a non-bottlenecked population at mutation-drift equilibrium ($H_E > H_{Eq}$) was assessed in BOTTLENECK, version 1.2.02 (Cornuet and Luikart 1996) for nuclear markers. This test is performed with the assumption of the infinite alleles model of mutation (IAM) though microsatellites are expected to generally conform more closely to a step-wise mutation model (SMM; Shriver et al. 1993) or a two phase model of mutation (TPM) incorporating aspects of both the IAM and the SMM (Luikart et al. 1998). The appropriate mutation model can vary among loci of the same marker type as well (Slatkin 2002). In the present context, the use of the IAM is equal to the assumption that the sampled Iliamna populations are not bottlenecked because their observed heterozygosity closely matches that expected at mutation-drift equilibrium under the IAM (Cornuet and Luikart 1996). Significance of heterozygosity excess over all loci was assessed with a Wilcoxon sign-rank test (Cornuet and Luikart 1996, Luikart and Cornuet 1998).

3.4 Results

3.4.1 Genetic variation within populations

Nineteen of 41 allozyme loci surveyed were polymorphic (2 to 4 alleles each; Table 2) and all populations were in Hardy-Weinberg proportions at all polymorphic allozyme and microsatellite loci combined within marker type. Only one locus (*PGM2*) in a single population (KLSB) deviated significantly from Hardy-Weinberg proportions ($P<0.05$ after sequential Bonferroni correction).

Five haplotypes were observed in the ND1/ND2 region of mtDNA (Figure 2, Table 3). Three of these haplotypes were previously described among sockeye salmon by Churikov et al. (2001; our haplotype *AACA* = their haplotypes *L* and *Q*, *ABBA =H, ABBC=G*), while two others

(*ABDC, AAFA*) are new. The *ABDC* and *AAFA* haplotypes differ from the *ABBC* and *AACA* haplotypes by the presence of an additional *Ban* II cut site that results in the absence of a 340bp band and the presence of 190bp and 150bp bands (Figure 2). The *AAFA* haplotype could also have been derived from the *ABDC* haplotype with the gain of three cut sites found among other haplotypes (Figure 2).

There was no tendency toward gametic disequilibrium between any pair of loci or within any population. Significant gametic disequilibrium ($P<0.05$ after sequential Bonferroni correction across populations) was observed in only 7 of 440 pair-wise microsatellite locus comparisons and 2 of 252 possible pair-wise allozyme locus comparisons. Significant gametic disequilibrium was present in 2 of 39 comparisons between mtDNA and allozyme loci and was not observed between mtDNA and microsatellite loci. Gametic disequilibrium was significant in 7 of 385 possible pair-wise comparisons between microsatellite and allozyme loci. Overall, there was no evidence for biologically meaningful gametic disequilibrium (significant gametic disequilibrium in multiple loci within the same population or vice versa).

3.4.2 Genetic bottleneck effects

A lower proportion of polymorphic allozyme loci was observed in Lake Clark populations (0.41) than Six-mile Lake and Lake Iliamna populations (0.56; $P<0.05$). Mean allelic richness (A) was significantly reduced in Lake Clark populations relative to Six-mile Lake and Lake Iliamna populations for all three marker types ($P=0.02$; Table 1). Microsatellite H_E was not lower among Lake Clark populations than Six-mile Lake and Lake Iliamna populations ($P=0.12$). However, mtDNA H_E was lower among Lake Clark than Six-mile Lake and Lake Iliamna populations ($P=0.02$). Allozyme H_E was greater in all Lake Clark populations than all Six-mile Lake and Lake Iliamna populations (Table 1) and thus negated the need for a formal one-tailed test of the opposite expectation. These differences persisted when populations were pooled (Table 1).

In addition, fewer alleles and haplotypes were observed in Lake Clark fish than Six-mile Lake and Lake Iliamna despite a greater sample size of Lake Clark fish. Only 82 microsatellite alleles were observed in 496 Lake Clark fish while 102 were observed in 283 Six-mile Lake and Lake Iliamna fish. Thirty-three allozyme alleles were observed in the 400 Lake Clark fish sampled while 40 were present in 299 fish sampled from Six-mile Lake and Lake Iliamna. Finally, 3 mtDNA haplotypes were present in 126 Lake Clark fish sampled while 5 haplotypes were observed in 77 Six-mile Lake and Lake Iliamna fish sampled. In total, 118 alleles and haplotypes were detected among 1022 Lake Clark fish sampled while 147 alleles and haplotypes were detected in 659 fish sampled from Six-mile Lake and Lake Iliamna (alleles and fish sample sizes summed over loci). Alleles and haplotypes present in Lake Clark populations were generally a subset of those found commonly in Six-mile Lake and Lake Iliamna populations (Figure 2, Table 2, Table 3). Exceptions were a single allozyme allele at the *LDH-B2* locus (Table 2) and 16 of 120 alleles found in 9 of 11 microsatellite loci surveyed (data not shown). These alleles were all rare (frequency less than 0.10).

There were no mode-shifts in microsatellite allele frequency distribution in any population. Lake Clark populations had a lower mean proportion of rare (frequency <0.1) microsatellite alleles (0.54) than Six-mile Lake and Lake Iliamna populations (0.65; P=0.02). Sucker Bay Lake had a lower proportion of rare microsatellite alleles (0.45) than any other population surveyed (range = 0.53 to 0.69). All Lake Clark populations had greater mean microsatellite heterozygosity than Six-mile Lake and Lake Iliamna populations under the IAM (Figure 3). Significant heterozygosity excess was observed only in Sucker Bay Lake (H_E-H_{Eq}=0.118; P<0.01), but not in any other Lake Clark population (H_E-H_{Eq}=0.024 to 0.045; P=0.12 to 0.26). There was no evidence of microsatellite heterozygosity excess in any population under the SMM or TPM.

The Little Kijik River population of Lake Clark displayed a shift in allozyme allele frequency mode (proportion of rare alleles = 0.125, proportion of alleles with frequency between

0.2 to 0.3 = 0.313) and Lake Clark populations had a significantly reduced mean proportion of rare allozyme alleles (0.22) relative to populations of Six-mile Lake and Lake Iliamna (0.37; P=0.03). All the Lake Clark populations displayed an excess of allozyme heterozygosity relative to Six-mile Lake and Lake Iliamna under the IAM (Figure 3). This effect was statistically significant in two populations (LKR, H_E-H_{Eq}=0.087; UTLK, H_E-H_{Eq}= 0.098; P<0.05).

3.4.3 Genetic divergence among populations

Twenty-seven of 28 pair-wise population comparisons showed significant differences in microsatellite allele frequencies over all loci (P<0.01; Ramstad et al. 2004). The only populations with statistically equal microsatellite allele frequencies were LKR and KLSB, proximate populations that are part of the same small tributary lake system to Lake Clark (Figure 1). F_{ST} ranged from 0.001 (LKR and KLSB) to 0.077 (TCI and UTLK; SBL and LKR; Ramstad et al. 2004). R_{ST} values (range = 0.002 to 0.110; results not shown) were generally similar to F_{ST} values and overall F_{ST} and R_{ST} values (all populations and loci) were 0.048 and 0.042, respectively. High F_{ST} and R_{ST} values suggested strong divergence between Lake Clark populations and those in Six-mile Lake and Lake Iliamna and between the Sucker Bay Lake population and all other populations. Principal components analysis supported this pattern (Figure 4). The first principal component grouped Six-Mile Lake and Lake Iliamna populations and differentiated them from Lake Clark populations. The second principal component differentiated the Sucker Bay Lake population from all other populations surveyed.

Significant differences in allozyme allele frequencies were observed in 18 of 21 pair-wise population comparisons (P<0.001 to 0.03; Table 4). Populations with equal allozyme allele frequencies were the two Iliamna populations (FDI and TCI) and Kijik Lake populations (LKR and KLSB; KR and LKR). Pair-wise F_{ST} between populations ranged from zero (FDI and TCI; LKR and KLSB) to 0.112 (TCI and KR; TCI and UTLK; Table 4). The pattern of genetic

divergence at allozyme loci was similar to that of microsatellite loci with the greatest F_{ST} values between fish spawning in Lake Clark and Lake Iliamna.

Principal components analysis of allozyme data suggested the Tazimina River population of Six-mile Lake was more similar genetically to Lake Clark than Lake Iliamna populations (Figure 4). However, this finding was largely due to a single allozyme locus (*ALAT*) with a strong influence in the principal components analysis (PC1 loading for *ALAT* allele **100* = -0.576, **91* = 0.717; next highest PC1 loading is for *mAAT-1* allele **-100* = 0.273). With removal of this locus, PCA of the remaining 18 allozyme loci suggested the TAZ population is more similar genetically to Iliamna populations (distance on the PC1 axis from TAZ to nearest Iliamna population, FDI=0.025) than to Lake Clark populations (distance on the PC1 axis from TAZ to nearest Lake Clark neighbor, KLSB=0.084). The *ALAT* locus also displayed disproportionately large F_{ST} values in comparisons among populations of different lakes (mean *ALAT* F_{ST} = 0.164 $\pm$ 0.059, 95% CI; mean F_{ST} all other loci = 0.077 $\pm$ 0.022, 95% CI) but not when comparisons among populations of the same lake are also considered (mean *ALAT* F_{ST} = 0.111 $\pm$ 0.052, 95% CI; mean F_{ST} all other loci = 0.054 $\pm$ 0.021, 95% CI).

Significant differences in mtDNA haplotype frequencies were present in 18 of 28 pair-wise population comparisons (P=0 to 0.05; Table 5). F_{ST} values ranged from zero (four comparisons) to 0.427 (TAZ and KR) and suggested strong divergence between fish spawning in Lake Clark and those spawning in Six-mile Lake and Lake Iliamna (Table 5). Principal components analysis of the mtDNA data closely approximated that of the microsatellite data in that the first principal component differentiated between fish spawning in Lake Clark and those spawning in Lake Iliamna and Six-mile Lake (Figure 4). Unlike the microsatellite data, however, mtDNA suggested similarity between the Sucker Bay Lake populations and all other Lake Clark populations.

3.5 Discussion

We found that (1) patterns of genetic variation at allozyme and mtDNA markers support the presence of a founder effect associated with the colonization of Lake Clark by sockeye salmon. (2) Further reduced microsatellite variation in Sucker Bay Lake sockeye salmon is likely due to persistent low *Ne* and isolation since the initial founder effect. And (3) variation at allozyme loci and mtDNA supports the conclusion that Six-mile Lake sockeye salmon are more similar genetically to Lake Iliamna than Lake Clark sockeye salmon.

3.5.1 Lake Clark founder effect

Lake Clark sockeye salmon populations have reduced genetic diversity at microsatellite loci, allozyme loci, and mtDNA relative to Six-mile Lake and Lake Iliamna populations. This is evidenced by lower allelic richness, fewer total numbers of alleles, a lower mean proportion of rare microsatellite and allozyme alleles, and a reduced proportion of polymorphic allozyme loci in Lake Clark than Six-mile Lake and Lake Iliamna sockeye salmon populations.

Heterozygosity of Lake Clark populations (H_E) exceeds that expected at mutation-drift equilibrium (H_{Eq}) relative to populations in Six-mile Lake and Lake Iliamna. With microsatellite data, this effect is evident in all Lake Clark populations (but only statistically significant in Sucker Bay Lake) and reflects the deficit of microsatellite alleles in Lake Clark sockeye salmon. Allozyme heterozygosity excess is evident in all Lake Clark populations as well, and is statistically significant in the Little Kijik River and Upper Tlikakila River populations. This trend toward allozyme heterozygosity excess is still evident when the outlier locus *ALAT* is removed and reflects not only reduced allelic diversity but also greater allozyme heterozygosity in Lake Clark than Six-mile Lake and Lake Iliamna populations. Achieving statistical power >0.80 to detect a bottleneck with this test requires 10 polymorphic loci per population (Luikart and Cornuet 1998). In this study, numbers of polymorphic allozyme loci per population range from 7 to 12. However, our findings of bottleneck effects based on allozyme heterozygosity are

conservative because at low statistical power it is more likely that a bottlenecked population will be identified as non-bottlenecked than vice versa.

Taken together, these results suggest that a common founding reduced the genetic variation of all Lake Clark populations relative to Six-mile Lake and Lake Iliamna populations of sockeye salmon. The observation that alleles and haplotypes present in Lake Clark populations were generally a subset of those found commonly in Six-mile Lake and Lake Iliamna populations further supports this hypothesis, as does the difference in A and H_E between pooled Lake Clark and pooled Six-mile Lake and Lake Iliamna populations. Had Lake Clark populations experienced bottlenecks independently, allele frequencies would have drifted independently among populations and pooling them would have produced A and H_E values similar to those observed in the pooled Six-mile Lake and Lake Iliamna populations. The fact that genetic bottleneck signals persist when Lake Clark populations are pooled (reduced A, increased allozyme H_E) suggests these populations experienced a common genetic bottleneck effect rather than multiple independent bottlenecks. A similar pattern was observed among introduced New Zealand Chinook salmon populations (*O. tshawytscha*) which have reduced allozyme and mtDNA variation relative to putative source populations (Quinn et al. 1996).

Reduced microsatellite variation and lack of mutation-drift equilibrium suggest that Lake Clark was colonized by sockeye salmon within the last 100 to 400 hundred sockeye salmon generations (~400 to 1400 years; Ramstad et al. 2004). This timeframe is not surprising given that Lake Clark was created by glacial retreat approximately 12 to 15 thousand years ago and is geologically young (Stilwell and Kaufman 1996). Once a population has lost genetic variation due to drift, it can only be restored by mutation or immigration. Recovery of genetic variation by mutation can take thousands of generations even with the high mutation rate of microsatellites (Crow and Aoki 1984; Birky et al. 1989; Waples 1998; Kinnison et al. 2002). The similar F_{ST} and R_{ST} values among Lake Clark populations suggest a minor role for mutation in promoting

genetic variation since a recent, common founding of Lake Clark sockeye salmon (Wenburg et al. 1998).

3.5.2 Sucker Bay Lake

The Sucker Bay Lake population exhibits a strong genetic bottleneck signal (reduced variation, strong divergence) at microsatellite loci relative to all other populations surveyed (Ramstad et al. 2004). However, this bottleneck signal could not be assessed at allozyme loci (no data are available), and Sucker Bay Lake fish have similar mtDNA variation to all other Lake Clark populations. In addition, the Sucker Bay Lake population exhibits similar haplotype frequencies and divergence from Six-mile Lake and Lake Iliamna fish as other Lake Clark populations. Caution is required when interpreting these results as mtDNA is a single locus while the microsatellite data are from eleven independent loci. However, the contrasting results between the mtDNA and the microsatellite data may lend some insight into the nature of the bottleneck signal in Sucker Bay Lake sockeye salmon.

Mitochondrial DNA is expected to show greater divergence among populations and lower variation than nDNA because of its reduced effective population size (Birky et al. 1983, 1989). An acute, brief bottleneck could greatly reduce mtDNA haplotype diversity while having little effect on nDNA allelic diversity (Wilson 1985; Birky 1991; Hurtado et al. 2004). This is opposite of the pattern observed in Sucker Bay Lake. The discordance between nDNA and mtDNA bottleneck signals in Sucker Bay Lake is likely due to persistent low N_e and isolation since sharing in a common founding with other Lake Clark populations. A similar pattern has been observed in post-fur trade populations of sea otters (*Enhydra lutris*) where further reduction of nDNA variation relative to mtDNA variation could also be explained by the differential effects of drift on nuclear and mitochondrial DNA (Larson et al. 2002).

Aerial estimates of numbers of spawning fish suggest that Sucker Bay Lake census population size is typically low relative to other Lake Clark populations and fluctuates

tremendously from year to year (<100 to >7,000 spawners annually between 1955 and 1998; Regnart 1998). In addition, recent evidence from tagging studies suggests isolation of the Sucker Bay Lake population (low straying, different spawning times) from other Lake Clark spawning populations (Young 2004). If these observations reflect historical patterns, they also support the hypothesis that persistent low *Ne* and isolation have promoted greater loss of nuclear variation in Sucker Bay Lake than other Lake Clark populations.

Greater divergence between populations in nDNA than mtDNA could also be due to greater female immigration or skewed sex ratio in favor of females. However, the bias would have to be extreme (between 7 and 15 females per male; Birky et al. 1989) or female gene flow would homogenize populations in both nuclear and mitochondrial DNA (e.g. Kittles et al. 1999). There is no evidence, for female-biased dispersal among spawning populations of sockeye salmon. Nuclear and mitochondrial divergence among Cook Inlet populations is equivalent after correction for the lower *Ne* of mtDNA (Allendorf and Seeb 2000) and Taylor et al. (1997) suggested restricted female gene flow in non-anadromous *O. nerka*. There is similarly no evidence for a highly skewed sex ratio among Sucker Bay Lake sockeye salmon (personal observation, K. Ramstad). Thus, it is unlikely that the discordance between nuclear and mitochondrial DNA divergence between Sucker Bay Lake and other Lake Clark populations is due to different reproductive contributions of males and females.

3.5.3 Six-mile Lake

All three marker types surveyed show divergence between sockeye salmon spawning in Lake Clark and those spawning in Lake Iliamna. Significant differences in allele and haplotype frequencies, high F_{ST} values between Lake Clark and Lake Iliamna populations, and principal components analysis (PCA) of variation at 11 microsatellite loci, 19 allozyme loci and mtDNA support this pattern.

All three marker types surveyed also support the genetic similarity between the Tazimina River population of Six-mile Lake and Lake Iliamna populations. A single, highly influential allozyme locus (*ALAT*) suggests the Tazimina River population is more similar genetically to Lake Clark than to Lake Iliamna populations. With the removal of this outlier locus, the remaining 18 allozyme loci support the similarity between Six-mile Lake (Tazimina River) and Lake Iliamna sockeye salmon.

It has been suggested that variation at allozyme loci is more likely to be influenced by natural selection than variation at microsatellite loci (e.g., Karl and Avise 1992; Pogson et al. 1995). Therefore, the exceptionally high F_{ST} values and PCA loadings at the *ALAT* locus may be due to selection at this locus or another to which it is tightly linked. Slatkin and Muirhead (1999) have demonstrated theoretically that slight deviations from neutrality can strongly affect both the allele frequencies and the probability of allelic loss at a given locus. However, the great divergence at *ALAT* may also be caused by genetic drift as fluctuations in effective population size over time are expected to increase the variance of F_{ST} among loci (Beaumont and Nichols 1996). Drift is suggested when outlier behavior of a locus is coupled with evidence of a genetic bottleneck effect while selection is supported if the same loci exhibit similar outlier patterns in multiple and distant sampling locations (Luikart et al. 2003). For example, Allendorf and Seeb (2000) observed an outlier allozyme locus (*sAH*) in their study of sockeye salmon populations of Cook Inlet, Alaska. Had the same locus been an outlier in this study, selection would be supported as the cause of the outlier behavior. However, different loci behave as outliers in these two studies and in both cases are associated with populations that appear to have gone through recent genetic bottlenecks. In contrast, the *LDH-B2* locus is likely under selection as it has exhibited outlier behavior in multiple, distant sampling locations with allele frequencies correlated with differences in spawning habitat (Varnavskaya et al. 1994a). Large variation in allele frequencies at the *ALAT* locus have been observed among sockeye salmon populations in both this and other studies (Varnavskaya et al. 1994a, 1994b; Wood et al. 1994). In other studies,

however, variation at the *ALAT* locus did not provided results that were discordant with other loci or were correlated with genetic bottleneck signals. Thus, without additional information, it is impossible to distinguish between the alternative explanations of selection or drift in promoting the outlier behavior of the *ALAT* locus in this study.

The data presented above, and the observation that a population at the confluence of Lake Clark and Six-mile Lake is genetically similar to Lake Iliamna populations (Ramstad et al. 2004), suggest that sockeye salmon of Six-mile Lake (Tazimina River) are genetically similar to Lake Iliamna sockeye salmon. In addition, Tazimina River does not display the reduced genetic variation common to Lake Clark populations at the varied markers surveyed here, or in the broader analyses of Ramstad et al. (2004) or Habicht et al. (2004). We conclude that sockeye salmon spawning in Six-mile Lake have a stronger genetic affinity with Lake Iliamna than Lake Clark sockeye salmon.

3.5.4 Implications and conclusions

This study demonstrates the importance of using a variety of genetic marker types to test for bottleneck effects and determine genetic population structure. The combination of microsatellite and mtDNA data suggests that isolation and low *Ne* have promoted further loss of genetic variation in the Sucker Bay Lake population since sharing in a common founder event with other Lake Clark populations. Thus, this combination of data types provides greater understanding of the nature of the bottleneck effect in the Sucker Bay Lake population than either marker type could have provided alone. In addition, similar results at two additional marker types strongly support the pattern of genetic population structure previously determined with microsatellite data.

Investigators should be cautious when using heterozygosity as an indicator of genetic bottleneck effects. This is because heterozygosity is not as sensitive to drift as allelic diversity (Allendorf 1986), and there is large stochastic variance in heterozygosity among loci, particularly after a reduction in population size (Leberg 1992; Tarr et al. 1998). When a population

experiences a bottleneck, a reduction in heterozygosity is expected *on average* across loci. However, for loci with two alleles, there is a nearly equal probability that heterozygosity will increase or decrease at each locus due to random genetic drift of allele frequencies. This is because heterozygosity is maximized when the alleles are equally frequent. For example, Tarr et al. (1998) assayed variation in a recently founded Laysan finch (*Telespyza cantans*) population and its founding population using nine microsatellite loci with 2-5 alleles per locus. They observed that four of the nine loci exhibited greater H_E in the bottlenecked population than in the founding population though mean H_E overall was reduced.

In this study, eight of 19 allozyme loci exhibited a greater mean H_E in Lake Clark populations than in Six-mile Lake and Lake Iliamna populations resulting in increased mean H_E over all loci and in every Lake Clark population surveyed. This increase was unexpected, given the observed reduction in H_E at microsatellite loci and mtDNA, and could have been interpreted as evidence that Lake Clark sockeye salmon did not experience a genetic bottleneck effect. However, increased mean allozyme H_E is common among all surveyed Lake Clark populations and coupled with a reduction in allelic diversity. This provides evidence of heterozygosity excess and further suggests a common founding event among Lake Clark sockeye salmon. The effects of genetic drift on heterozygosity can be complex, and it is necessary to assess variation at many loci, particularly with higher numbers of alleles, to detect and quantify these effects (Tarr et al. 1998). Similarly, it is important to assess changes in heterozygosity relative to changes in allelic diversity when assessing evidence of genetic bottleneck effects.

Detection of outlier loci is critical to the accurate assessment of both genetic bottleneck effects and genetic population structure. It is not uncommon to find an outlier locus that profoundly affects results in studies including 20 or fewer loci (Allendorf and Seeb 2000; Landry et al. 2002; Luikart et al. 2003). In this study, the Tazimina River population of Six-mile Lake grouped with the bottlenecked Lake Clark populations when the *ALAT* locus was included but grouped with the non-bottlenecked populations in Lake Iliamna when *ALAT* was not included.

Repeating analyses with and without the *ALAT* locus provided evidence of the Lake Clark founder event and the genetic similarity between Six-mile Lake and Lake Iliamna populations. Other studies provide similar examples where outlier loci profoundly affect interpretation of genetic variation relative to geographic distribution, phenotypic variation, and phylogeny (Wilding et al. 2001; Landry et al. 2002).

Is mitigative management warranted for Lake Clark sockeye salmon?

It is unlikely that Lake Clark sockeye salmon are experiencing deleterious bottleneck effects because their founding event was associated with post-glacial colonization and is not recent (<100 generations; Luikart et al. 1998). In addition, the Lake Clark sockeye salmon founding event has resulted in only a modest reduction in heterozygosity (<5% at microsatellite loci) and there is no evidence in the literature of deleterious effects associated with such a small reduction in heterozygosity (Keller and Waller 2002). There are currently at least 32 spawning populations of sockeye salmon in Lake Clark without known barriers to dispersal among them (Young 2004). Given time, genetic variation of these populations will likely increase and mutation-drift equilibrium will be reached.

Isolation and low *Ne* have apparently promoted further loss of genetic variation in the Sucker Bay Lake population. It is unclear if an increase or further erosion of genetic variation among Sucker Bay Lake sockeye salmon is more likely given time. Conservative management should seek to maintain or increase effective population size, minimize disturbance of the Sucker Bay Lake population, and maintain connectivity among all sockeye salmon spawning populations of Lake Clark.

Table 3-1 Sample description and size (*N*), mean heterozygosity (H_E), and mean allelic/haplotypic diversity (*A*) for microsatellite loci, allozyme loci, and mtDNA of Lake Clark, Six-mile Lake, and Lake Iliamna sockeye salmon populations. Allelic/haplotypic diversity is the number of alleles or haplotypes per population, standardized to the lowest sample size per locus and population. Sampling dates available upon request from corresponding author.

	Sample description			Microsatellites[a]			Allozymes			Mitochondrial DNA		
	Site		Lake	*N*	H_E	*A*	*N*	H_E	*A*	*N*	H_E	*A*
1	TCI	Talarik Creek	Iliamna	97	0.500	6.4	100	0.097	1.7	24	0.746	4.0
2	FDI	Fuel Dump Island	Iliamna	87	0.483	7.1	99	0.101	1.7	30	0.662	3.9
3	TAZ	Tazimina River	Six-mile	99	0.507	6.3	100	0.098	1.7	23	0.708	4.0
Six-mile Lake and Lake Iliamna populations pooled				283	0.507	9.3	299	0.101	2.1	77	0.708	5.0
4	SBL	Sucker Bay Lake	Clark	100	0.502	4.2	---	---	---	23	0.609	3.0
5	KR	Kijik River	Clark	99	0.484	5.1	100	0.115	1.5	23	0.237	2.0
6	LKR	Little Kijik River	Clark	98	0.457	4.9	100	0.110	1.5	26	0.542	3.0
7	KLSB	Kijik Lake South Beach	Clark	100	0.452	4.9	100	0.107	1.6	26	0.517	2.0
8	UTLK	Upper Tlikakila River	Clark	100	0.480	5.0	100	0.120	1.5	28	0.362	3.0
Lake Clark populations pooled				496	0.488	6.7	400	0.114	1.7	126	0.473	3.0

[a]From Ramstad et al. 2004

Table 3-2 Allozyme allele frequencies in sockeye salmon of Lake Clark, Six-mile Lake, and Lake Iliamna. Frequencies are given for the most common allele for diallelic loci. Loci for which the frequency of the common allele is at least 0.99 in all populations are not listed. Refer to Table 1 for population names.

		mAH-1,2			*mAH-3*	*LDH-B2*			*mAAT-1*	*TPI-1,2*		
Sample		**100*	**75*	**133*		**100*	**110*	**85*		**-100*	**54*	**-173*
1	TCI	0.85	0.125	0.025	1	0.93	0.07	-	0.923	0.995	-	0.005
2	FDI	0.783	0.187	0.03	1	0.894	0.106	-	0.948	0.99	0.005	0.005
3	TAZ	0.845	0.14	0.015	1	0.855	0.145	-	0.969	0.985	0.015	-
5	KR	0.795	0.17	0.035	0.98	0.82	0.18	-	0.765	1	-	-
6	LKR	0.855	0.11	0.035	0.979	0.855	0.145	-	0.833	1	-	-
7	KLSB	0.825	0.17	0.005	0.99	0.91	0.08	0.01	0.8	1	-	-
8	UTLK	0.779	0.189	0.032	1	0.765	0.23	0.005	0.67	1	-	-

Table 3.2 - continued

Sample		*ALAT*				*PGM-1*	*PGM-2*	*MPI*			
		**100*	**91*	**108*	**95*			**100*	**105*	**91*	**96*
1	TCI	0.372	0.541	0.015	0.071	0.805	0.758	0.98	0.015	-	0.005
2	FDI	0.414	0.525	-	0.061	0.832	0.778	0.995	-	0.005	-
3	TAZ	0.725	0.181	-	0.093	0.697	0.775	1	-	-	-
5	KR	0.781	0.073	-	0.146	0.64	0.815	1	-	-	-
6	LKR	0.707	0.131	-	0.162	0.716	0.73	1	-	-	-
7	KLSB	0.735	0.15	-	0.115	0.72	0.755	1	-	-	-
8	UTLK	0.708	0.078	-	0.214	0.773	0.815	1	-	-	-

Table 3-3 Sample size (*N*) and composite mtDNA haplotype frequencies of Lake Clark, Six-mile Lake, and Lake Iliamna sockeye salmon populations. Refer to Table 1 for population names.

Sample		*N*	*ABDC*	*AACA*	*ABBA*	*ABBC*	*AAFA*
1	TCI	24	0.208	0.292	0.375	0.125	-
2	FDI	30	0.233	0.500	0.233	0.033	-
3	TAZ	23	0.130	0.478	0.217	-	0.174
4	SBL	23	0.565	0.261	0.174	-	-
5	KR	23	0.870	0.130	-	-	-
6	LKR	26	0.615	0.308	0.077	-	-
7	KLSB	26	0.538	0.462	-	-	-
8	UTLK	28	0.786	0.179	0.036	-	-

Table 3-4 Allozyme pair-wise F_{ST} (below diagonal) and number of loci with significant allele frequency differences after sequential Bonferroni correction (above diagonal) between Lake Clark, Six-mile Lake, and Lake Iliamna sockeye salmon populations. Comparisons without significant allele frequencies differences over all loci (P>0.05 Fisher's combined probability test) are noted as NS. No allozyme data is available for Sucker Bay Lake (population 4, SBL), refer to Table 1 for population names.

	1 TCI	2 FDI	3 TAZ	5 KR	6 LKR	7 KLSB	8 UTLK
TCI	---	NS	1	4	1	2	3
FDI	0	---	2	3	2	2	3
TAZ	0.067	0.060	---	2	1	1	2
KR	0.112	0.106	0.024	---	NS	1	1
LKR	0.074	0.072	0.008	0.008	---	NS	1
KLSB	0.075	0.072	0.013	0.007	0	---	3
UTLK	0.112	0.104	0.050	0.011	0.019	0.020	---

Table 3-5 Pair-wise F_{ST} of mtDNA (below diagonal) and significance of haplotype frequency differences (above diagonal) between Lake Clark, Six-mile Lake, and Lake Iliamna sockeye salmon populations. NS = not significant at P>0.05; *significant at P<0.05; **significant at P<0.01; ***significant at P<0.001. Refer to Table 1 for population names.

	1 TCI	2 FDI	3 TAZ	4 SBL	5 KR	6 LKR	7 KLSB	8 UTLK
TCI	---	NS	NS	*	***	**	***	***
FDI	0.014	---	NS	NS	***	*	**	***
TAZ	0.033	0	---	**	***	***	**	***
SBL	0.085	0.088	0.138	---	*	NS	*	NS
KR	0.367	0.369	0.427	0.109	---	NS	*	NS
LKR	0.146	0.120	0.175	0	0.080	---	NS	NS
KLSB	0.163	0.083	0.138	0.022	0.196	0	---	*
UTLK	0.288	0.285	0.344	0.038	0	0.015	0.112	---

Figure 3-1 Map of Lake Clark, Six-mile Lake, and Lake Iliamna with sample sites shown. Sites are numbered from downstream to upstream, refer to Table 1 for population names.

Figure 3-2 Network of haplotypes detected in this study. Haplotypes represent one BstU I site, two Ban II sites, and one Sau96 I site and are coded as ABDC=1111, AACA=0000, ABBA=1100, ABBC=1101, and AAFA=0010. Arrows indicate direction of site gain, circle size indicates haplotype frequency over all fish sampled, and shading within circles indicates proportion of fish from Six-mile and Iliamna Lakes (open) and Lake Clark (shaded) with that haplotype. The dashed arrow indicates an alternative, less parsimonious derivation of the AAFA haplotype from the ABDC haplotype.

Figure 3-3 Relationship between mean expected heterozygosity observed (H_E) and expected at mutation-drift equilibrium (H_{Eq}) for 11 microsatellite loci (circles) and 13 allozyme loci (triangles) in Lake Clark (black), Six-mile Lake (grey), and Lake Iliamna (open) sockeye salmon populations. Lake Clark populations have greater H_E than H_{Eq} for both markers due to genetic drift associated with a common founding event. Refer to Table 1 for population names.

Figure 3-4 Principal components analysis for Lake Clark (black), Six-mile Lake (grey) and Lake Iliamna (open) sockeye salmon populations. Analyses are of 11 microsatellite loci, 13 allozyme loci, nuclear markers combined, and mtDNA. Percentages in parentheses indicate amount of variation explained by each principal component. Refer to Table 1 for population names.

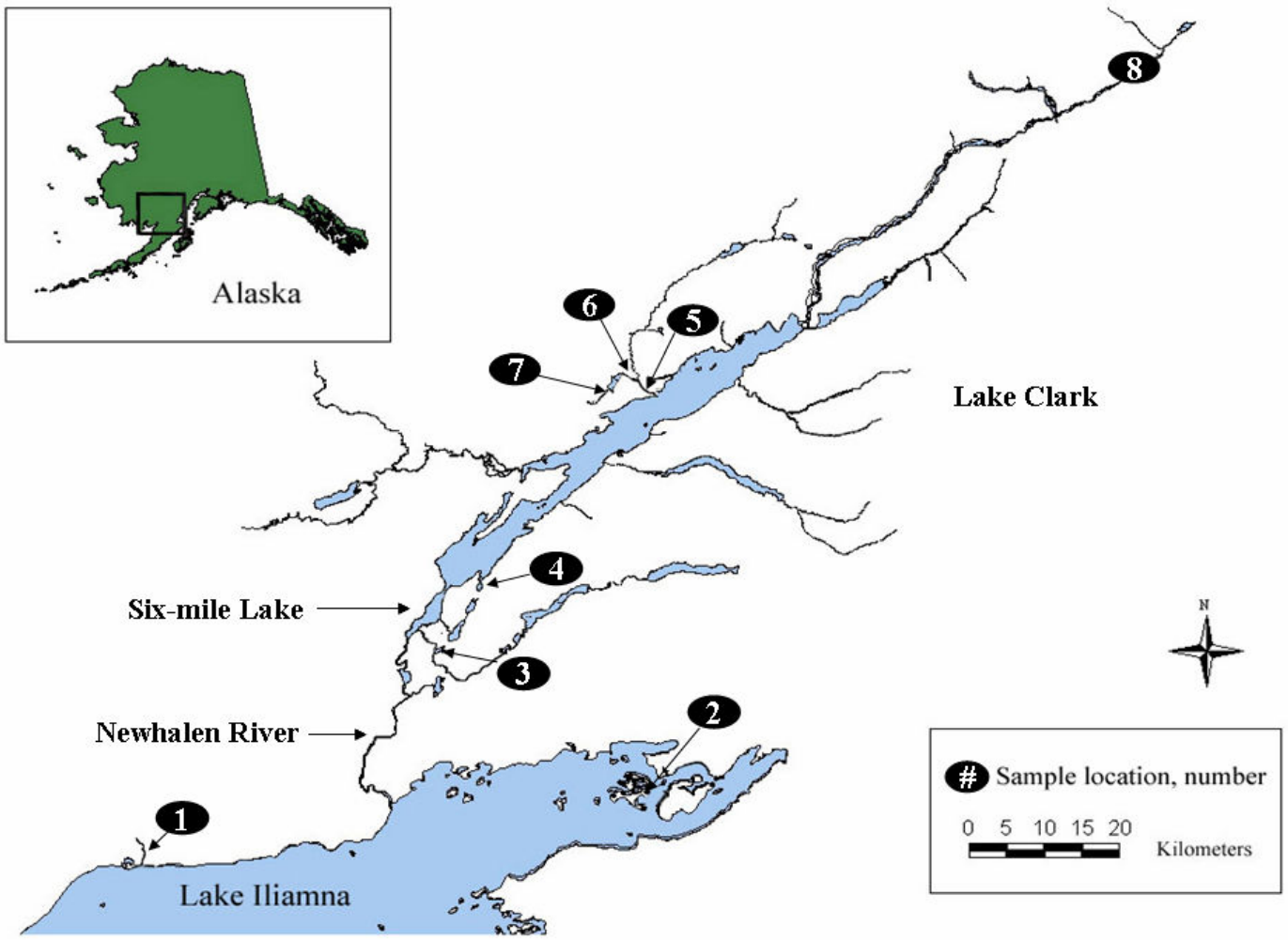
Alaska
8
6
5
7
Lake Clark
4
Six-mile Lake
3
2
Newhalen River
1
Lake Iliamna
N
Sample location, number
0 5 10 15 20
Kilometers

Figure 3-1

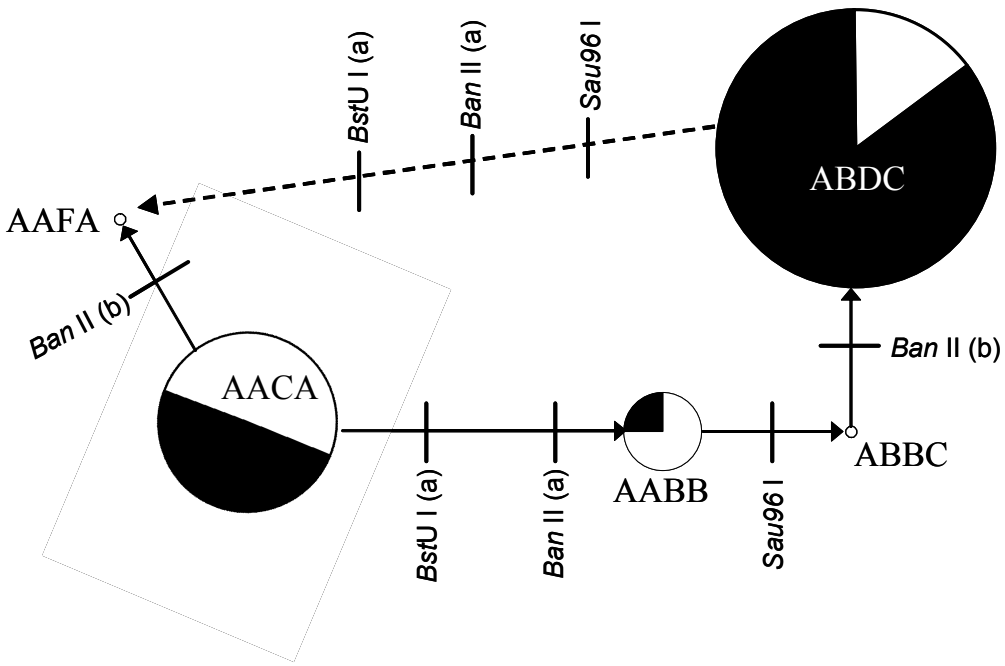
BstU I (a)
Ban II (a)
Sau96 I
ABDC
AAFA
Ban II (b)
AACA
Ban II (b)
ABBC
BstU I (a)
Ban II (a)
AABB
Sau96 I

Figure 3-2

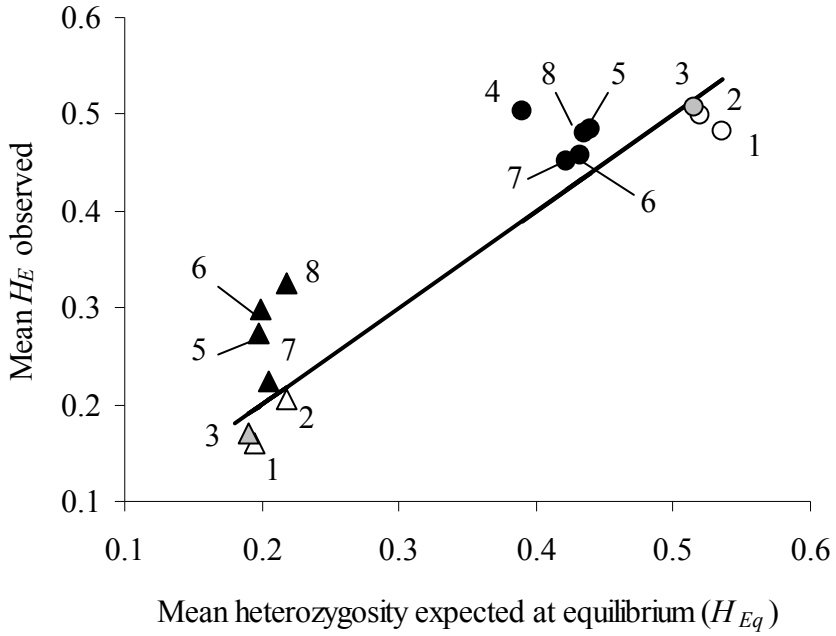
0.6
0.5
0.4
0.3
0.2
0.1
Mean H_E observed
0.1
0.2
0.3
0.4
0.5
0.6
Mean heterozygosity expected at equilibrium (H_{Eq})
4
8
5
3
2
1
7
6
6
8
5
7
3
2
1

Figure 3-3

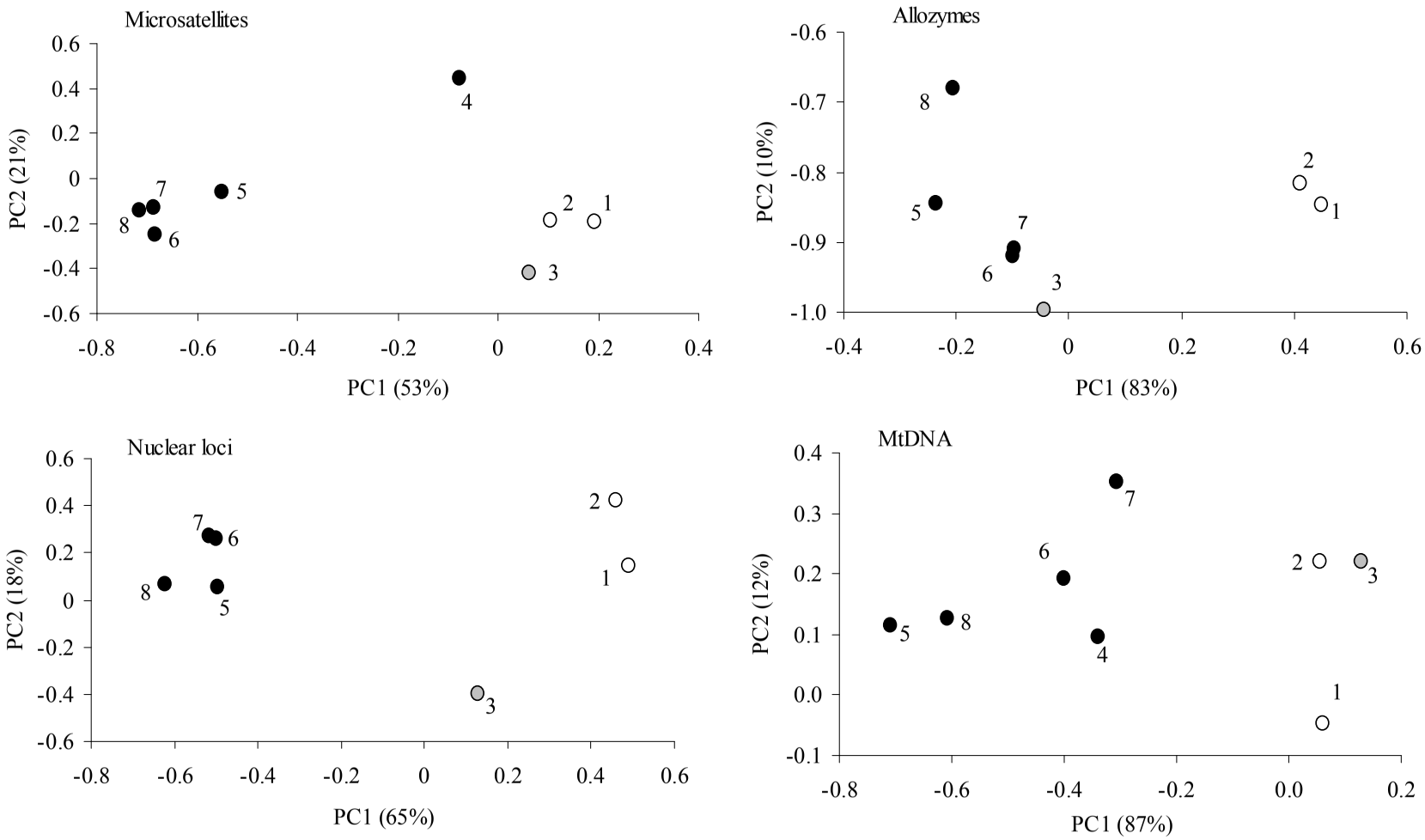
Microsatellites
PC2 (21%)
PC1 (53%)
Allozymes
PC2 (10%)
PC1 (83%)
Nuclear loci
PC2 (18%)
PC1 (65%)
MtDNA
PC2 (12%)
PC1 (87%)

Figure 3-4

Chapter 4 - Evidence for local adaptation of sockeye salmon to beach, tributary, and glacial spawning habitats

4.1 Abstract

Adaptive divergence is expected among salmonid populations because they spawn in diverse habitats and experience spatially and temporally restricted gene flow. However, salmonids are also prone to founder effects, so phenotypic divergence among populations may be due to genetic drift. To test if sockeye salmon (*Oncorhychus nerka*) populations are adapted to beach, tributary, and glacial spawning habitats, we examined variation in heritable phenotypic traits associated with spawning in thirteen populations of wild sockeye salmon in Lake Clark, Alaska. Lake Clark sockeye salmon populations were commonly founded between 100 and 400 hundred generations ago and exhibit low genetic divergence at 11 microsatellite loci (F_{ST} < 0.024) that is uncorrelated with spawning habitat type. We found significant phenotypic divergence among populations that was associated with spawning habitat differences, but not with neutral genetic divergence. For example, female body color was lighter and egg color was darker in glacial than non-glacial habitats. This is due possibly to reduced selection for red spawning color in glacial habitats and an apparent trade-off between body and egg color in females. Mean P_{ST} (phenotypic divergence among populations) exceeded neutral F_{ST} for most phenotypic traits indicating that phenotypic differences among populations could not be explained by genetic drift alone. Plasticity is an unlikely source of phenotypic differences because Lake Clark sockeye salmon spend nearly all their lives in a common environment. These data suggest that Lake Clark sockeye salmon populations are adapted to spawning in beach, tributary, and glacial habitats and provide the first evidence of a glacial spawning phenotype among sockeye salmon.

4.2 Introduction

Phenotypic variation among wild conspecific populations has long fascinated naturalists and evolutionary ecologists (Darwin 1859, 1871). What is the source of this variation? What processes maintain it? How can we conserve it? There are four primary sources of phenotypic divergence among individuals and populations: mutation, genetic drift, selection, and phenotypic plasticity. Mutation is the ultimate source of all genetic variation, but is generally a weak force in evolution unless it is coupled with genetic drift or selection (Volpe 1985; Avise 1994). Genetic drift promotes genetic divergence among populations that have consistently low effective population size (N_e) or have experienced genetic bottleneck effects (acute reductions in N_e, Avise 1994). Selection promotes genetic divergence, or local adaptation, among populations when restricted gene flow is coupled with different selection regimes (Endler 1986; Stearns 1992).

Phenotypic divergence among populations in different environments may or may not reflect genetic divergence. For example, genetically similar populations may diverge phenotypically due solely to environmental differences through phenotypic plasticity (the range of expression of the genotype in interaction with the environment, Stearns 1992). Conversely, similar phenotypes among populations can mask strong genetic differences due to counter gradient selection (genetic effects on a trait oppose or compensate for environmental effects such that phenotypic divergence among populations is minimized, e.g., Craig and Foote 2001).

In a common environment, phenotypic divergence among populations is largely due to the balance of drift, selection, and migration (gene flow). Effective population size, selection strength (s), and migration rate (m) interact to determine if selection will be effective in changing population allele frequencies (Wright 1931; Li 1978; Allendorf 1983). In the absence of genetic drift (large N_e), selection will have a significant effect on allele frequencies when $s > m$ (Wright 1931; Allendorf 1983). Thus, selection can affect allele frequencies at any level of migration short of total panmixia provided the strength of selection is sufficient. Selection will be ineffective in the presence of significant genetic drift, or when $s < 1 / N_e$ (Li

1978; Allendorf 1983). Thus, adaptive divergence is expected among large, relatively isolated populations experiencing different selection regimes and divergence due to drift is expected among small, isolated populations (Wright 1931; Allendorf 1983).

It is essential to differentiate between drift and selection in promoting phenotypic divergence among populations because failure to recognize the effect of one will lead to erroneous conclusions about the other. For example, ascribing the effects of drift to selection may mislead researchers to overestimate local adaptation, promote "just-so" stories to explain the observed pattern, and conduct studies with no hope of increasing our understanding of natural selection in the wild (Gould and Lewontin 1979; Adkison 1995). Failure to recognize genetic drift could result in serious conservation consequences as well because populations experiencing strong genetic drift may have reduced evolutionary potential and probability of persistence (Luikart et al. 1998; Soulé and Mills 1998; Briskie and Mackintosh 2004).

We can formally test the hypothesis that phenotypic divergence among populations is due to selection against the alternative null that divergence is due to neutral genetic drift by comparing the metrics F_{ST} and Q_{ST} (Merilä and Crnokrak 2001; McKay and Latta 2002). When calculated with neutral genetic markers, F_{ST} reflects the amount of genetic divergence among populations that is due to the balance of neutral genetic drift and gene flow (Wright 1951). In contrast, Q_{ST} reflects divergence among populations in quantitative traits due solely to additive genetic effects (Spitze 1993). If quantitative traits have an additive genetic basis and are not under selection, Q_{ST} is expected to equal F_{ST} (Wright 1951). Therefore, if F_{ST} differs significantly from Q_{ST}, the phenotypic divergence observed among populations cannot be explained by genetic drift alone and selection must be acting to shape phenotypes.

Proper estimation of Q_{ST} requires control of environmental and non-additive genetic effects on phenotype (Merilä and Crnokrak 2001; McKay and Latta 2002). If it can be assumed that these effects are negligible, then Q_{ST} can be approximated as simply the phenotypic divergence observed among populations. Authors have often presented the observed phenotypic divergence as Q_{ST} when common garden experimentation was not possible (Saint-Laurent et al. 2003 and references therein). Because this can lead to

substantial confusion, we have termed the observed phenotypic divergence among populations P_{ST} to differentiate it from Q_{ST} and make explicit that equating the two measures assumes that environmental and non-additive genetic effects are negligible in the system under study.

Here we compare P_{ST} and neutral F_{ST} to determine the relative effects of genetic drift and selection in promoting phenotypic divergence among populations of sockeye salmon (*Oncorhynchus nerka*) spawning in different habitat types of Lake Clark, Alaska (Figure 1). Rapid adaptive divergence has been observed among sockeye salmon introduced to Lake Washington (~ 13 generations, Hendry et al. 2000b) and chinook salmon (*O. tshawytscha*) introduced to New Zealand rivers (< 30 generations, Kinnison et al. 1998; Quinn et al. 2000). This study represents a similar natural experiment because Lake Clark sockeye salmon populations shared in a common founding event approximately 400 to 1400 years ago (100 to 400 hundred generations, Ramstad et al. 2004). Thus, adaptive phenotypic divergence among Lake Clark populations would require divergent selection since their common founding. Environmental effects are assumed to be negligible because Lake Clark sockeye salmon spend nearly all of their lives in a common environment, as detailed in the next section. In addition, all phenotypic traits considered in this study are associated with spawning, are fully developed prior to fish arriving at their spawning habitats (Hendry et al. 1999; Hamon and Foote 2000; Hamon et al. 2000; Hendry et al. 2000a), and have an additive genetic basis in salmonids (see references below).

4.2.1 Natural History of Sockeye Salmon

Sockeye salmon typically rear in freshwater for 1 to 2 years, migrate to sea as smolt, spend 1 to 4 years at sea, and return to their natal freshwater systems to spawn once and die (Burgner 1991). Though this life history exposes sockeye salmon to a wide variety of habitats, fish from the same natal lake spend most of their lives together in a common environment. For example, Lake Clark sockeye salmon rear as juveniles in the same nursery lake (Schlenger 1996), collectively outmigrate to sea as smolt (Orrell 1963; Woolington et al. 1990), spend

their adult lives in a common oceanic environment (French et al. 1976; Burgner 1980), and return synchronously to Lake Clark to spawn (Burgner 1980; Jensen and Mathisen 1987).

Fish mature during their homeward natal migration and develop dorsal humps, bright red spawning color, and elongated snouts with large, sharp teeth. These traits are more exaggerated in males than females. Maturation is also marked by a shift in the relative proportion of the visual pigments rhodopsin and porphyropsin, which increases sensitivity to red wavelengths (Beatty 1966; Novales-Flamarique 2000). Red body color is derived from carotenoids obtained through diet while at sea, initially sequestered in the flesh, and then transferred to the skin and eggs during maturation (Crozier 1970; Goodwin 1984). During spawning, females select and compete for breeding sites where they dig a series of nests (redds) and defend them until they die or are driven off their territory. In contrast, males compete with one another for access to females, but do not take part in redd construction or defense (Foote 1990; Quinn and Foote 1994). A single male cannot wholly monopolize a female and small males will sneak matings when possible (Gross 1985; Foote and Larkin 1988).

Specific natal homing and spawning habitat differences within nursery lakes promote reproductive isolation, genetic structuring, and phenotypic divergence among populations of sockeye salmon (Ricker 1972; Quinn 1985; Quinn and Dittman 1990). There is often significant genetic and phenotypic divergence among populations within lakes that spawn in different types of habitat (ecological isolation) or differ in their time of return or spawning (temporal isolation; Ricker 1972; Wood 1995; Woody et al. 2000; Ramstad et al. 2003; Hendry and Day 2005). Sockeye salmon are excellent colonizers of newly created habitats and can quickly establish spawning populations with few individuals (Milner 1987; Milner and Bailey 1989; Milner et al. 2000) because their homing fidelity exists in a dynamic balance with a tendency to stray (Quinn 1984, 1985). Thus, sockeye salmon are vulnerable to genetic drift due to founder effects.

The morphology, behavior, and life history of sockeye salmon are shaped by both sexual and natural selection (see reviews in Taylor 1991; Wood 1995). In females, large body

size is correlated with territory acquisition, territory defense, and fecundity (van den Berghe and Gross 1989; Fleming and Gross 1994). In males, large body size is correlated with acquisition and defense of females (Foote 1990; Fleming and Gross 1994; Quinn and Foote 1994; Hamon and Foote 2005). Snout length, spawning color intensity, and dorsal hump size are positively correlated with breeding competition (Fleming and Gross 1989, 1994; Quinn and Foote 1994). Males and females use their snouts as weapons in intrasexual aggression by biting and ramming competitors (Darwin 1871; Quinn and Foote 1994), spawning color appears to indicate social status for both sexes (Fleming and Gross 1989, 1994), and males use their dorsal humps for lateral body size displays to other males (Quinn and Foote 1994; but see Hamon and Foote 2005). Males preferentially compete for intensely colored, large bodied females that are ready to spawn (Foote 1988; Foote and Larkin 1988; Craig and Foote 2001; Foote et al. 2004) while females indirectly select large males by delaying spawning when attended by small males (Foote and Larkin 1988; Berejikian et al. 2000).

Sockeye salmon phenotypic traits often correlate with differences in spawning habitat. Egg size is positively correlated with substrate size (Quinn et al. 1995), male dorsal hump size is positively correlated with spawning habitat water depth (Blair et al. 1993; Woody et al. 2000; Quinn et al. 2001) and probability of bear predation (Ruggerone et al. 2000; Quinn et al. 2001), and spawning time is correlated with incubation thermal regime (Brannon 1987). Many of these traits have been shown to have an additive genetic basis in salmonids (egg size - Gall and Neira 2004, morphology - Taylor and McPhail 1985; Funk et al. 2005, age at maturity - Gall et al. 1988; Hankin et al. 1993, spawning time - Siitonen and Gall 1989; Quinn et al. 2000, flesh color – Withler 1986, ability to convert carotenoids to red colored tissue/spawning color - Craig and Foote 2001) and parallel correlations in multiple lake systems between phenotype and spawning habitat type suggest phenotypic differences are adaptive (Wood 1995). This has prompted definition of spawning ecotypes (populations that reside in and are adapted to distinct environments, Turesson 1922) of sockeye salmon. For example, the beach spawning ecotype of sockeye salmon is characterized by larger eggs, deeper bodies, smaller size at age, younger age at maturity, and later spawning times than the

inlet tributary ecotype (Rogers 1987; Blair et al.1993; Quinn et al. 1995; Wood 1995; Hamon et al. 2000; Hendry et al. 2000b; Quinn et al. 2001).

In contrast to beach and tributary spawning populations, evidence of local adaptation of sockeye salmon to glacial spawning habitats (recently uncovered by retreating glaciers, turbidity > 5 nephelometric turbidity units (NTU) at peak spawning; Koenings et al. 1986, 1990) has never been assessed. Indeed, most studies of salmonid ecology have been conducted in non-glacial habitats (< 5 NTU) and little is known of the ecology of salmonids inhabiting glacial waters (but see Lorenz and Eiler 1989; Eiler et al. 1992; Young 2004). Glacial habitats are characterized by elevated turbidities, low temperatures, unstable flow regimes, and silty substrates (Stanford and Ward 1992; Murphy et al. 1997) while non-glacial habitats are typically characterized by clear water with moderate temperature and flow, little silt, and moderate to fine sized gravel (Burgner 1991).

Turbidity is an optical property of water wherein suspended sediment and dissolved matter absorb and scatter light rather than transmit it in straight lines (APHA et al. 1980). Elevated turbidity dramatically reduces light penetration in both lakes and streams and is associated with reduced primary productivity, low species diversity, and impaired growth and vision of fishes (Lloyd et al. 1987; Barrett et al. 1992). Fish that spawn in glacial and non-glacial habitats will likely differ phenotypically in response to different selection pressures associated with these different environments. For example, silty substrates may promote reduced egg size (Quinn et al. 1995), colder incubation temperatures may promote earlier spawning time (Brannon 1987), and reduced visibility due to high turbidity may relax selection for visual sexual signals (Barrett et al. 1992; Seehausen et al. 1997) in glacial spawning fish. Given that glacial spawning habitats have been recently colonized, genetic drift due to founder effects may also promote genetic and phenotypic divergence among populations spawning in glacial and non-glacial habitats.

4.2.2 Objectives

The objective of this study was to test for evidence of local adaptation of sockeye salmon to beach, tributary, and glacial spawning habitats of Lake Clark, Alaska. We addressed three primary questions: (1) What is the pattern of phenotypic variation among spawning populations of Lake Clark sockeye salmon? (2) Is phenotypic divergence among populations correlated with differences in spawning habitat? (3) Is phenotypic divergence among populations (P_{ST}) correlated with neutral genetic divergence (microsatellite F_{ST})? If phenotypic divergence among populations is not correlated with differences in their spawning habitats but is correlated with neutral genetic divergence, then genetic drift is likely the cause of the observed phenotypic variation. In contrast, a correlation between spawner phenotype and spawning habitat type when neither factor is correlated with neutral genetic divergence would suggest that selection is the primary force in driving phenotypic divergence among spawning populations.

4.3 Methods

Lake Clark is one of two large lake systems in the Kvichak River watershed of Bristol Bay, Alaska (Figure 1). The world's largest and most lucrative commercial salmon fisheries take place in Bristol Bay, and Kvichak River sockeye salmon historically dominated the harvest (ADFG 2002; Fair 2003). Lake Clark sockeye salmon often comprise a significant portion of the fish spawning annually in the Kvichak watershed. For example, 30 thousand to 8.4 million sockeye salmon, representing between one and 75 percent of Kvichak spawning fish, spawned in Lake Clark annually between 1979 and 1998 (Rogers et al. 1999).

Lake Clark is geologically young (12 to 15 thousand years old, Stilwell and Kaufman 1996) and has spawning habitats that were deglaciated as recently as 200 years ago (P. Heiser, pers. comm. 2002). Spawning habitats in Lake Clark are highly heterogeneous and include glacial and non-glacial beach, inlet tributary, and outlet tributary habitats (Demory et al. 1964; Brabets 2002). In this study, we formally compared fish spawning in (1) beach and

inlet tributary habitats, (2) glacial and non-glacial beach habitats, and (3) glacial and non-glacial inlet tributary habitats. We excluded outlet tributary populations from comparisons among habitat types because there are no glacial outlet tributaries in the Lake Clark system. We included outlet tributary populations in analyses that treated populations independently, however, to increase the spawning habitat diversity represented and neutral genetic divergence among study populations. For example, the Lake Clark Outlet (OUT) population is highly divergent from all other Lake Clark populations in neutral microsatellite allele frequencies because it apparently was not part of a founder event associated with the colonization of Lake Clark by sockeye salmon (Figure 1; Ramstad et al. 2004). Inlet tributaries are referred to simply as tributaries throughout the text unless otherwise stated.

4.3.1 Spawning Habitat Measures

Turbidity (NTU) was measured with a Hach® Pocket Turbidimeter (Loveland, Colorado, USA) at peak spawning time for 13 spawning sites (Figure 1, Table 1). For 10 of these 13 sites, water depth (m)was measured every meter and substrate size composition assessed by Wolman Pebble Count (Kondolf and Li 1992) along three to five randomly chosen transects crossing the wetted width of tributaries and the wetted beach shore to a depth of approximately 1.5 meters directly offshore. A StowAway TidbiT datalogger (Onset© Computer Corporation, Bourne, Massachusetts, USA) was anchored with rebar in the substrate of 11 habitats and recorded hourly intergravel incubation temperatures. Retrieval of dataloggers was attempted annually and hourly temperatures were averaged within habitats between 14 October and 30 May to determine mean incubation temperatures.

4.3.2 Phenotypic Measures

Phenotypic traits of sockeye salmon from 13 spawning populations throughout Lake Clark and Six-mile Lake and from all spawning habitat types defined above were measured in 2000 and 2001 (Figure 1, Table 2). Fish were captured on their spawning grounds by beach seine and tangle net. Morphological measurements were taken on approximately 20 fish per sex from four populations in 2000 (SBL - Sucker Bay Lake, LRK - Little Kijik River, LLCB -

Little Lake Clark Beach, LTLK - Lower Tlikakila River) and 30 fish per sex from 13 populations in 2001. Measurements of hypural length (HL - mid-eye to posterior edge of the hypural plate), body depth (BD - anterior insertion of the dorsal fin to belly at a 90º angle to the lateral line), and snout length (SN - tip of snout to mid-eye) were taken on the left side of each fish with calipers to the nearest mm.

Eggs of ripe and spawning females were collected from 11 to 42 fish from 13 populations in 2001. Eggs were immediately placed in 5% buffered formalin and stored for 63 to 125 days prior to processing. Salmonid eggs can be stored in this manner for at least five months without increase in egg weight (Fleming and Ng 1987). Approximately 30 eggs per fish were collectively weighed to the nearest gram by the same technician and balance after removal of excess storage solution.

Spawning body color was assessed for approximately 30 fish per sex from 13 populations in 2001. Skin color on the left lateral dorsal hump was subjectively categorized as orange, red, or pink by one of two observers; scores were repeatable both within and between observers ($P \geq 0.89$). Egg color was categorized as the closest matching plate of plates 1 through 5 of the Hoffman-La Roche Color Card for Salmonids (Roche Vitamins and Fine Chemicals Division, Hoffman-La Roche Inc., Nutley, NJ) by the same observer. Egg color measures were conducted without any biased expectation of darker eggs in specific habitat types because the observer was unfamiliar with the Lake Clark system. Hoffman-La Roche color scores are highly correlated with red measures given by a Minolta CR-100 chromameter (a*) and carotenoid concentration (Smith et al. 1992; Craig and Foote 2001).

Mean peak spawning time of each population was taken from Ramstad et al. (2004). This date is the estimated mean date of peak spawning across years based on the presence of gravid females during sampling.

4.3.3 Statistical Analyses

We first characterized spawning habitat variation by comparing abiotic habitat characteristics among habitat types with one-way analysis of variance (ANOVA). Substrate counts are

presented as proportions by size categories that sum to one within sites, but are independent among sites.

Body depth, snout length, and egg size covary with body size and were adjusted to common body lengths prior to statistical comparisons. Individual trait values were $\log_e$ transformed and compared among populations and habitat types by analysis of covariance with $\log_e$ HL as the covariate in SPSS© v.12.0 (SPSS, Inc., Chicago, Illinois, USA, Zar 1984). When group slopes were equal, traits were adjusted to the grand sample mean body size (519mm male morphology, 500mm female morphology, 507mm egg size) using the common within group slope. Differences among groups will vary based on fish body size when groups have unequal slopes. Thus, when group slopes were unequal, individual trait measures were adjusted to three different body sizes (male morphology: 480mm, 519mm, 560mm; female morphology: 484mm, 500mm, 524mm; egg size: 480mm, 507mm, and 530mm) with the following equation:

$$T_{adj} = T_{obs}\left(\frac{L_{adj}}{L_{obs}}\right)^{b}$$

where T_{adj} is the adjusted trait size, T_{obs} is the observed trait measure, L_{adj} is the hypural length to which the trait is being adjusted, L_{obs} is the hypural length observed, b is the individual group slope.

We used principal components analysis (PCA) to initially establish the presence of phenotypic divergence among fish spawning in different habitat types. Males (N = 317 males, 59 to 90 per habitat type) and females (N = 176 females, 32 to 59 per habitat type) were analyzed separately in MINITAB, version 11 (State College, PA) using the correlation matrix of phenotypic traits (males: BD and SN adjusted to 519mm HL, spawning color, and spawning time; females: BD and SN adjusted to 500 mm HL, egg size adjusted to 507mm HL, spawning color, egg color, and spawning time). Significance of divergence among

habitat types was assessed by comparison of 95% confidence intervals of principal component scores.

Following multivariate analysis, we investigated phenotypic differences among populations and habitats types for individual traits. Adjusted morphological traits and egg size were compared among populations and habitat types with one-way analysis of variance (ANOVA) followed by Tukey post-hoc multiple comparisons. Spawning and egg color were compared among habitat types and to each other by log-likelihood ratio (*LR*), or G statistics, for contingency tables with exact significance calculated using a Monte Carlo method. Egg color scores are interval data and were compared among populations with one-way ANOVA and Tamahane's T2 post-hoc multiple comparisons. Egg size and egg color within females were compared among populations and within habitat types with two-way ANOVA. Mean peak spawning time (number of days from 1 Aug) was compared among habitat types with one-way ANOVA. Likelihood ratio analysis and ANOVA were conducted in SPSS©. Corrections for multiple comparisons were not applied beyond the post hoc tests described above.

Correlations between mean spawner phenotypes and spawning habitat parameters were assessed with simple regression. Two-tailed tests of significance were used in all analyses except regression analyses testing predictions that specify direction of the effect (e.g. substrate size is positively correlated with egg size).

Correlations among phenotypic divergence among populations, neutral genetic divergence among populations, and spawning habitat type were assessed with simple and partial Mantel tests (Smouse et al. 1986) in FSTAT version 1.2 (Goudet 1995) according to Manly (1991). Phenotypic divergence among populations was calculated as P_{ST} for body depth, spawning color, and egg color. Morphological traits with unequal slopes among populations (SN, egg size) were excluded from this analysis as results would vary with fish body size. Spawning time P_{ST} could not be calculated because spawning time was estimated for populations and not individual fish. For continuous data (body depth), phenotypic variance components were estimated with one-way ANOVA in SPSS© and P_{ST} was calculated

as Q_{ST} but substituting twice the phenotypic variance among individuals within populations for $2\sigma^2_{GW}$ and the phenotypic variance among populations for σ^2_{GB} (McKay and Latta 2002; Saint-Laurent et al. 2003).

For nominal (spawning color) and interval (egg color) data, phenotypic variance components and P_{ST} were calculated using the ANOVA framework developed for categorical genetic data by Cockerham (1973). Traits were treated as haploid loci, phenotypic categories within traits were coded as alleles, and pairwise P_{ST} was calculated as pairwise F_{ST} in FSTAT version 1.2 (Goudet 1995) according to Weir and Cockerham (1984). In the haploid case, there is no variance among alleles within individuals. Thus, only the variances in allele frequencies among and within populations are estimated and a ratio of the among population and the total variances taken as an estimate of F_{ST} (Weir 1996). Because the data are not normally distributed, the resulting statistic does not follow an F distribution and may not be used to test for differences in group means (as is typically done with an ANOVA). However, when used with phenotypic data, the ratio represents the proportion of total phenotypic variation among individuals that is due to differences among the populations, or P_{ST}.

Neutral genetic divergence among populations was calculated as pair-wise microsatellite F_{ST}, computed in FSTAT according to Weir and Cockerham (1984), and taken from Ramstad et al. (2004). Two spawning habitat matrices were defined as BT (beach or tributary) and GNG (glacial or non-glacial) and coded for populations spawning in similar (0) and different (1) habitat types. P_{ST} and F_{ST} were log transformed after zero values were reassigned a value of 0.0001 based on the smallest pair-wise F_{ST} detected in all population comparisons.

4.4 Results

4.4.1 Spawning Habitat Variation

We found significant variation in the physical parameters of the 13 spawning habitats sampled (Table 1). Beaches and tributaries did not differ in mean turbidity ($F_{1,9}$ = 0.30, P = 0.87). However, water depth was deeper on average at beach than tributary spawning sites ($F_{1,7}$ = 5.94, P = 0.05) and beaches had a higher proportion of course substrate than tributary spawning sites ($F_{1,7}$ = 4.29, P = 0.08; Table 1). Priest Rock Creek (PRC) was unusual in that it had a higher (Table 1) and more stable temperature throughout the year (data not shown) than nearly all other habitats surveyed. With the exception of PRC, outlet tributaries and beaches had higher mean incubation temperatures (2.9 $\pm$ 0.6 ºC 95% CI) than inlet tributaries (1.5 $\pm$ 0.6 ºC, $F_{1,4}$ = 45.71, P < 0.01).

Turbidity during spawning was significantly elevated in glacial habitats relative to non-glacial habitats (outlet tributaries included; $F_{1,11}$ = 41.64, P < 0.001; Table 1). Differences in turbidity were marginally significant for beach ($F_{1,3}$ = 8.00; P = 0.07) and highly significant for tributary ($F_{1,4}$ = 56.67; P < 0.01) habitats. Lack of data precluded testing for differences in depth and substrate size between glacial and non-glacial beaches. However, glacial tributaries tended to be shallower (0.39 $\pm$ 0.14m 95% CI) than non-glacial tributaries (0.52 $\pm$ 0.23m; $F_{1,4}$ = 4.86, P = 0.09) and did not differ in substrate size composition from non-glacial tributaries (fine substrate: $F_{1,4}$ = 0.15, P = 0.72; moderate : $F_{1,4}$ = 0.78, P = 0.43; course: $F_{1,4}$ = 0.45, P = 0.54).

It was not possible to test for differences in incubation temperature between glacial and non-glacial habitats because dataloggers in glacial habitats were rarely deployed and recovered (2 recovered of 8 deployed) relative to those in non-glacial habitats (13 recovered of 18 deployed). Dataloggers in glacial habitats typically did not remain buried in the stream channel throughout the incubation period, but were later found in streamside locations nearby. This suggests glacial spawning habitats of Lake Clark are less stable than non-glacial habitats and experience the significant scour, flooding, and freezing associated with unstable flow

regimes that are typical of glacial riverine habitats (Stanford and Ward 1992; Murphy et al. 1997).

4.4.2 Phenotypic Variation

Principal components analysis revealed that specific combinations of phenotypic traits are found in different habitat types for both males and females (Figure 2). Beach and tributary spawning fish were differentiated by the first principal component (PC) for males (not significant between non-glacial beaches and glacial tributaries) and the second PC for females (not significant between glacial beaches and non-glacial tributaries; Fig 2). Fish spawning on beaches tended to have deeper bodies, longer snouts (males only), and later spawning times than fish spawning in tributaries. Glacial and non-glacial spawning fish were differentiated by the second PC for males (not significant for glacial beaches and non-glacial tributaries) and the first PC for females (Fig 2). Fish spawning in glacial habitats tended to have shallower bodies (males only), longer snouts (females only), lighter spawning color, darker egg color, and later spawning times than fish spawning in non-glacial habitats.

Morphology

Male and female morphology were analyzed separately because sexes differed in BD and SN ($P < 0.03$). Years were pooled within sexes because interannual variation was significant for only female SN between two populations (SBL and LKR; $P \leq 0.04$). There were significant differences, but also broad overlap, among populations for both BD and SN within both sexes.

Male BD increased with body size in all populations with a significant difference in elevation ($F_{12, 445} = 25.81$, $P < 0.001$) but no difference in slope ($F_{12, 433} = 1.15$, $P = 0.32$) among population regression lines. After adjustment to a common body size (519mm hypural length), Upper Tlikakila River (UTLK), LTLK, and PRC males had the shallowest bodies and Kijik Lake South Beach (KLSB), LKR, LLCB, and Chi Point Beach (CHI) males had the deepest bodies (Table 2). Male SN increased with body size in all populations, but regression

slopes differed among populations ($F_{12, 432} = 3.22$, $P < 0.001$). Regardless of the body size to which traits were adjusted, Tazimina River (TAZ), PRC, and Lake Clark Outlet (OUT) males had the shortest snouts and CHI, Hatchet Point Beach (HPB), and LKR males had the longest snouts (data not shown). Populations were sometimes extreme in multiple morphological traits. For example, males spawning in PRC had small body sizes, shallow bodies, and short snouts, while males spawning in LKR and LLCB had deep bodies and long snouts.

Female BD increased with body size in all populations with a significant difference among regression lines in elevation ($F_{12, 455} = 14.60$, $P < 0.01$) but not slope ($F_{12,443} = 1.50$, $P > 0.10$). Similar to the males, females of the KLSB, LLCB, and LKR had the greatest female body depths of all populations surveyed after adjustment to a common hypural length (500mm, Table 2). Female SN increased with body size in all populations, but regression slopes differed ($F_{12,442} = 1.90$, $P = 0.04$). Little Kijik River females had the longest snouts and KLSB females had the shortest snouts regardless of body size to which SN was adjusted.

Egg size

Egg size and body size were positively correlated in eight populations surveyed but were not correlated in five others (Currant Creek (CC), CHI, Kijik River (KR), LTLK, and UTLK). Thus, lines of regression between egg and body size differed among populations in slope ($F_{12,304} = 3.27$, $P < 0.01$). Priest Rock Creek and UTLK females consistently had the smallest eggs and HPB the largest eggs over all body sizes to which egg size was adjusted. Difference in mean egg size among all other populations varied radically based on body size.

Spawning and egg color

Male and female spawning color were analyzed separately because sexes differed significantly in spawning color frequencies ($LR_2 = 119.1$, $P < 0.01$). Males were almost exclusively red (93 to 100% per population) with rare occurrences of pink (3 to 7%; Table 2), and male color frequencies were similar among populations ($LR_{12} = 12.2$, $P = 0.17$). Females displayed red, pink, or orange body color with significant differences among populations in

color frequencies ($LR_{24} = 322.3$, $P < 0.001$). Females with orange body color were extremely rare (frequency less than 1%). Thus, color differences among females were summarized as simply the frequency of pink females by population (Table 2). Nine of 13 populations had a majority of red females (85 to 100%) while four populations (CC, LLCB, LTLK, UTLK) had a majority of pink females (57 to 97%; Table 2).

Eggs varied in shade from light to dark orange and egg color (shade of orange) differed significantly among populations ($LR_{48} = 195.90$, $P < 0.001$). Tazimina River, PRC, and HPB populations had lighter mean egg color and the KLSB population had a darker mean egg color score than other populations surveyed (Table 2). Egg size and egg color were independent. Two-way ANOVA revealed that egg weight differed significantly among habitat types ($F_{4, 309} = 3.3$, $P = 0.01$) but not among egg color categories ($F_{4,309} = 1.0$, $P = 0.43$) within habitat types. In addition, there was broad overlap in mean egg weight among populations and habitat types within each egg color category. For example, PRC and HPB females had some of the lightest mean egg color scores, but PRC had the smallest and HPB the largest mean egg size among populations.

Female body color and egg color were negatively correlated ($F_{1,188} = 11.0$, $P < 0.01$) and the distribution of egg color scores between red and pink females differed significantly ($LR_3 = 13.08$, $P < 0.01$). Eggs were lighter in color among red females (mean egg color scores glacial: 2.13 ± 0.35 95% CI; non-glacial: 2.20 ± 0.21) than pink females (2.69 ± 0.23; $F_{2,187} = 5.52$, $P < 0.01$; Figure 3).

Spawning time

Peak spawning occurred between 25 August and 12 October for surveyed populations between 1999 and 2001 (Table 2). Tazimina River and SBL populations spawned earliest and HPB, PRC, LLCB, and LTLK populations spawned latest.

4.4.3 Comparison of Phenotypic and Habitat Variation

Beach and tributary habitats

Morphology.— Beach spawning males and females had significantly deeper bodies and longer snouts than tributary spawning males and females. Slopes of regression of female SN on body size differed between beach and tributary populations (Table 3). However, beach spawning females had greater SN than tributary spawning females regardless of body size to which SN was adjusted ($F_{1,387} \geq 15.74$, $P < 0.01$, data not shown). Differences in BD and SN were greater between males than females. Beach spawning males had 9% greater BD and 4% greater SN than tributary spawning males while beach spawning females had 6% greater BD and 3% greater SN than tributary spawning females. Body depth increased with water depth for both sexes; the relationship was significant among males ($F_{1,8} = 3.80$, $P = 0.04$, $R^2 = 0.32$) but not females ($F_{1,8} = 1.05$, $P = 0.17$, $R^2 = 0.12$). Snout length was not correlated with water depth in either sex ($P > 0.19$).

Egg size.— Beach spawners had 8% larger eggs at all body sizes than tributary spawners. Egg size increased with body size in both beach and tributary fish, with a significant difference in elevation but not slope between regression lines (Table 3). Egg size was larger among beach spawners due primarily to PRC, a tributary population with exceptionally small eggs. Egg size was still larger among beach than tributary spawning fish after the removal of PRC (mean egg size beaches: 0.115gm, 0.112 – 0.117 95% CI; tributaries: 0.108, 0.106 – 0.110), though the difference was only marginally significant ($P = 0.09$). Egg size and proportion of fine substrate were negatively correlated ($F_{1,8} = 10.24$, $P < 0.01$, $R^2 = 0.56$). This relationship was driven by PRC and UTLK, two tributary habitats with small mean egg size and a preponderance of fine substrate.

Spawning and egg color. — Spawning color did not differ between males ($LR_1 = 1.3$, $P = 0.34$) but differed significantly between females ($LR_2 = 9.3$, $P < 0.01$) spawning in beach and tributary habitats. The difference in female color reflected a higher frequency of red and a lower frequency of pink females in beach (red = 78%, pink = 22%) than tributary populations

(red = 63%, pink = 36%; Table 2). Female body color was not correlated with either water depth or substrate size ($P \geq 0.82$).

Beach spawning females had significantly darker eggs than tributary spawning females ($LR_4 = 12.5$, P = 0.01; Table 2) due to the darker eggs of the KLSB population. Beach and tributary populations did not differ in egg color when KLSB was removed ($LR_4 = 5.1$, P = 0.30) and egg color was not correlated with either water depth (P = 0.81) or substrate size (P = 0.43).

Spawning time. — Mean peak spawning date did not differ between beach and tributary spawning populations ($F_{1,9} = 0.03$, P = 0.87; Table 2). Mean incubation temperature and mean peak date of spawning were not correlated (P = 0.20).

To summarize, salmon populations spawning on Lake Clark beaches had deeper bodies, longer snouts, larger eggs, and higher frequencies of red females than salmon populations spawning in Lake Clark tributaries. Spawning site water depth was positively correlated with fish body depth and proportion of fine substrate was negatively correlated with egg size. Correlations with measured habitat characteristics could not account for differences in snout length or spawning color between beach and inlet tributary populations.

Glacial and non-glacial habitats

Morphology.—Glacial beach spawning fish had similar BD and greater SN than non-glacial beach spawning fish (Table 3). Regression lines of BD on body size did not differ in slope or intercept between glacial and non-glacial beach spawning males or females. However, slopes of SN regressed on body size differed between males and were similar between females spawning in glacial and non-glacial beach habitats. Glacial beach males had snouts 1 to 5% longer (3 to 7mm) than non-glacial beach males when adjusted to all but the smallest body sizes (480mm: $F_{1,187} = 0.83$, P=0.36; 519.1mm: $F_{1,187} = 14.20$, $P < 0.01$; 560mm: $F_{1,187} =$

45.32, $P < 0.01$). Glacial beach spawning females had snouts approximately 3% longer (2mm) than non-glacial beach spawning females at all body sizes.

Males spawning in glacial inlet tributaries had shallower bodies and longer snouts than males spawning in non-glacial inlet tributaries but glacial and non-glacial tributary females did not differ in BD or SN (Table 3). Lines of regression for male BD and SN on body size had similar slopes but different intercepts between glacial and non-glacial tributary populations. After correction to equal body size, males spawning in glacial tributaries had approximately 4% reduction in BD (9mm) and 5% increase in SN (5mm) relative to males spawning in non-glacial tributaries (Table 3). Among females, BD and body size regression lines had equal slopes and intercepts while SN and body size regression slopes differed between glacial and non-glacial tributary populations. Mean SN was greater among glacial than non-glacial tributary females after correction to multiple body sizes, but differences were not significant ($P > 0.47$). Body depth and SN were not correlated with turbidity in either males or females overall ($P \geq 0.13$), but SN was correlated with turbidity among tributary males ($P = 0.05$).

Egg size.—Egg size did not differ between glacial and non-glacial beach spawning populations, but the relationship between egg and body size differed significantly between glacial and non-glacial tributary populations (Table 3). Regression lines of egg size on body size for glacial and non-glacial beach populations did not differ in slope or intercept. However, slopes of egg size regressed on body size differed between glacial and non-glacial tributary populations because egg size increased with body size in two non-glacial tributary populations (TAZ, PRC) but was independent of body size in another non-glacial (KR) and all three glacial tributary populations. Thus, mean egg size was greater in glacial than non-glacial tributary populations at small body size (480mm: $F_{1,182} = 6.08$, $P = 0.02$) and vice versa at large body size (though not statistically significant; 530mm: $F_{1,182} = 2.2$, $P = 0.14$). There was no correlation between mean egg size and turbidity at any body size to which egg sizes were adjusted ($P \geq 0.29$).

Spawning and egg color.—Males spawning in glacial and non-glacial habitats did not differ in body color (beach: $LR_1 = 5.7$, P = 0.06; tributary: $LR_1 = 1.3$, P = 1.0). Males were primarily red; pink body color was rare among males (3 to 7% per population) and observed only in glacial habitats (Table 2). In contrast, there were significant differences between glacial and non-glacial spawning populations in female body color frequencies (beach: $LR_1 = 77.1$, $P < 0.001$; tributary: $LR_2 = 132.2$, $P < 0.001$). The majority of females in glacial habitats were pink (beach = 67%, tributaries = 72%), while all of the non-glacial spawning females were red (Table 2).

Egg color did not differ between glacial and non-glacial beach populations ($LR_3 = 5.1$, P = 0.18). However, glacial tributary populations had significantly darker eggs than non-glacial tributary populations ($LR_4 = 45.3$, $P < 0.01$) due to lighter colored eggs in the PRC and TAZ populations than other tributary populations surveyed (Table 2).

Turbidity was highly correlated with female body color ($F_{1,11} = 42.06$, $P < 0.001$, $R^2 = 0.79$; Figure 4A) among all populations surveyed and with mean egg color among inlet tributary populations ($F_{1,4} = 8.05$, P = 0.02, $R^2 = 0.67$; Figure 4B). Egg color still increased with turbidity when all populations were considered, but the correlation was not significant (P = 0.23; Figure 4B).

Spawning time. —Peak spawning occurred later among glacial (3 Oct, 95% CI 17 Sept to 20 Oct) than non-glacial habitats (17 Sept, 6 to 28 Sept). This trend was evident but not significant within beach and tributary habitats types ($P \geq 0.13$) and was marginally significant overall ($F_{1,9} = 3.52$, P = 0.09; Table 2). Mean peak spawning date was positively correlated with turbidity ($F_{1,11} = 3.47$, P = 0.04, $R^2 = 0.24$; Figure 4C).

In summary, salmon populations spawning in glacial habitats had longer snouts, higher frequencies of pink females, and later spawning times than salmon populations spawning in non-glacial habitats. In addition, salmon populations spawning in glacial tributaries had

shallower bodied males, different egg size allometries, and darker colored eggs than salmon populations spawning in non-glacial tributaries. Spawning site turbidity was correlated with male SN (tributary habitats only), female body color, egg color (tributaries only), and spawning time (beach and tributary habitats pooled). Differences in water turbidity could not explain differences observed in additional morphological traits or egg size.

Comparison of phenotypic and neutral genetic divergence

Mean P_{ST} was not correlated with mean F_{ST} for male or female BD or male body color ($P \geq 0.19$) but was correlated with mean F_{ST} for female body color and egg color ($P \leq 0.06$). Body depth P_{ST} was correlated with the BT habitat matrix (males: $P < 0.01$, females: $P = 0.06$) but not the GNG habitat matrix ($P > 0.30$ males and females). A model including both the BT and GNG habitat matrices explained the most variation in body depth P_{ST} of all models considered ($R^2 = 0.13$ males, 0.05 females), but little more than that explained by the BT matrix alone ($R^2 = 0.12$ males, 0.04 females). In contrast, spawning color was not significantly correlated with the BT habitat matrix ($P \geq 0.18$ males and females) but was correlated with the GNG habitat matrix ($P < 0.01$ males and females). The GNG habitat matrix explained little if any variation among males ($R^2 < 0.01$) and the majority of variation among females ($R^2 = 0.63$) in spawning color P_{ST}. Female body color was more closely associated with the GNG habitat difference ($P < 0.001$, $R^2 = 0.63$) than F_{ST} ($P = 0.06$, $R^2 = 0.05$), and adding F_{ST} to a model including only the GNG habitat matrix did not increase R^2 appreciably ($R^2 = 0.67$ for model with both GNG and F_{ST}). Egg color was not correlated with either habitat matrix ($P \geq 0.23$), regardless of whether we first removed the variation explained by F_{ST}.

Body depth P_{ST} exceeded F_{ST} in comparisons between and within beach and tributary habitat categories for both males and females (Figure 5A). Mean body depth P_{ST} was greater between beach and tributary populations than among populations of similar habitat types. This was significant for both beach and tributary populations in males and for tributary

populations in females. The greatest body depth P_{ST} was observed between beach and tributary spawning males.

Male and female spawning color P_{ST} was undefined among non-glacial populations because all fish were red. Male spawning color P_{ST} was significantly less than F_{ST} in comparisons of glacial and non-glacial populations but similar to F_{ST} in comparisons among glacial populations (Figure 5B). Female spawning color P_{ST} exceeded F_{ST} in comparisons between glacial and non-glacial populations and among glacial populations. In females, spawning color P_{ST} was significantly greater in comparisons of glacial and non-glacial populations than in comparisons of glacial populations but the two P_{ST} values were similar in males. Egg color P_{ST} significantly exceeded F_{ST} in all comparisons and was significantly greater among non-glacial populations than among glacial populations (Figure 5B).

4.5 Discussion

Our data suggests local adaptation of Lake Clark sockeye salmon to beach, tributary, and glacial spawning habitats. Phenotypic divergence among populations is correlated with differences in spawning habitat, independent of neutral genetic divergence among populations, and, for most traits, greater than expected based on genetic drift alone. Phenotypic plasticity is an unlikely source of phenotypic differences among populations because Lake Clark sockeye salmon rear as juveniles in a common nursery lake (Schlenger 1996), collectively outmigrate to sea as smolt (Orrell 1963; Woolington et al. 1990), spend their adult lives in a common oceanic environment (French et al. 1976; Burgner 1980), and return synchronously to Lake Clark to spawn (Burgner 1980; Jensen and Mathisen 1987). Thus, there is little opportunity for populations to experience different environments. In addition, the phenotypic traits measured for this study have an additive genetic basis, are directly related to spawning, and are fully developed prior to fish arriving at spawning habitats they have only previously encountered during their earliest developmental stages 3 to 4 years prior.

4.5.1 Lake Clark founder event

Eleven of the thirteen populations included in this study exhibit a common genetic bottleneck signal due to a founder event associated with the colonization of Lake Clark by sockeye salmon (Ramstad et al. 2004). Genetic bottleneck effects can decrease variation in quantitative traits with a strong additive genetic basis (Wright 1969; Crow and Kimura 1970; Lande 1980) and increase variation in quantitative traits with strong epistatic or dominant genetic effects (Goodnight 1987; Willis and Orr 1993; Armbruster et al. 1998). If the Lake Clark founder event had a lasting effect on quantitative trait variation, we should observe phenotypic divergence between those populations that were commonly founded and those that were not (OUT and TAZ). We found no evidence of such a pattern. Thus, genetic drift due to the Lake Clark founder event cannot explain the observed patterns of phenotypic divergence among populations of Lake Clark sockeye salmon. In addition, the genetic bottleneck associated with colonization of Lake Clark is mild (decrease in microsatellite heterozygosity of < 5%) and old (> 100 sockeye salmon generations; Ramstad et al. 2004) and should not affect the ability of populations to adapt locally.

Commonly founded populations have provided some of the best evidence of rapid adaptive divergence in salmon because phenotypic divergence due to selection must have arisen since populations became established (Quinn et al. 1996; Kinnison et al. 1998; Hendry et al. 2000b; Quinn et al. 2000). These studies suggest that local adaptation can occur quickly (e.g. 13 generations in Lake Washington sockeye salmon) and be present among populations that exhibit little neutral genetic divergence (Hendry et al. 2000b; Stockwell et al. 2003).

4.5.2 Beach and tributary habitats

Body depth and egg size differed significantly between sockeye salmon spawning in beach and tributary habitats of Lake Clark. Mean body depth was positively correlated with water depth and fish spawning on beaches had deeper bodies than those spawning in tributaries. Mean egg size was positively correlated with substrate size and fish spawning on beaches had larger eggs than fish spawning in tributaries. This pattern has also been found among sockeye

salmon in other freshwater systems (Blair et al. 1993; Quinn et al. 1995; Hamon et al. 2000; Hendry et al. 2000b; Quinn et al. 2001).

Differences in body depth between beach and tributary spawning populations is hypothesized to be due to differential trade-offs between sexual and natural selection. Sexual selection favors large males with deep bodies (Darwin 1871; Foote 1990; Fleming and Gross 1994; Quinn and Foote 1994; Hamon and Foote 2005). However, bear predation is higher in tributary than beach habitats and tends to select against large, deep bodied males (Quinn and Kinnison 1999; Gende at al. 2001; Quinn et al. 2001). In addition, deep bodied males may have difficulty gaining access to shallow spawning tributaries (Hamon and Foote 2005). Thus, males with deep bodies are selected against in shallow tributary spawning habitats while beach spawning populations appear to freely develop exaggerated dorsal humps (Blair et al. 1993; Quinn et al. 2001).

Mean egg size differences among beach and tributary spawning populations have been attributed to oxygen limitation and selection on body size of emergent fry. Redds constructed in fine substrate (e.g., tributaries) have lower porosity, poorer water circulation, and lower dissolved oxygen concentration than redds constructed in course substrate (Chapman 1988). Smaller eggs may be favored under these conditions because they have reduced metabolic oxygen demands, have more efficient oxygen uptake due to their high surface area to volume ratio, and produce small emergent fry able to navigate the small interstitial spaces of the redd (Phillips et al. 1975; Hausle and Coble 1976; Hutchings 1991). The opposite is expected for eggs in course substrate redds (e.g., beaches) where high oxygen availability allows for larger eggs with smaller surface area to volume ratios and large emergent young.

We observed what appears to be the smallest mean egg size yet documented for this species in the Priest Rock Creek population of Lake Clark (83.8 mg adjusted to hypural length of 450mm, Burgner 1991; Hilborn et al. 2003). Priest Rock Creek is a tributary with exceptionally fine substrate (66% < 4 mm) owing to a series of beaver dams that reduce water flow. The stable, warm water temperature of Priest Rock Creek suggests significant influence

of upwelling, spring-fed ground water which could combine with small egg size to compensate for the smothering effects of fine substrate (Quinn et al. 1995).

Taken together, these data support the view that sockeye salmon in Lake Clark have adapted locally to beach and tributary spawning habitats. The parallel pattern of divergence among beach and tributary spawning populations in multiple freshwater systems cannot be due to random genetic drift. Additionally, divergence in male and female body depth is significantly greater than can be explained by neutral genetic drift alone and greater between than within beach and tributary habitat types. The data also suggest heterogeneity in strength and/or direction of selection within beach and tributary habitats. Body depth P_{ST} exceeds neutral F_{ST} in population comparisons within beach and tributary habitat types and correlation with spawning habitat type explains only 4 to 12% of the variation in body depth P_{ST}.

4.5.3 Glacial habitats

In this study we present the first evidence of local adaptation of sockeye salmon to glacial spawning habitats. In Lake Clark and generally, glacial habitats have elevated turbidities, less stable flow regimes, siltier substrates, and lower temperatures than non-glacial habitats (Burgner 1991; Stanford and Ward 1992; Murphy et al. 1997). Fish spawning in glacial habitats in Lake Clark had longer snouts, different egg size allometries, lighter spawning color, darker egg color, and later spawning times than fish spawning in non-glacial habitats of Lake Clark. Male snout length, female spawning color, egg color, and spawning time were all significantly correlated with spawning habitat turbidity. Spawning color P_{ST} was greater than F_{ST} for females and less than F_{ST} for males in comparisons between glacial and non-glacial habitats. This suggests that selection favors different phenotypes among females spawning in glacial (pink spawning color) and non-glacial habitats (red spawning color) and the same phenotype among males (red spawning color) in both types of habitat.

In Pacific salmon, spawning time differences among populations are thought to be adaptive because they are positively correlated with incubation temperature and, thus, allow young to emergence synchronously at optimum foraging time in spring (Brannon 1987). In

sockeye salmon, warmer incubation temperatures typically promote later spawning times in beach and outlet tributary habitats than inlet tributary habitats (Wood 1995). Spawning time did not differ among Lake Clark beach, outlet, and inlet tributary populations, but was later in glacial than non-glacial populations. Our data will not allow us to test if this difference is correlated with incubation temperature. However, we suggest three mutually inclusive hypotheses that may explain the later spawning times of glacial populations. First, glacial populations tend to spawn in areas of upwelling groundwater (Lorenz and Eiler 1989) which likely promotes relatively warm and constant incubation temperature and allows glacial populations to spawn later but still synchronize their emergence time with other Lake Clark populations. Second, glacial habitats may have lower or similar incubation temperatures and later optimum emergence times than non-glacial spawning habitats. Finally, spawning time in glacial habitats may be more tightly constrained by a window of opportunity for adults to spawn rather than for young to emerge. For example, spawning in glacial tributaries of Lake Clark coincides with, and may be constrained by, an annual dramatic decrease in turbidity (Young 2004).

Snouts are used as intrasexual weapons by both male and female sockeye salmon, and snout length is positively correlated with spawning density (Fleming and Gross 1989). We have no reason to believe, however, that spawning density is greater in glacial than non-glacial populations of our study. Further, redd size tends to decrease and spawning color intensity increase with spawning competition (Mathiesen 1962; Fleming and Gross 1989), but sockeye salmon construct larger redds (Lorenz and Eiler 1989) and have lighter spawning color in glacial than non-glacial habitats. These data suggest that fish spawning in glacial habitats may perceive low spawning density because their ability to see competitors is reduced. Thus, difference in breeding competition is an unlikely explanation for why fish in glacial habitats have longer snouts than fish in non-glacial habitats. More likely, low visibility in glacial habitats reduces the ability of individuals to avoid aggressive encounters through visual displays of body size and status and promotes increased investment in the development of weapons. Reduced body depth among males spawning in glacial tributaries

(relative to non-glacial tributaries) is consistent with this view as is the lighter spawning color among females of glacial populations.

4.5.4 Significance of spawning and egg color

Carotenoids are lipid soluble antioxidant pigments that protect tissues from oxidative damage and help regulate immune response (Olson and Owens 1998; Blount et al. 2000; but see Hartley and Kennedy 2004). Animals cannot synthesize darotenoids *de novo* but must acquire them through diet, which is a costly process because they are a limited resource that is relatively rare in nature. Thus, carotenoid-based sexual signals are thought to be honest signals of the fitness and status of individuals (Olson and Owens 1998). It is also important to provision eggs with carotenoids to improve the immune response and survival of young (Blount et al. 2000; Blount et al. 2002). Many organisms concentrate carotenoids in their eggs at the expense of their own body color and, potentially, effective immune response (Green 1965; Royle et al. 2003).

Sockeye salmon deposit carotenoids in both their skin and eggs in preparation for spawning. Deposition of carotenoids in the skin results in red spawning color that is important for mate recognition and mate choice (Craig and Foote 2001; Foote et al. 2004). Both males and females have a keen ability to see red while spawning (Novales-Flamarique 2000), and males preferentially mate with the reddest female available (Foote et al. 2004). Lighter spawning color among glacial fish has not been previously reported, but has been previously observed in female sockeye salmon of the Taku River (D. Martin, pers. comm. 2005) and both male and female pink salmon (*Oncorhynchus gorbuscha*) in Wolf Point Creek of Glacier Bay (C. Kondzela, pers. comm. 2005). In Lake Clark, male spawning color is generally red regardless of spawning habitat type, female spawning color is red in non-glacial habitats, and female spawning color is light pink in glacial habitats. Why might we observe such a pattern?

Differences in diet and/or ability to utilize carotenoids can cause differences in spawning color (red and white Chinook salmon - Withler 1986, sockeye salmon and kokanee

- Craig and Foote 2001, piscivorous and planktivorous kokanee – Miller et al. *in press*) but are not likely the cause of spawning color differences between glacial and non-glacial salmon populations. Glacial and non-glacial habitats may differ in the amount of dietary carotenoids available, but the primary source of carotenoids in sockeye salmon is their diet while at sea (Craig and Foote 2001), and we have no reason to believe glacial females are segregated from other sockeye salmon while at sea. In addition, ability to use dietary carotenoids to produce red spawning color is a genetically based trait that has been shown to vary among populations and morphs within species, but not between sexes within populations (Withler 1986; Craig and Foote 2001).

Our data are consistent with an apparent trade-off between body and egg color in female sockeye salmon (Crozier 1970; Craig and Foote 2001). Thus, color differences between glacial and non-glacial spawning females could be due to (1) relaxed selection for red spawning color, (2) selection for lighter spawning color, and/or (3) selection for greater egg carotenoid concentration in glacial habitats.

Selection for red spawning color may be relaxed in glacial habitats because visual signals in general are not effectively transmitted in glacially turbid waters. We did not test the visual acuity of salmon or measure the spectral properties of glacial and non-glacial waters. However, we did observe that glacial spawning fish were not able to see us, our fishing nets, or seemingly each other, as well as fish spawning in non-glacial habitats. Red body color is a common signal in freshwater fishes but becomes ineffective when increased turbidity renders it invisible (Seehausen et al. 1997). In addition, visual signals are only effective when they contrast strongly with their background and it is possible that red does not contrast well against milky blue glacial waters. Clear freshwater transmits UV blue light most effectively and contrasts strongly with red and yellow light. Tannin stained and turbid waters do not contrast well with red color and loss of red nuptial color has been observed in three-spine stickleback (*Gasterosteus aculeatus*) and bluefin killifish (*Lucania goodie)* males that inhabit these types of habitats (Boughman 2001; Fuller 2002). In addition, variation in vision physiology is associated with spawning behavior in sticklebacks (Boughman 2001) and

has an genetic basis in blue killifish (Fuller et al. 2005) suggesting that sensory systems themselves evolve in response to selection.

Alternatively, glacial habitats may select for lighter spawning color through predation. Bears may preferentially prey on red fish if pink spawning color does not contrast with the light, milky blue color of glacial waters as much as red spawning color. Black bears (*Ursus americanus*) have color vision (Bacon and Burghardt 1976), so it is likely that Lake Clark brown bears (*Ursus arctos*) use color vision when fishing for salmon. Predation has been implicated in promoting different amounts of red nuptial color in guppy (*Poecilia reticulate*, Endler 1995; Endler and Houde 1995) and *Diaptomus* copepod (Hairston 1979) populations. Differentiating between relaxed sexual selection for red and increased natural selection for pink spawning color will require analysis of the spectral properties of glacial water, and in particular, the ability of sockeye salmon and brown bears to differentiate shades of red in glacial water.

Finally, female body color may differ between glacial and non-glacial spawning populations as a by-product of selection on egg carotenoid concentration. While there are no heritability estimates available for egg color or egg carotenoid concentration *per se*, the ability of sockeye salmon to turn tissues red with carotenoids is highly heritable, does not differ between sexes, and is not due to maternal effects (Craig and Foote 2001). Recall also that plasticity is an unlikely source of phenotypic variation among Lake Clark sockeye salmon populations. These observations suggest that carotenoid partitioning among tissues is itself a trait under additive genetic control and, therefore, responsive to selection. Eggs and pre-emergent fry in glacial redds may experience lower dissolved oxygen concentrations and increased levels of free radicals because glacial habitats tend to have fine substrate and poor inter-gravel water circulation. These conditions could result in selection for higher carotenoid concentration in eggs and a differential partitioning of carotenoids to body and egg color between fish spawning in glacial and non-glacial habitats.

We currently cannot differentiate among the three hypotheses outlined above. However, the most parsimonious explanation is simply relaxed selection for bright red visual

signals in glacial habitats. Why, then, do we observe duller spawning color among only *females* in glacial habitats? First, males may indeed have lighter spawning color in glacial than non-glacial habitats with the difference being subtle and undetectable visually. The human eye cannot distinguish among red hues above a relatively low threshold of carotenoid concentration and males may differ in spawning color beyond this threshold (Smith et al. 1992). Secondly, similar spawning color selection would likely affect males and females differently because males have no alternative use for their carotenoids while females must partition their carotenoids between themselves and their young. These observations suggest that local adaptation of sockeye salmon to glacial spawning habitats involves reduced intensity of visual signals and increased weapon size. Because visual displays are not effective in avoiding aggressive encounters, individuals must arm themselves with effective weapons.

4.5.5 Conclusions

Phenotypic divergence among spawning populations of sockeye salmon is likely the product of local adaptation. The data provide additional evidence of beach and tributary ecotypes already reported in the literature, as well as the first evidence of a glacial ecotype of sockeye salmon. Local adaptation to glacial spawning habitats suggests that part of the reason sockeye salmon are excellent colonizers is that they can adapt quickly to highly unstable, geologically young habitats. As E. O. Wilson stated (1965), "To be a superb colonizer, one must not only scatter a large fraction of each generation into lethal habitats; one must also adapt to the comparatively unstable habitats…that form the main ports of entry into new regions". Glacial habitats are ephemeral and will slowly become non-glacial and lose their turbidity over geologic time. Sockeye salmon currently spawning in glacial habitats may slowly take on the characteristics of non-glacial spawners, reverting to red spawning color, as visibility increases in glacial habitats.

Table 4-1 Mean (SD) spawning habitat characteristics for 13 sockeye salmon populations in Lake Clark, Alaska. Substrate composition is presented as percent fine (< 4mm), moderate (4 to < 64mm), and course (≥ 64mm) in size; incubation temperature was measured in spawning substrate between 14 Oct and 30 May. Standard deviations within sites represent interannual variation for habitat characteristics measures in multiple years. Habitat types are coded as beach (B), inlet tributary (IT), or outlet tributary (OT) and glacial (G) or non-glacial (NG).

Sample site description				Turbidity (NTU)	Depth (m)	Percent substrate			Incubation temperature (˚C)
						Fine	Moderate	Course	
B1	SBL	Sucker Bay Lake	NG	2.7 (1.0)	0.72	11	41	49	3.0 (1.0)
B2	CHI	Chi Point Beach	NG	2.4 (0.2)	-	-	-	-	-
B3	KLSB	Kijik Lake South Beach	NG	0.7 (0.6)	0.48	16	80	4	-
B4	HPB	Hatchet Point Beach	G	5.7 (1.5)	-	-	-	-	-
B5	LLCB	Little Lake Clark Beach	G	11.6 (1.3)	0.90	2	32	66	-
Beach mean				4.6 (4.3)	0.70 (0.21)	10 (7)	51 (25)	40 (32)	-
IT1	TAZ	Tazimina River	NG	0.4 (<0.1)	0.63	11	75	14	1.3
IT2	KR	Kijik River	NG	2.2 (2.4)	0.44	17	70	13	1.8
IT3	PRC	Priest Rock Creek	NG	1.6 (0.1)	0.51	66	32	2	4.5 (0.3)
IT4	CC	Currant Creek	G	7.0	0.44	24	52	24	-
IT5	LTLK	Lower Tlikakila River	G	6.2 (2.3)	0.38	24	56	20	1.5 (0.4)
IT6	UTLK	Upper Tlikakila River	G	8.1	0.34	73	26	1	-

Table 4-1 continued.

Sample site description				Turbidity (NTU)	Depth (m)	Percent substrate			Incubation temperature (˚C)
						Fine	Moderate	Course	
OT1	OUT	Lake Clark Outlet	NG	2.9 (0.1)	-	-	-	-	2.6
OT2	LKR	Little Kijik River	NG	0.6 (0.3)	0.47	10	87	3	3.0 (0.9)
Glacial mean				7.7 (2.4)	0.51 (0.26)	31 (30)	41 (15)	28 (27)	-
Non-glacial mean				1.7 (1.0)	0.54 (0.11)	22 (22)	64 (22)	14 (18)	2.7 (1.1)

Table 4-2 Summary of phenotypic variation among 13 sockeye salmon populations of Lake Clark, Alaska. Body depths represent trait means adjusted to hypural lengths of 500mm for females and 519mm for males. Spawning color values represent the frequency of pink body color, egg color is the mean of interval color rank from light (1) to dark (5) orange, and spawning time is the estimated mean peak date of spawning from Ramstad et al. 2004. Standard deviations of means are in parentheses and habitat types are coded as beach (B), inlet tributary (IT), or outlet tributary (OT) and glacial (G) or non-glacial (NG). Refer to Table 1 for population names.

Sample site description			Adjusted body depth (mm)		Spawning color		Egg	Spawning
			♂	♀	♂	♀	color	time
B1	SBL	NG	200.4 (9.8)	136.2 (7.6)	0	0	1.8 (0.6)	30-Aug
B2	CHI	NG	207.7 (9.4)	135.1 (6.4)	0	0	3.1 (0.8)	24-Sep
B3	KLSB	NG	205.9 (9.3)	144.8 (9.6)	0	0	3.4 (0.6)	25-Sep
B4	HPB	G	202.4 (13.5)	134.6 (6.9)	0.03	0.15	1.5 (0.5)	12-Oct
B5	LLCB	G	206.9 (11.8)	142.4 (8.9)	0.07	0.93	2.9 (0.9)	7-Oct
IT1	TAZ	NG	189.1 (11.2)	130.7 (9.0)	0	0	1.3 (0.4)	25-Aug
IT2	KR	NG	196.2 (12.3)	136.3 (10.3)	0	0	2.3 (0.9)	20-Sep
IT3	PRC	NG	186.5 (10.2)	129.5 (5.7)	0	0	1.3 (0.6)	11-Oct
IT4	CC	G	188.1 (9.2)	129.1 (9.1)	0	0.97	2.8 (0.9)	25-Sep
IT5	LTLK	G	185.6 (12.5)	130.6 (7.3)	0	0.63	2.3 (0.9)	7-Oct
IT6	UTLK	G	178.6 (7.8)	132.8 (6.9)	0.03	0.57	2.4 (0.8)	25-Sep
OT1	OUT	NG	191.8 (14.1)	132.2 (10.3)	0	0	2.0 (0.9)	13-Sep
OT2	LKR	NG	206.0 (12.4)	143.2 (9.9)	0	0	2.5 (0.8)	18-Sep

Table 4-3 Difference in body depth, snout length, and egg size between Lake Clark sockeye salmon spawning in beach and tributary, glacial and non-glacial beach, and glacial and non-glacial inlet tributary habitats. Significance was tested by analysis of covariance with $\log_e$ transformed body length as the covariate and habitat type as a factor. Where trait size increased similarly with body size between groups, traits were adjusted to a common body size within groups and group means are given with 95% confidence intervals in parentheses. When trait size differences between groups varied with body size, results represent significance of interaction effects between body and trait size.

Variable	Beach and tributary	Glacial and non-glacial	
		Beaches	Tributaries
	Males		
Body depth (mm)			
Beach, Glacial	204.0 (202.4 - 205.6)	203.6 (201.1 - 206.0)	184.2 (182.0 - 186.4)
Tributary, Non-glacial	186.8 (185.3 - 188.3)	202.8 (200.8 - 204.8)	192.7 (190.0 - 195.4)
Na	190, 190	80, 110	110, 80
F	206.5**	0.3	19.3**
Adjusted body size (mm)	519	517	521
Snout length (mm)			
Beach, Glacial	107.1 (106.1 - 108.2)	NA	105.1 (103.9 - 106.4)
Tributary, Non-glacial	102.6 (101.6 - 103.6)	NA	100.5 (98.9 - 102.1)
N	189, 189	80, 109	110, 79
F	37.0**	5.6*	17.7**

Table 4-3 continued.

Variable	Beach and tributary	Glacial and non-glacial	
		Beaches	Tributaries
	Females		
Body depth (mm)			
Beach, Glacial	138 (136.6 - 139.4)	138.1 (136.2 - 140.1)	130.6 (129.0 - 132.2)
Tributary, Non-glacial	130.7 (129.7 - 131.8)	136.7 (135.1 - 138.4)	132.4 (130.6 - 134.3)
N	190, 200	80,110	110, 90
F	71.7**	1	2
Adjusted body size (mm)	499	497	501
Snout length (mm)			
Beach, Glacial	NA	71.6 (70.3 - 72.9)	NA
Tributary, Non-glacial	NA	69.3 (68.2 - 70.5)	NA
N	189, 200	80,109	110, 90
F	8.1**	7.3**	5.2*
Egg size			
Beach, Glacial	0.114 (0.112 - 0.117)	0.114 (0.111 - 0.117)	NA
Tributary, Non-glacial	0.106 (0.105 - 0.107)	0.113 (0.110 - 0.116)	NA
N	104, 184	50, 54	104, 80
F	37.1**	0.2	20.4**
Adjusted body size (mm)	505	503	NA

[a]Beach and tributary or glacial and non-glacial, respectively. $P < 0.05$*, $P < 0.01$**

Figure 4-1 Map of Lake Clark, Six-mile Lake, and Lake Iliamna with sampling sites shown. Sites are numbered from downstream to upstream, and coded for spawning habitat type (B = beach; IT = inlet tributary; OT = outlet tributary) and genetic population structure (open = Iliamna/Six-mile Lake group; grey = Sucker Bay Lake; black = Lake Clark group), refer to Table 1 for population names.

Figure 4-2 Principal components analysis of morphological (body depth, snout length, spawning color), life history (egg size), and behavioral (spawning time) traits of male and female sockeye salmon in Lake Clark, Alaska. Points represent mean component scores by spawning habitat type coded as non-glacial beaches (◇, N = 89 males, 42 females), non-glacial inlet tributaries (□, N = 79 males, 43 females), glacial beaches (◆, N = 59 males, 32 females), and glacial inlet tributaries (■, N = 90 males, 59 females). Percentages in parentheses indicate amount of variation explained by each principal component; bars represent 95% confidence intervals.

Figure 4-3 Mean egg color score for female sockeye salmon with red (black points) and pink (grey points) spawning color in glacial and non-glacial habitats of Lake Clark, Alaska. Bars represent 95% confidence intervals, egg color scores are the closest matching of plates 1 (lightest) through 5 (darkest) of the Hoffman La-Roche color card for salmonids (Roche Vitamins and Fine Chemicals Division, Hoffman-La Roche Inc., Nutley, NJ).

Figure 4-4 Correlation between turbidity during spawning and (A) percent of female sockeye salmon with pink body color, (B) mean egg color (by visual color score, statistics and dashed line for inlet tributary populations only), and (C) relative peak spawning time (number of days since 1 Aug) by habitat type.

Figure 4-5 Comparison of mean pair-wise P_{ST} and F_{ST} of sockeye salmon spawning within and between (A) beach and tributary and (B) glacial and non-glacial habitats of Lake Clark,

Alaska. Significant deviation of P_{ST} from F_{ST} is noted with an asterisk (*). Letters represent significant differences among P_{ST} values and are based on 95% confidence intervals (bars).

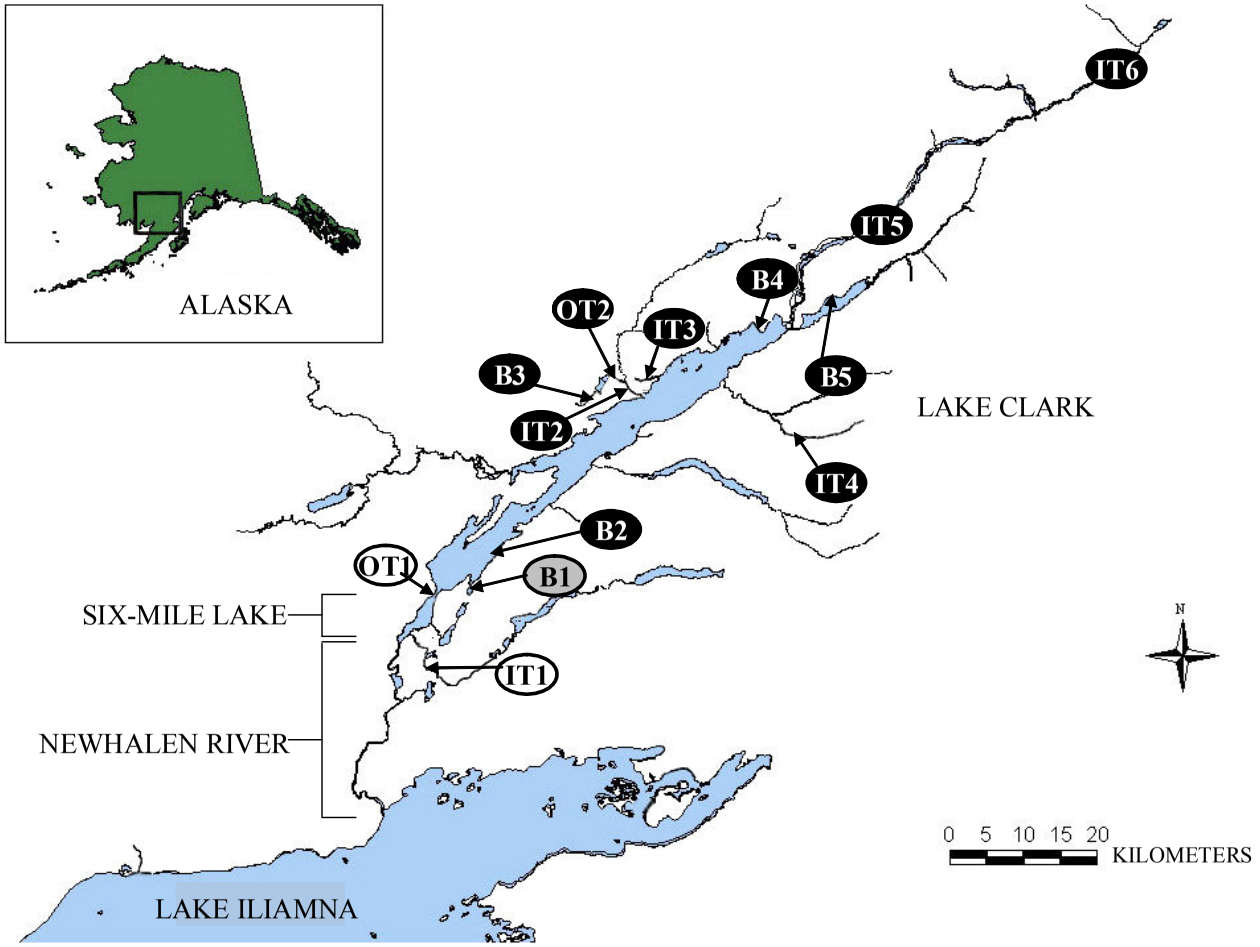
ALASKA
IT6
IT5
B4
OT2
IT3
B3
B5
LAKE CLARK
IT2
IT4
B2
OT1
B1
SIX-MILE LAKE
IT1
NEWHALEN RIVER
LAKE ILIAMNA
N
0 5 10 15 20
KILOMETERS

Figure 4-1

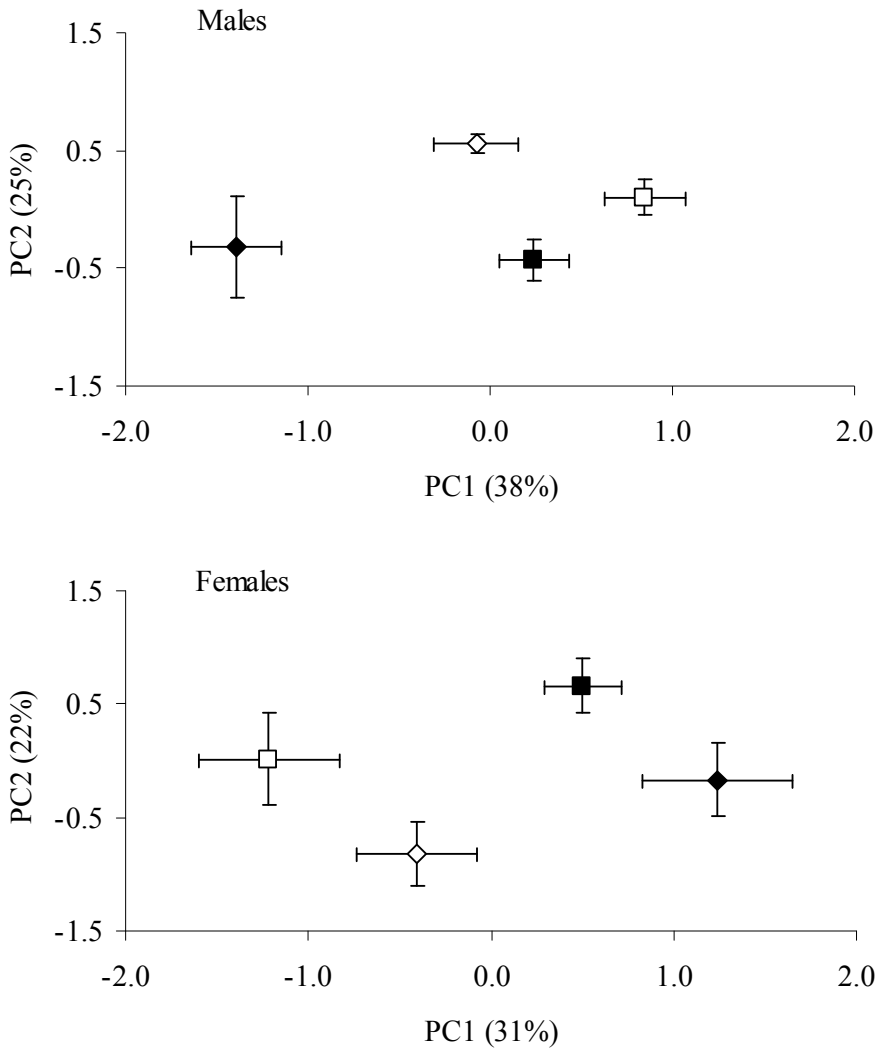
Males
1.5
0.5
-0.5
-1.5
PC2 (25%)
-2.0
-1.0
0.0
1.0
2.0
PC1 (38%)
Females
1.5
0.5
-0.5
-1.5
PC2 (22%)
-2.0
-1.0
0.0
1.0
2.0
PC1 (31%)

Figure 4-2

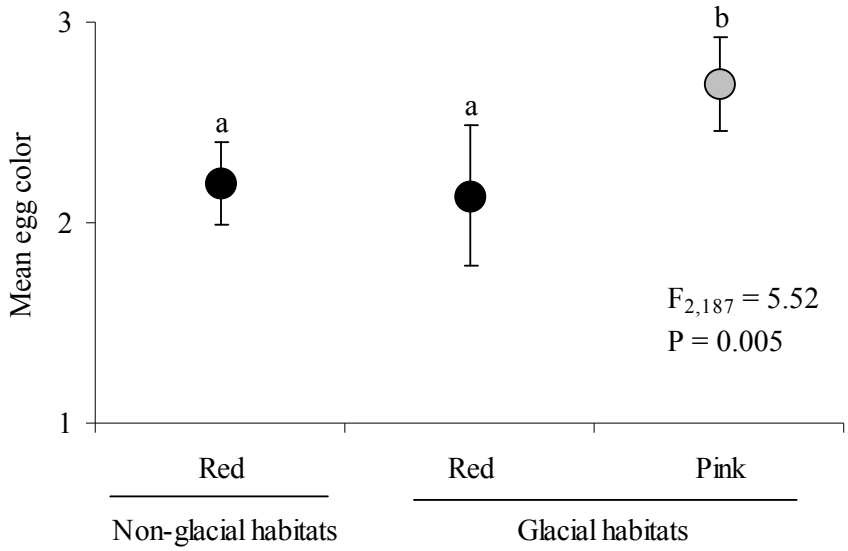
3
2
1
Mean egg color
a
a
b
$F_{2,187}$ = 5.52
P = 0.005
Red
Red
Pink
Non-glacial habitats
Glacial habitats

Figure 4-3

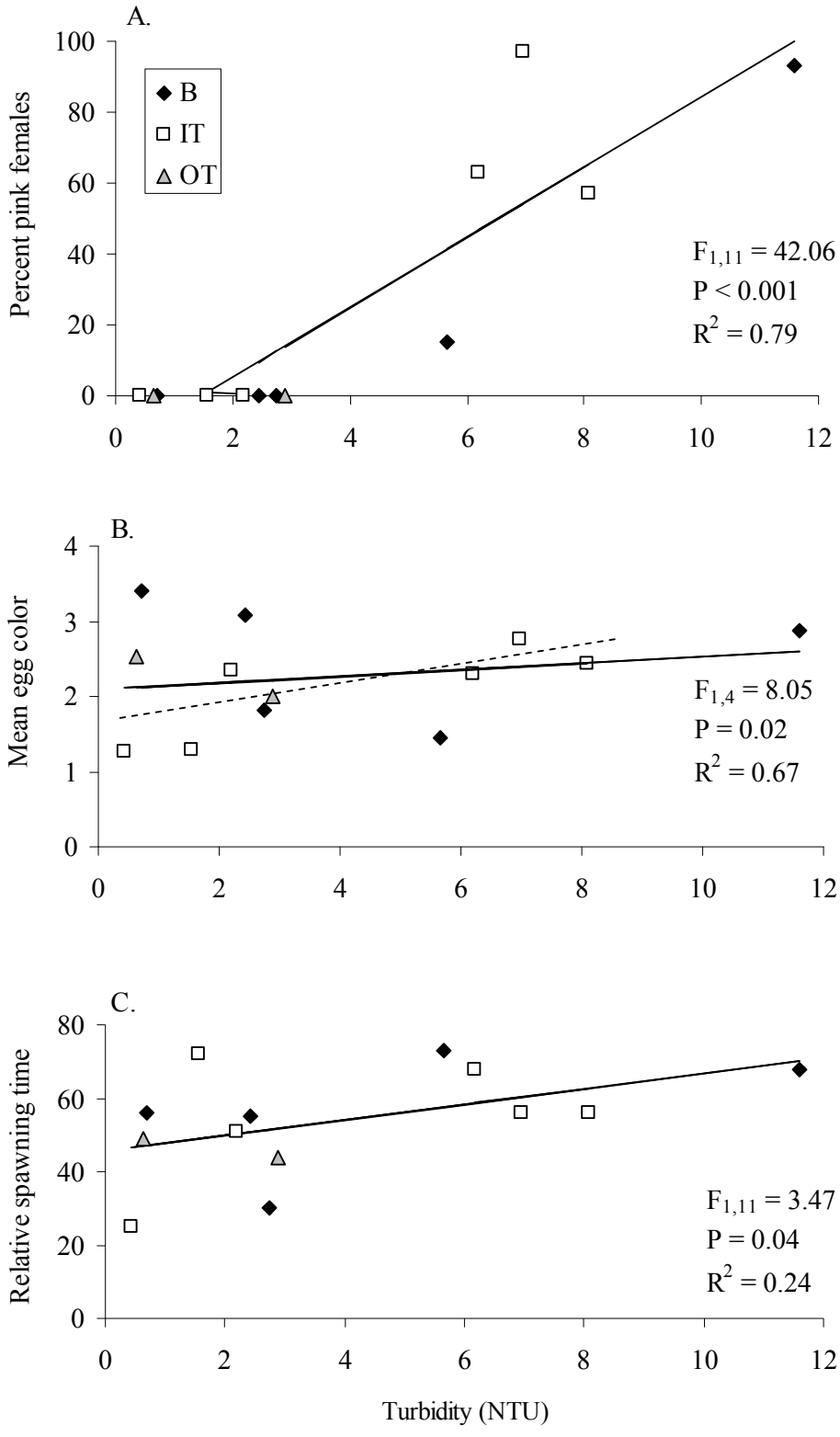
A.
Percent pink females
B
IT
OT
$F_{1,11}$ = 42.06
P < 0.001
R^2 = 0.79
B.
Mean egg color
$F_{1,4}$ = 8.05
P = 0.02
R^2 = 0.67
C.
Relative spawning time
$F_{1,11}$ = 3.47
P = 0.04
R^2 = 0.24
Turbidity (NTU)

Figure 4-4

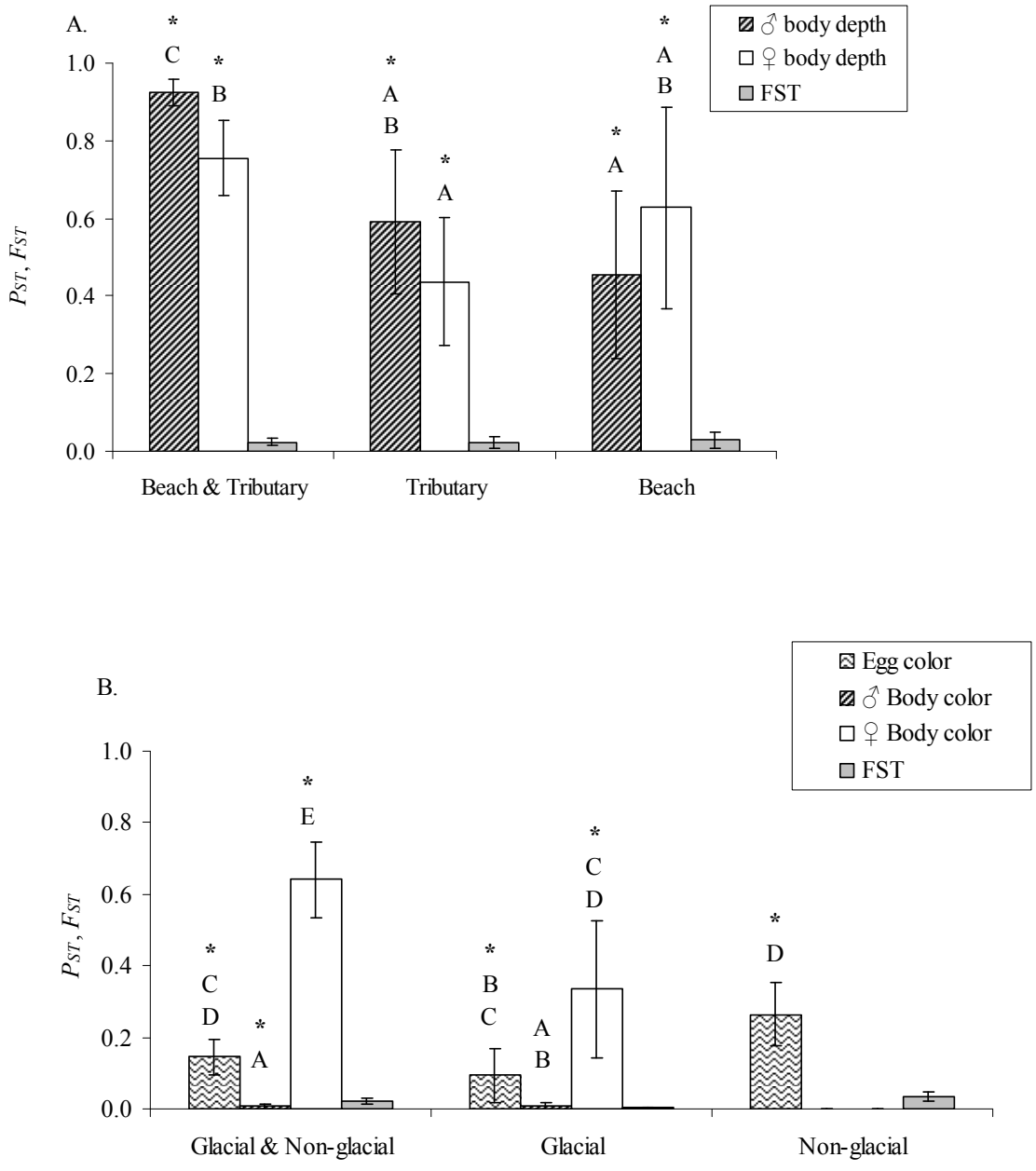
A.
♂ body depth
♀ body depth
FST
P_{ST}, F_{ST}
1.0
0.8
0.6
0.4
0.2
0.0
Beach & Tributary
Tributary
Beach
B.
Egg color
♂ Body color
♀ Body color
FST
P_{ST}, F_{ST}
1.0
0.8
0.6
0.4
0.2
0.0
Glacial & Non-glacial
Glacial
Non-glacial

Figure 4-5

Chapter 5 - Species and cultural conservation in New Zealand: Māori traditional ecological knowledge of tuatara

5.1 Abstract

Traditional ecological knowledge (TEK) can be highly informative and complement scientific knowledge. Integrating both types of information has increased understanding and improved management of many species. This has been especially true for abundant species that interact frequently with the indigenous peoples of their native range (e.g., through harvest). Isolated or declining species have been the subject of such studies less frequently. Here we describe Māori TEK of tuatara, the last living representatives of the reptilian Order Sphenodontia. Māori are indigenous to New Zealand, having settled it 800 to 1000 years ago. Tuatara are endemic to New Zealand, have been declining in numbers since human settlement, and are now restricted to 37 offshore islands. We conducted semi-directed interviews of elders of Te Atiawa, Ngati Koata, and Ngati Wai Iwi (similar to tribes), the guardians of several islands currently inhabited by tuatara. The detail and volume of tuatara TEK was less than that recorded in similar studies of abundant or accessible species. In addition, traditional knowledge of the cultural significance of tuatara was more common and detailed among the elders surveyed than traditional knowledge of tuatara biology or ecology. The TEK collected, however, provided the first evidence of seven former sites of tuatara occupation, suggested five additional sites that tuatara may presently occupy, contained novel hypotheses for scientific testing, and described tuatara cultural roles that have not been previously reported. This study illustrates that some TEK persists as species decline, even in the absence of direct and ongoing contact, and may serve as a valuable source of ecological information.

5.2 Introduction

Traditional ecological knowledge (TEK) is knowledge that is acquired through extensive and long term observation of an area or species (Huntington 2000). Though TEK is not unique to indigenous individuals or communities (Huntington 2000; Pierotti and Wildcat 2000), indigenous TEK is often highly valuable owing to its localized and long term nature (Turner et al. 2000). A broad range of ecological and evolutionary concepts are found in TEK. For example, the O'odham and Comcáac peoples of Arizona, USA and Sonora, Mexico, recognize predator and prey, ectoparasite and host, forager and forage, mimic and model, and dweller and dwelling ecological relationships (Nabhan 2000). Traditional ecological knowledge is not limited to those species that are actively harvested by or economically valuable to indigenous communities (Nabhan 2000; Kimmerer 2002).

Western scientific ecological knowledge and TEK are similar in a number of important ways. Both types of knowledge are based on observations of the natural world, suggest hypotheses about how the natural world works, are tested, shared with a community, interpreted within a cultural context, and used as a basis of resource management (Berkes 1998; Pinkerton 1998; Berkes et al. 2000; Kimmerer 2002). Acquisition and use of knowledge, however, differs markedly between scientific and traditional contexts. Scientific hypotheses are tested formally by the scientific method while TEK hypotheses are tested through application to the livelihood and survival of indigenous peoples (Huntington 2000). Results of scientific studies are published in peer reviewed journals and presented at meetings of scientific societies, while TEK is transmitted orally within and among families and tribes by stories, song, art, and ceremony (Berkes et al. 2000; Turner et al. 2000).

Western science may have much to learn from TEK. Traditional ecological knowledge can contain novel ecological information, resource management practices, and ecological hypotheses (Berkes et al. 2000; Kimmerer 2002). Indeed, scientists have sometimes reported discoveries that have long been known among indigenous people, such as the intoxicating effects of thornappple (*Datura*) alkaloids on nectar feeding hawkmoths

(*Manduca* spp.; Grant and Grant 1965; Nabhan 2000) or the effect of grizzly bear (*Ursus arctos horribilis*) foraging on the growth and persistence of glacier lilies (*Erythronium grandiflorum*; Tardiff and Stanford 1998; Turner et al. 2000). Traditional ecological knowledge may provide insight into past ecosystem states because it is older than most monitoring programs. For example, Dena'ina Athabaskans of the Lake Clark area of Alaska recall when a local dry streambed was once a watered creek full of sockeye salmon (Stickman et al. 2003). Finally, indigenous people have had lasting impacts on current ecology through species introductions (Matisoo-Smith et al. 1998), selective cultivation and harvest of wild plants (Deur 2002), and nutrient cycling shifts (Douglas et al. 2004). Knowledge of this pre-history could be crucial to western scientists trying to understand current ecology of "wild" populations and landscapes.

In addition to learning from indigenous TEK, western scientists often need to cooperate and collaborate with indigenous communities to initiate and continue scientific research. Indigenous communities control access to many wildlands where studies of unperturbed ecosystems are best conducted. For example, Native American land holdings (Mexico, USA, and Canada) contain more wildlands than all national parks and nature conservancy lands combined (Nabhan 2000). Additionally, ongoing research may be disrupted by indigenous communities if their cultural values are not respected. For example, the Kuna Indians of Panama closed a research station of the Smithsonian Tropical Research Institute in San Blas in 1997 because they felt that scientists were disregarding the research guidelines developed by the Kuna (Mauro and Hardison 2000). When western scientists and TEK practitioners recognize the value of each others work, dialogue and cooperation are encouraged and our understanding and conservation of species is improved (Kimmerer 2002). These outcomes have been realized in systems ranging from marine reserve design and bonefish (*Albula* spp.) conservation in the tropical nation of Kiribati (Johannes and Yeeting 2001; Drew 2005) to participatory land management with reindeer herders in Northern Sweden (Sandström et al. 2003).

Collaboration between western scientists and TEK practitioners can be difficult. Western scientists may be frustrated with the difficulty of verifying observations from the past, lack of replication or random sampling of TEK, the need to be culturally sensitive, difficulty in insuring a right to publish their findings, inability to separate fact from folklore, and the time and personal commitment required to establish collaborative relationships with indigenous communities (Ford and Martinez 2000; Huntington 2000; Mauro and Hardison 2000). Ecologists may also be unfamiliar or uncomfortable with delving into social science methods or with cross-cultural interactions (Huntington 2000). International law acknowledges that indigenous people have rights to their traditional resources, including their TEK, because they are necessary for their survival (Posey 1996; Mauro and Hardison 2000; Huntington 2000). As such they are entitled to benefit from the use of their TEK and control its dissemination (Anaya 1996). Thus, indigenous communities and resource managers may not be interested in working with western scientists owing to concerns that collaborative relationships will not be reciprocal or respectful, their TEK will be misunderstood or misapplied, and control of their TEK or management rights may be diminished (Huntington 2000; Kimmerer 2002).

Regardless of the difficulties outlined above, western scientists and TEK practitioners share a common goal of species conservation and it is crucial that they work together to conserve both species and cultures. Species extinction rates are one to 10 thousand times higher presently than in the past (Singh 2002). At the same time, there is a growing awareness that the indigenous TEK of the world is rapidly disappearing. Of approximately 6,000 distinct languages in the world, 300 are spoken by roughly 95% of the world's people and 3000 are spoken by communities of 10,000 people or less (Maffi 1998). It is projected that 90% of the world's languages will be extinct or nearly so within the next 100 years (Cox 1997; Stork 1999). To the extent that local knowledge is reflected in local language, TEK is similarly eroding. Transmission of TEK between generations has been reduced in many communities as well because it is not perceived as necessary for individual survival (Gadgil et al. 2000). For example, in the Kaihad Village of Himachal Pradesh of India, individuals less

than 30 years of age were unable to identify, characterize or mention uses for any local flowering plants while individuals that were 30 to 50 years old could identify approximately 25%, characterize 4% and mention uses for 1%. Individuals 50 years and older could identify 70%, characterize 40% and mention uses for 5% of local flowering plants (Gadgil et al. 2000).

In this study, we record the TEK of Māori elders to learn about the biology, ecology, and cultural significance of the tuatara (*Sphenodon* spp.). The objectives of this study are three fold. First, we aim to learn more about tuatara biology and ecology. Second, we hope to document the cultural significance of tuatara to Māori. Finally, we hope to learn something of the nature of TEK itself through comparing this case study to others in the literature.

5.2.1 Natural history of New Zealand and tuatara

New Zealand is a South Pacific nation comprised of two main islands surrounded by many small islands. It has been isolated from neighboring land masses since breaking free of the Gondwana supercontinent approximately 80 million years ago (Cooper and Millener 1993). Long term isolation and a lack of native mammalian predators promoted a high level of endemism in New Zealand, particularly of flightless birds and reptiles (Daugherty et al. 1993). New Zealand was the last major land mass to be colonized by humans (Gibbons 2001; Hurles et al. 2003). The indigenous people of New Zealand are the Māori, a Polynesian people that settled New Zealand approximately 800 to 1000 years ago (Hurles et al. 2003). European colonization began approximately 230 years ago with the arrival of Captain Cook in New Zealand in 1769 and the subsequent signing of the Treaty of Waitangi in 1840 (Buick 1972; Holdaway 1989).

Tuatara are endemic to New Zealand and the last living representatives of an entire order of ancient reptiles, the Sphenodontia (Daugherty et al. 1990; Daugherty and Cree 1990). They are considered a living fossil, having changed little morphologically in over 220 million years (Daugherty and Cree 1990). Tuatara have a host of unusual biological attributes including a rudimentary “third eye” or pineal gland on the top of their heads, lack of a male

copulatory organ, temperature-dependent sex determination, and a life span of approximately 100 years (Daugherty and Cree 1990; Nelson et al. 2004; N. Nelson, unpublished data). Tuatara live in burrows, often with breeding shorebirds, or muttonbirds (Daugherty and Cree 1990).

Both Māori and European settlers introduced predatory mammals to New Zealand resulting in a wave of extinction of endemic species (Holdaway 1989; Atkinson and Cameron 1993). Sub-fossil evidence suggests that tuatara were once found throughout New Zealand (Crook 1975), but were extirpated from the main islands in the early 1900's and are presently found on only 37 offshore islands (Daugherty and Cree 1990; N. Nelson, unpublished data; Figure 1). Two species of tuatara are presently recognized: *S. punctatus* and *S. guntheri*, the latter having only a single naturally occurring population that is listed as vulnerable by the IUCN and endangered by the New Zealand Threat Classification System (Daugherty et al. 1990; Hitchmough 2002).

This study is timely for conservation of both Māori culture and tuatara. There is growing concern that Māori TEK may be lost over time with the passing of elders. At the same time, heroic conservation actions are being taken to insure the persistence of tuatara (Gaze 2001). Our focus on a rare species of conservation concern sets this study apart from many other TEK studies which focus on species that are abundant and frequently interact with an indigenous community (Gadgil et al. 2000; Stickman et al. 2003; but see Nabhan 2000). There is no active or recent subsistence harvest for tuatara, tuatara have been neither abundant nor accessible for at least 100 years, and there are no living Māori elders that have had active and regular involvement with tuatara throughout their lives. Specifically we ask, does TEK persist as species decline?

5.3 Methods

We recorded TEK through structured, semi-directed interviews of Māori elders that were based on a survey developed collaboratively by university ecologists and iwi (tribe or tribal)

resource managers. The survey included 45 questions that were both open form (respondent expressed their response in their own words) and open form combined with closed from (multiple choice presented with opportunity to add comments or additional categories). Survey questions asked about distribution, harvest, life history, behavior, habitat use, and cultural significance of tuatara (e.g., Where did tuatara live in the past and when? What eats tuatara?).

Interviews lasted from 30 to 90 minutes each and were recorded in digital audio (iRiver iHP-120 audio recorder in MP3 format at 256 kilobytes per sec) and, when permission was granted, video (Sony DCR-TRV33 camcorder on mini digital video tape in SP mode and 16 bit audio). Audio interview records were transcribed using the program WAVpedal v5.05 (Programmers' Consortium Inc., Vienna, VA) and interview transcripts were checked for accuracy by those interviewed. Audio, video, and transcript interview records formed a digital TEK archive for participating elders and iwi. A flexible informed consent allowed participants to determine which part(s) of their interview could be shared with the public, their iwi, or their family. Iwi trust councils also reviewed the interview transcripts and provided approval for sharing this information with the public.

Elders interviewed for this study represent iwi from three different areas of New Zealand. Te Atiawa and Ngati Koata iwi currently have territories in the Marlborough Sounds region of the south island of New Zealand. Te Atiawa are guardians of North Brother Island which is home to the only remaining natural population of *S. guntheri*. Ngati Koata are guardians of Stephens Island which is home to world's largest population of *S. punctatus*. Nga Puhi and Ngati Wai have territories in the Hauraki Gulf area of the north island of New Zealand; Ngati Wai are guardians of Little Barrier Island and the Mokohinau Islands (including Burgess and Fanal Islands). Of these islands, only Little Barrier currently has a population of tuatara (*S. punctatus)*.

Elders interviewed from Te Atiawa and Ngati Koata were selected by their iwi because they have experience with and knowledge of their iwi's territory, history, and culture. Whetu McGregor, an elder from Nga Puhi and Ngati Wai Iwi, was asked to participate in this

study based on her public record of activism in the conservation of tuatara and Little Barrier Island. Eighteen elders were interviewed between 26 February and 19 June 2004, and 16 agreed to publicly share their TEK. Participating elders comprised 5 women and 11 men that range in age from their 30's to their 70's; seven participants represent Te Atiawa, eight represent Ngati Koata, and one elder is from Nga Puhi/Ngati Wai Iwi. Interview responses were compiled and summarized as relative percentages of responses for each question. Not all questions were answered by all respondents, but all interviews included in this study were included entirely (no portions of interviews were excluded from this report by the elders).

5.4 Results

The elders interviewed speak with varying levels of experience and authority about both tuatara and the islands they inhabit. The majority of participating elders had direct and personal experience with islands inhabited by tuatara (60%) and with tuatara themselves (93%). Some elders had little personal association with the islands under study, but most had lived in the area (Marlborough Sounds, Hauraki Gulf) since birth and had extended families there for 150 to 200 years.

5.4.1 Tuatara biology and ecology

Nearly all respondents named at least one location where tuatara were found in the past (87%; past = 900 AD when Kupe explored New Zealand to within the last 50 years) and one location where tuatara are found presently (93%; Figure 1). In general, locations were in the Marlborough Sounds and Hauraki Gulf areas that respondents were personally familiar with, and several sites were given as having tuatara both in the past and present. Eighteen sites of past tuatara occupation were identified by the elders, including 16 islands or island groups and two mainland sites. The most commonly cited locations were Stephens Island (15%), D'Urville Island (15%), the Trios Islands (8%), French Pass (8%), Cooper's Island (8%), and North Brother Island (6%). The elders identified 11 locations, including 10 islands or island

groups, where tuatara are found presently. The most commonly cited areas were Stephens Island (29%), North Brother Island (16%), and the Trios Islands (11%)

Nearly every elder named at least one characteristic of the types of habitats in which tuatara live (100%) or build their nests (73%). The most common habitat characteristic given (both living and nesting sites) was in burrows in the ground or under vegetation (21 and 31%), followed by closed or covered areas (9 and 10%), with muttonbirds (6 and 7%), and either near or in sunny locations (4 and 10%). Additionally, elders indicated that tuatara live under or around buildings (6%) and in dry areas (6%) and that tuatara nest in soft ground (14%). Most elders agreed that tuatara like these places because they are warm (29 and 31%), protected (19 and 26%), and have a food source nearby (19 and 25%). The elders felt that tuatara share burrows with muttonbirds because tuatara eat the birds and their young, gain shelter from the burrow, gain warmth from the birds, and are relieved of having to dig their own burrows. Most elders felt that tuatara living (42%) and nesting (50%) habitat preferences had changed over the years due to introduced predators, habitat destruction, human habitation, and relocation of tuatara populations to different islands.

The majority of elders (57%) thought the maximum age of tuatara was between 150 and 300 years, while 29% thought tuatara live approximately 100 years and 7% thought tuatara live less than 100 years. Maximum body size of tuatara was estimated by the elders to range from 35 cm to 1 m. Twenty one percent of the elders were not sure how big tuatara get, 35% thought tuatara grow to be greater than half a meter in length, 21% thought tuatara grew to approximately one half meter in length, and 14% thought tuatara grew to be less than one half meter in length. Most elders did not think that maximum tuatara body size (40%) or age (50%) had changed over the years.

Seventy three percent of elders named at least one food item in the tuatara diet. The most common food items listed were insects (57%; including wetas, bugs, and beetles), muttonbirds and their eggs (17%), and "anything they can capture" (9%). Similarly, 79% of elders named at least one predator of tuatara. The most common predators given were introduced mammals (55%), seabirds (18%), and hawks (14%). Most of the elders felt that

tuatara diet (40%) and predators (50%) had changed over the years due to relocation of tuatara to different islands, extinction of native food species, and the introduction of mammalian predators.

The elders also indicated that changes in the land (92%) and climate (64%) had affected tuatara. Land changes given were the use of fire to clear land (35%) and the introduction of predators (20%). Climate changes included more erratic weather with unpredictable seasons (28%), more southerly winds (17%), and colder temperatures (17%).

5.4.2 Cultural significance of tuatara

Most elders were not sure what the word tuatara meant (50%) and weren't aware of any origin stories of the tuatara (53%). Twenty nine percent of the elders said that the word tuatara refers to the rough skin and spiny back of the creature while one elder suggested the name means "here before Tara" and refers to the ancient nature of tuatara with tua meaning before and tara referring to an early Māori explorer of the same name.

Twenty percent of the elders explained the origin of tuatara in terms of cultural tradition, either as having been created by Ioa Matua Nui te Kore (the Supreme being, high god) or, more commonly, as a descendent of Tū-ta-wehiwehi (the ancestor of reptiles; Figure 2). Figure 2 represents the genealogy of tuatara and is based on one elder's recounting of the origin of tuatara. Additional individuals were added to the genealogy based on Roberts et al. 1995 and Orbell 1995 to provide broader context. The origin of reptiles (also called mokomoko and ngarara) is often told as a story wherein the brothers Ika-tere and Tū-te-wehiwehi disagree as to whether they should flee to the sea or forest to escape the wrath of their great uncle (usually either Tawhirimatea or Tū-mata-uenga). Tū-te-wehiwehi, the ancestor of reptiles, flees to the forest and warns his brother "Your children will be food for men." Ika-tere, ancestor of the fishes, flees to the sea and replies "And yours will be the ugliest thing on the face of the earth." This story was given as the basis for this genealogy and is likely why elders identify tuatara as the children of Tū-te-wehiwehi.

The majority of elders rejected the idea that either Māori or Europeans used to harvest tuatara (Māori 67%; European 69%) or tuatara eggs (Māori 73%; European 64%) for subsistence purposes. Reasons given for this include fear of tuatara, reverence and respect for tuatara, little meat on adult tuatara, small size of tuatara eggs, and the presence of ample alternative food sources (e.g., muttonbirds). Several elders added that tuatara may have been eaten by Māori as part of ritual, however, either to show bravery or gain knowledge.

Four respondents described catching tuatara while muttonbirding either to show their bravery when young or as part of the traditional muttonbird harvest. Whetu McGregor described how tuatara were used by her family and Ngati Wai Iwi to keep their bodies cool while they collected muttonbirds on Burgess and Fanal Islands in the Hauraki Gulf. Muttonbirding took place on these islands for two weeks annually at the height of summer (late November, early December). As a young teenager, Whetu watched as the muttonbirders, primarily the women, collected tuatara from burrows in the morning and placed them under their shirts and against their skin to keep them cool while they collected muttonbirds throughout the day. At the end of the day, the tuatara were returned to their burrows with a thank you prayer.

> *"I used to go out with my family…to harvest muttonbirds. The tuatara were quite plentiful on the island and the harvesters…would put them next to their skin in their clothes…They would be able to harvest all day with the tuatara keeping their body cool. That was a very, very important part of Māori association with the tuatara. That was a very special reptile to them…Without it, you couldn't harvest."*
>
> – Whetu McGregor, Nga Puhi/Ngati Wai

We asked how tuatara are used and what they signify in Māori arts including songs, proverbs, stories, and carvings. We were unable to record any songs or proverbs that include

tuatara. Most elders (43%) said tuatara are not found in waita and none of the elders could recall any tuatara songs specifically. Similary, most elders (50%) indicated that tuatara are not in proverbs, and none of the elders could recall any proverbs involving tuatara specifically. In contrast, the majority of elders had stories to relate of sites in the Cook Strait area that are inhabited by tuatara (62%) and had observed tuatara in traditional carvings and tattooing (80%). We recorded four stories of the Māori explorer Kupe and the Cook Strait area and four traditional meeting houses with tuatara carvings. The most common story related by the elders was of the creation of North Brother Island. After defeating Muturangi's octopus, Kupe flung the eyes of the octopus into Cook Strait where they formed (or in some accounts landed on) the North and South Brothers Islands.

In Māori stories and carvings, tuatara often signify calamity and death or a warning that something monstrous is about to occur. Tuatara are typically portrayed as guardians and used in this way as a social control. For example, carvings of tuatara can be found on wakatupapaku, or boxes containing bones of the dead, and women used to sometimes tattoo reptiles (presumably some of which represent tuatara) near their genitals. As one Te Atiawa Iwi member stated:

> *"They* [tuatara] *were often used in our stories as boundaries. As perimeters that signify tapu* (sacred or restricted) *and indicate…that if you cross that boundary, there is mana there. There's an authority, there's a power, and there's going to be repercussions."* – Rōpata Taylor, Te Atiawa

Seventy one percent of the elders related ways in which tuatara figure prominently in Māori culture in addition to those we specifically asked about (subsistence, songs, proverbs, stories, carvings). Twenty percent of elders noted that tuatara were sometimes kept as pets and one elder related that Māori used to fashion tuatara bones into jewelry. The elders related that some iwi considered tuatara a form of taniwha (reptile-like water monster), a bad omen, or linked with the malevolent supernatural being Whiro who is typically symbolized by the

eye of a reptile. In addition, islands inhabited by tuatara were tapu themselves. For example, tradition restricted viewing North Brother Island to only the highborn or after first passing, depending on the iwi. North Brother Island was viewed as tapu because of the story of Muturangi's octopus and because it was inhabited by tuatara. Stephens Island was viewed as tapu because spiritual leaders spent time there to gain insight through nature and because it was inhabited by tuatara.

The elders also related that tuatara are regarded as guardians of the stream of knowledge and that this is made evident by their biology. The tuatara's third eye is evidence that tuatara are from and can see another dimension. As Ben Hippolite (Ngati Koata) put it, *"…we honestly believed, and we told our children…that when the tuatara is quite young you can see the three eyes."* In addition, the long life of the tuatara allows accumulation of knowledge, and the deep lineage of the tuatara means they have inhabited New Zealand and been accumulating knowledge since very early times. Indeed, the lack of a male copulatory organ in tuatara is viewed by some Māori as evidence that tuatara were required on Earth in such a hurry that they were sent to Earth by Io Matua unfinished.

5.4.3 Nature of traditional ecological knowledge

In general, there was greater volume and more detailed traditional knowledge of cultural traditions involving tuatara than biology or ecology of tuatara. The majority of the elders' knowledge came from personal observation (49% biological; 48 % cultural) and stories handed down from their elders (29% biological; 40% cultural). Popular and scientific media and interaction with scientists comprised 17% of their biological TEK sources but only 7% of their cultural TEK sources. The remainder of the elders' TEK (5% both biological and cultural) came from hearsay from island lighthouse keepers, University studies, and private family memoirs.

When asked if this traditional knowledge was being lost over time, 41% of the elders said yes, 24% said somewhat, 12% said no, and 24% said that it is unlikely that Māori ever had much traditional knowledge of tuatara. Reasons given for this include the naturally

secretive nature of tuatara, the isolation and protection of tuatara and the islands they inhabit, and the tapu nature of tuatara.

All of the elders expressed their support for tuatara conservation and said they would like to see more tuatara dispersed throughout New Zealand. The majority of elders (86%) were also agreeable to tuatara being taken out of New Zealand, but only under strict conditions. The elders expressed support specifically for continued total protection of tuatara, breeding and translocation programs, island and habitat restoration, careful management and research, public education, iwi participation in management, and a desire to see tuatara left in peace.

5.5 Discussion

5.5.1 Tuatara biology and ecology

Traditional knowledge and western scientific knowledge of tuatara biology were largely similar (e.g., tuatara diet and predators) but also differed from each other in a number of important ways. There is currently no scientific evidence (e.g. subfossil remains) to suggest that tuatara used to occupy seven of the sites listed by the elders as places where tuatara were found in the past (Victory, Titi, Motuara, Cooper's, Long, Allports, and Mabel Islands; Cree 1993; personal communication P. Gaze 2006). Thus, TEK provides the only evidence to date that tuatara inhabited these islands. Caution is required in interpreting these data, however, as Cooper's Island (mentioned by four elders and called Motungarara in Māori meaning "lizard island") was the only site mentioned by more than one elder. Similarly, five sites listed by elders as places where tuatara can be found presently are not currently known by the New Zealand Department of Conservation to be occupied by tuatara (D'Urville, Victory, Chetwodes, and Cooper's Islands, French Pass area of the mainland) despite recent searches of these areas for signs of tuatara (personal communication P. Gaze and M. Aviss 2006). Thus, TEK appears to provide the only evidence that tuatara may still occupy these sites. Again, caution is required in interpreting these data because all of these sites have introduced

mammalian predators (Baldwin 1983; personal communication M. Aviss 2006) making them inhospitable for tuatara (Crook 1973; Cree et al. 1995), and only two of these sites (D'Urville and Cooper's Islands) were mentioned by more than one elder.

It is possible, however, that there are small, extant populations of tuatara yet to be confirmed through formal scientific surveys. Such a population was discovered living in the presence of rats on Little Barrier Island in 1991 (Ombler 2004). Islands for tuatara translocation are selected based partially on evidence of former tuatara occupation (Cree 1993). Thus, results of this study will help managers prioritize islands for tuatara searches and translocations.

Differences between TEK and western science represent important opportunities for research. For example, most of the elders related that tuatara use the same burrow or similar habitats for both nesting and living. In contrast, behavior studies on Stephens Island suggest that tuatara dig separate nest holes in habitats that are more open and sunny than the habitats in which tuatara live (Cree 1993; Thompson et al. 1996). The elders also suggested that tuatara gain warmth from muttonbirds in their burrows, but this interaction has never been scientifically tested. In addition, TEK may exaggerate maximum tuatara size and age. The oldest known tuatara is 81 years old and a maximum age for tuatara of 100 years is likely (unpublished data N. Nelson). The largest tuatara observed are approximately half a meter in total body length (Dawbin 1982; Thompson et al. 1992). Most of the elders, however, described tuatara as being older (up to 300 years) and longer (up to one meter). This may be a cultural bias because tuatara are often viewed as similar to taniwha which are typically represented as huge in size (Orbell 1995). It should be noted that scientists do not yet know for certain how long tuatara can live; today's oldest living tuatara may well survive the scientists monitoring its age. Thus, differences between traditional and scientific knowledge suggest that tuatara nesting behavior, use of muttonbirds for warmth, maximum size, and maximum age could benefit from additional research.

5.5.2 Cultural significance of tuatara

The elders related a great deal of information concerning the traditional role of tuatara in their iwi's culture. Some of this information agrees with published records, some disagrees with published records, and some is reported here for the first time. Much of the TEK we recorded has been recounted elsewhere, including the origin stories of tuatara (Orbell and Moon 1985) and North Brother Island (Orbell 1995), the use of tuatara in traditional carvings (Phillips 1955), links between tuatara and taniwha (Downes 1937; Orbell and Moon 1985; Reed 1999) and reptiles and Whiro (Best 1924; Buck 1949), and the cultural role of tuatara as guardians (Downes 1937; Orbell and Moon 1985). However, there were some important differences between the traditional knowledge collected in this study and that found in the literature.

Historical records suggest that Māori feared reptiles because they live on land but are viewed as being fish-like (Orbell and Moon 1985). Lizards appear to have been more feared than tuatara because they were believed to deliver punishments for improper behavior in the form of disease and death (Downes 1937; Orbell and Moon 1985; Mead and Grove 2001). However, tuatara were still often portrayed as vengeful, bloodthirsty, cannibalistic, man-eating, jealous creatures that served harmful spirits (Beattie 1920; Hongi 1922; Barrow 1984; Beattie 1994). In addition, both lizards and tuatara could be the physical embodiment of spirits (Orbell and Moon 1985) and shared similar traits with taniwha (Downes 1937; Reed 1999; Beattie 1920). The majority of elders we interviewed, however, did not express fear of tuatara but respect and reverence. This apparent contradiction with published accounts of Māori attitudes toward tuatara may be due to differences among iwi in their relationship with tuatara, changes in attitudes over time, or a tendency for early writers and Māori to lump tuatara and lizards together simply as reptiles. The latter would confuse fear of lizards with fear of tuatara.

The elders rejected the notion that Māori or New Zealand Europeans ate tuatara for subsistence purposes, though this generalization is often found in the literature. A lack of oral history of subsistence on tuatara has been reported historically (Beattie 1994). However, Māori subsistence hunts of tuatara (Beattie 1920; Phillips 1955; Orbell and Moon 1985) have

also been reported historically and the tuatara sub-fossil remains have been found in pre-historic middens (Crook 1975). However, in the words of the elders:

> *"Ngati Koata never ate tuatara...I think it's a…piss poor assumption by scientists to consider 'cause there's bones* [in middens]*, we ate them. There was so much food around those days. Hell, we even ate each other. That was a better meal, so I'm told. We didn't have to eat tuatara."*
>
> - Jim Elkington, Ngati Koata

> *"*[Eating tuatara] *wasn't necessary. The tuatara was like a god to them. It protected them. It saved their lives when they went harvesting for food."*
>
> – Whetu McGregor, Nga Puhi/Ngati Wai

Again, this apparent contradiction may be due to differences among iwi in their relationship with tuatara or changes in attitude over time. Several elders indicated that it was likely people ate tuatara to show their bravery or gain knowledge and there are similar stories of people eating lizards to show their authority or power (Orbell and Moon 1985).

Additional roles for tuatara in Māori culture that have not been previously reported include being the guardian of knowledge and being an aid in traditional muttonbirding. Tuatara are a cold-adapted reptile and typically have a low body temperature (Daugherty and Cree 1990). Elders of Ngati Wai Iwi used to place tuatara in their shirts against their skin to cool themselves while collecting muttonbirds at the height of summer. While this story seems unlikely to some, at least one historical report describes Māori nestling tuatara against their skin (Beattie 1994) and other Ngati Wai elders that were not formally interviewed for this study corroborate this story. To our knowledge, this is the first account in the literature of these roles for tuatara in Māori culture. Traditional knowledge recorded in this study presents an opportunity to revise our current understanding of Māori attitudes toward tuatara. Not all

Māori fear tuatara, not all iwi subsisted historically on tuatara, and the cultural role of tuatara differs through time and among iwi.

5.5.3 Nature of traditional ecological knowledge

Knowledge of tuatara biology and ecology was not as widespread or detailed among the elders as knowledge of the cultural significance of tuatara. Similarly, other TEK studies reported a greater volume and detail of TEK that that reported here. For example, 18 Dena'ina Athabaskan elders of Lake Clark, Alaska identified 48 sockeye salmon (*Oncorhynchus nerka*) spawning sites and indicated reduced size and delayed timing of salmon runs in the last 10 years (Stickman et al. 2003). The same study recorded decadal abundance of a variety of fish species since the 1930's and contemporary distribution of an additional 9 fish species (Stickman et al. 2003). Similarly detailed information is found in the TEK of Sami reindeer (*Rangifer tarandus*) herders in Northern Sweden and was used to construct a GIS database of seasonal reindeer distribution, movement, and forage base (Sandström 2003).

Why might there be less TEK of tuatara? It is possible that Māori have never had detailed TEK of tuatara biology because they had no dependence on, didn't encounter, purposefully avoided, or were actively kept distant from tuatara. There is often a correlation between dependence on a species and volume and detail of TEK of that species (Gadgil et al. 2000), and a lack of subsistence harvest of tuatara might negate the need for TEK of their biology. However, some studies have found that indigenous communities acquire substantial TEK that is not directly related to obtaining economically important species (e.g., Nabhan 2000). Thus, lack of subsistence dependence on tuatara alone likely cannot explain why there is little TEK of tuatara biology.

Perhaps tuatara were not encountered often by Māori historically. Tuatara were possibly few in number on the mainland when Māori arrived in New Zealand. Exploring Polynesians appear to have introduced Pacific rats (*Rattus exulans*) to New Zealand up to 1000 years before Māori settlement (Holdaway 1996; Hurles et al. 2003). Thus, tuatara were

possibly declining on the mainland for 1000 years before cohabiting New Zealand with Māori and may have been rare on the mainland when Māori settled New Zealand. However, several studies have found detailed indigenous TEK of rare and endangered species such as the desert tortoise (*Gopherus agassizii*), green sea turtle (*Chelonia mydas*), Sonoran pronghorn antelope (*Antilocapra americana sonoriensis*) and desert bighorn sheep (*Ovis canadensis mexicanus*; Nabhan 1992; Nabhan 2000). In addition, the iwi that collaborated on this study have a history of actively spending time on tuatara islands. Thus, a lack of encountering tuatara alone also cannot explain why there is little, widespread TEK of tuatara biology.

Tuatara are tapu and live in tapu places. Therefore, people may have purposefully avoided or been kept from interacting with tuatara based on their role or status. Several elders suggested that interaction with tuatara and access to tuatara islands may have been limited to spiritual leaders, and it is not uncommon for TEK of a species to be limited to specific people within a community based on status or labor role (Turner et al. 2000). For example, certain birds are regarded as the property of specific Cheyenne priests that have gained knowledge of the birds through apprenticeship (Moore 1986). A priest will only talk about another priest's birds under strict conditions including that the qualified person to speak about the birds is deceased (Moore 1986). A similar arrangement surely would have limited experience with and knowledge of tuatara historically. It is interesting that presently, access to islands and tuatara is similarly controlled through legislation. Tuatara have been protected by law since 1895 (Cree 1993) and all of the islands presently inhabited by tuatara are protected reserves. This represents a formal conservation strategy to prevent public access to tuatara islands. Intense protection of tuatara, both through tapu historically and legal action presently, has likely distanced Māori from tuatara and accelerate the loss of traditional knowledge of tutara biology.

Most elders felt certain that more TEK of tuatara biology would have been known but has since been lost because it is otherwise unlikely that tuatara would figure so prominently in Māori culture. It is clear that Māori have been in New Zealand long enough (800 to 1000 years is roughly equivalent to estimates of occupation time of Dena'ina Athabaskans in Lake

Clark) to have gained substantial knowledge of native flora and fauna. Iwi collaborating on this study have been in their territories for shorter periods of time (e.g., Ngati Koata since the 1820's, Te Atiawa since the 1830's), but are highly knowledgeable about many aspects of local ecology (e.g., muttonbirding and fishing locations). Long term rarity and isolation of tuatara, lack of dependence on tuatara for survival, and protection of tuatara through tapu and legislation have likely all contributed to the paucity of traditional knowledge of tuatara biology.

5.5.4 Can TEK persist as species decline?

Traditional ecological knowledge does persist as species decline, but the detail and volume of biological TEK may decrease while cultural TEK is amplified. In the absence of personal interaction with tuatara, cultural constructs appear to have become exaggerated. Tuatara are literally larger than life in Māori tradition. This is similar to the "extinction of experience" described by Nabhan (2000) when mutually reinforcing connections between cultural and biological diversity are broken because youth do not encounter rare or extinct species.

At the extreme, exaggerated stories about species persist after their extinction. It is unclear how much of this traditional knowledge may inform science. At least, these stories may suggest the former presence of extinct species. For example, when Europeans arrived in New Zealand, all of the large flightless Moa species had already gone extinct but Māori continued to tell stories about the huge, powerful birds (Duff 1956). The presence of sub-fossilized bones corroborated the former presence of these birds in New Zealand. Similarly, when Europeans colonized New Zealand, Māori told stories of giant geckos called kawekaweau. A lack of specimens and scientific descriptions of kawekaweau suggested this creature was mythical, but a study skin found in 1979 at the Museum of Natural History in Marseille, France and bones later found at the Canterbury Museum in Otago, New Zealand suggest the kawekaweau did indeed once existed in New Zealand (Whitaker 1992). As one elder stated: "*At least if the taonga* (valued treasure) *disappears, we will still be able to talk about it in the future.*"

5.5.5 Does this mean we have to believe in sasquatch or taniwha?

No. As pointed out by Huntington (2000) "unquestioning acceptance of TEK is as foolish as its unquestioning rejection". Traditional stories and art may be some of the last sources of information on rare and extinct species and should not be rejected outright (Nabhan 2000). Sometimes TEK is wrong (just like science), but that does not mean that TEK never contains sound information (Huntington 2000). For example, the Cheyenne include mythical creatures (e.g. the thunderbird), meteorological events (e.g. tornadoes), and insects (e.g. butterflies and dragonflies) in their traditional bird taxonomy (Moore 1986). This clearly does not conform to any scientific species concept. But the same ethnotaxonomy provides an alternative view of the relationships between many extant birds and encodes knowledge of their morphology, behavior, and distribution that has been collected through observation over thousands of years.

5.5.6 Implications for conservation

This study has tangible outcomes for tuatara conservation. First, the TEK collected in this study presents the only evidence of past tuatara occupation in seven sites and suggests an additional five sites in which tuatara may possibly still be found. This information will help managers prioritize islands for tuatara searches and translocations. Secondly, TEK has suggested hypotheses for formal scientific testing such as maximum tuatara size and age, use of muttonbirds by tuatara to gain warmth, and use of burrows in which tuatara live for nesting. Finally, this study affirms that many Māori feel a strong personal connection with tuatara, are aware of the threats to the persistence of tuatara, and have a strong desire to conserve tuatara.

We are presently experiencing a species and cultural extinction crisis with profound losses of species (Singh 2002), indigenous languages (Cox 1997; Maffi 1998; Stork 1999), and TEK. Species and cultural diversity are correlated and these are not independent events (Sutherland 2003). Thus, if we wish to conserve TEK and cultural diversity, we must conserve species that figure prominently in traditions and maintain links between traditional

cultures and species. It should be encouraging that many elders feel it is not necessary for people to understand a species from a western scientific perspective to appreciate or want to protect that species. Understanding a species from many cultural perspectives can engender the same conservation ethic.

Figure 5-1 Distribution of tuatara (*Sphenodon* spp.) in New Zealand. Closed symbols represent sites occupied by tuatara in the past based on subfossil remains (●, from Crook 1975) and sites occupied by tuatara presently based on scientific surveys (▼, from Gaze 2001). Open symbols represent past (○) and present (▽) sites of tuatara occupation based on traditional ecological knowledge (TEK). Numbers refer to (1) Poor Knights Islands, (2) Hen and Chickens Islands, (3) Little Barrier Island, (4) Cuvier Island, (5) Mercury Islands, (6) Aldermen Islands, (7) Karewa Island, (8) Motunau Island, (9) Moutoki Island, (10) Moutohora Island, (11) Matiu-Somes Island, (12) Stephens Island, (13) Victory Island, (14) D'Urville Island, (15) French Pass, (16) Trios Islands, (17) Chetwodes Islands, (18) Titi Island, (19) Motuara Island, (20) Long Island, (21) Cooper's Island, (22) North Brother Island, (23) Allports Island, and (24) Mabel Island. Tuatara were translocated to Titi Island in 1995 (Gaze 2001), but TEK provides the only evidence to date that tuatara occupied Titi Island in the past. Thus this island is coded with a half open, half closed symbol. Figure modified from Hay et al. 2003.

Figure 5-2 Genealogy of tuatara as related by elders interviewed (modified from with additional information from Roberts et al. 1995, Orbell 1995).

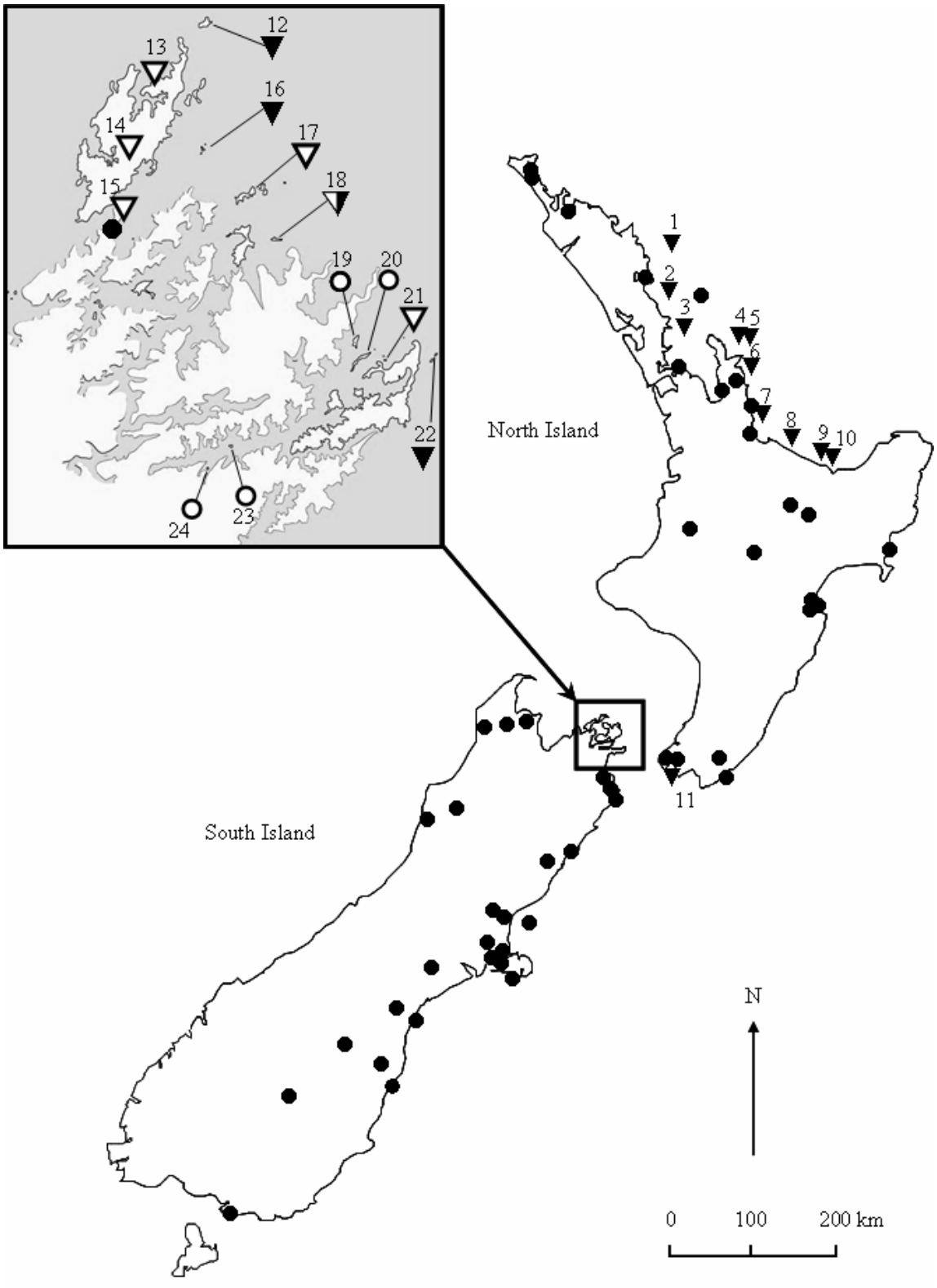
12
13
16
14
17
15
18
19
20
21
22
24
23
1
2
3
4 5
6
7
8
9 10
North Island
11
South Island
N
0
100
200 km

Figure 5-1

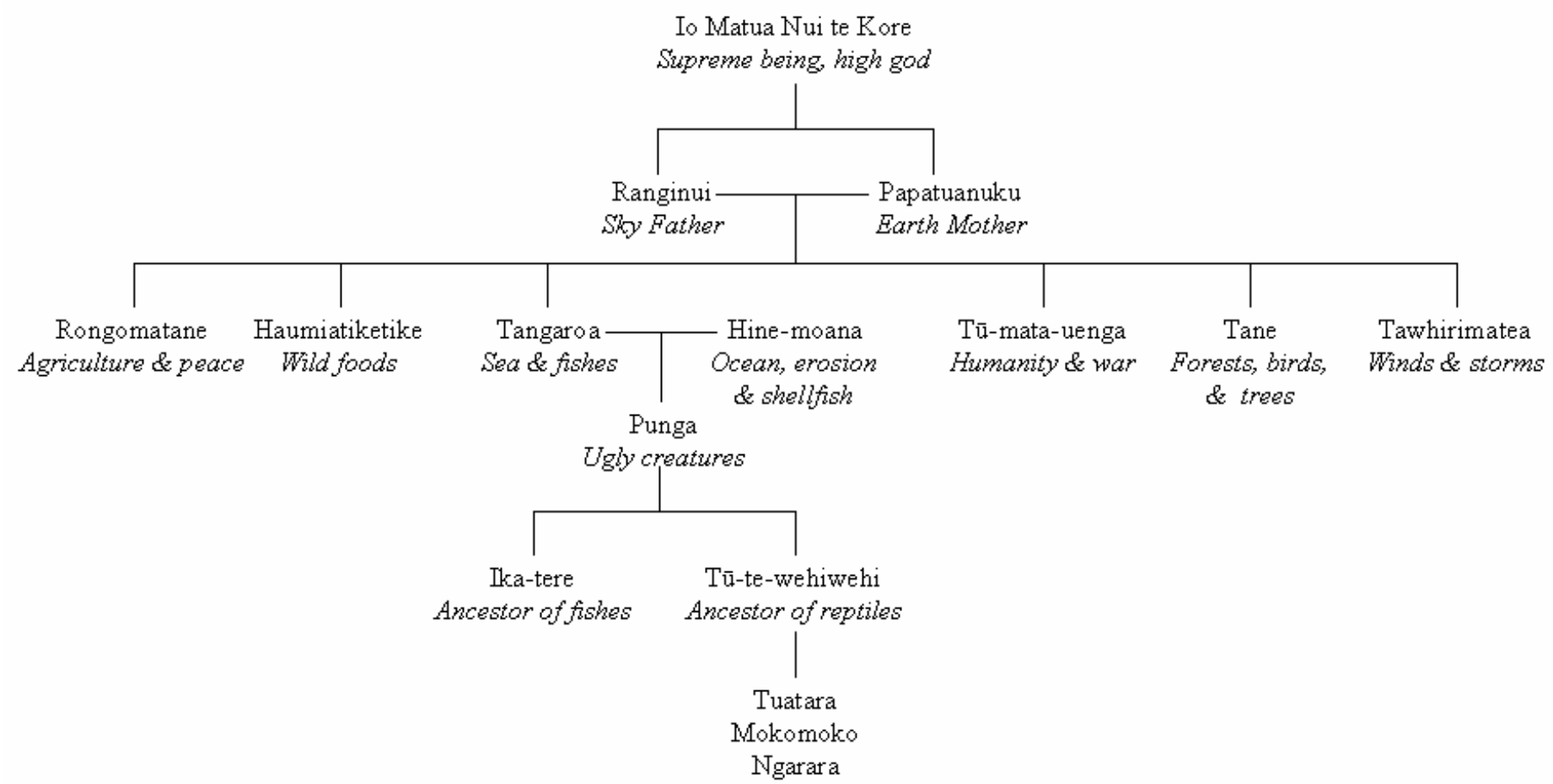
Io Matua Nui te Kore
Supreme being, high god
Ranginui
Sky Father
Papatuanuku
Earth Mother
Rongomatane
Agriculture & peace
Haumiatiketike
Wild foods
Tangaroa
Sea & fishes
Hine-moana
Ocean, erosion & shellfish
Tū-mata-uenga
Humanity & war
Tane
Forests, birds, & trees
Tawhirimatea
Winds & storms
Punga
Ugly creatures
Ika-tere
Ancestor of fishes
Tū-te-wehiwehi
Ancestor of reptiles
Tuatara
Mokomoko
Ngarara

Figure 5-2

Appendix A - Allele frequencies of 11 microsatellite loci among 15 populations of Lake Clark sockeye salmon.

Refer to Table 2 -1 for population names.

	FDI	TCI	TAZ	OUT	SBL	CHI	KR	LKR	KLSB	PRC	CC	HPB	LLCB	LTLK	UTLK
Oki1-1															
N	87	97	99	100	100	99	99	98	100	65	100	99	100	100	100
106	0.006	0.005	0.005	-	-	0.010	0.015	-	-	-	0.005	0.010	0.025	-	0.015
110	0.816	0.876	0.793	0.790	0.680	0.682	0.626	0.770	0.740	0.700	0.655	0.667	0.630	0.630	0.625
114	0.178	0.119	0.202	0.210	0.320	0.308	0.354	0.230	0.255	0.292	0.340	0.303	0.330	0.370	0.355
118	-	-	-	-	-	-	-	-	0.005	0.008	-	0.020	0.015	-	0.005
122	-	-	-	-	-	-	0.005	-	-	-	-	-	-	-	-
Oki1-2															
N	87	97	99	100	100	99	99	98	100	65	100	99	100	100	100
140	-	0.005	-	-	-	-	-	-	-	-	-	-	-	-	-
144	-	-	-	-	-	0.005	-	-	-	-	0.015	0.015	0.005	0.010	-
148	0.672	0.598	0.692	0.675	0.670	0.753	0.722	0.577	0.560	0.723	0.705	0.682	0.740	0.750	0.740
152	-	0.021	-	0.005	-	-	-	-	-	-	-	-	-	-	-
156	0.299	0.361	0.293	0.305	0.315	0.237	0.278	0.418	0.440	0.277	0.280	0.303	0.255	0.240	0.260
160	0.029	0.015	0.015	0.015	-	0.005	-	0.005	-	-	-	-	-	-	-
164	-	-	-	-	0.015	-	-	-	-	-	-	-	-	-	-

Appendix A continued.

	FDI	TCI	TAZ	OUT	SBL	CHI	KR	LKR	KLSB	PRC	CC	HPB	LLCB	LTLK	UTLK
Omy325															
N	87	97	99	100	100	99	99	98	100	65	100	99	100	100	100
118	-	0.005	-	-	-	-	-	-	-	-	-	-	-	-	-
134	-	-	0.020	-	-	-	-	-	-	-	-	-	-	-	-
138	-	-	0.005	-	-	-	-	-	-	-	-	-	-	-	-
140	0.184	0.103	0.030	0.080	0.005	0.056	0.061	0.020	0.025	0.008	0.040	0.086	0.04	0.035	0.08
142	-	0.010	-	-	-	-	-	-	-	0.031	0.010	-	-	-	-
144	0.006	-	-	-	-	-	-	-	-	-	-	-	-	-	-
146	-	-	-	0.005	-	-	-	-	-	-	-	-	-	-	-
148	-	0.005	0.005	-	-	0.010	-	-	-	-	0.035	0.020	0.005	0.020	0.010
150	0.017	-	-	0.005	0.005	0.010	0.030	0.036	0.040	0.023	0.020	0.030	0.025	0.015	0.040
152	0.115	0.211	0.187	0.110	0.200	0.328	0.318	0.449	0.340	0.431	0.390	0.263	0.290	0.385	0.355
154	0.080	0.010	0.005	0.020	0.095	0.076	0.101	0.051	0.095	0.077	0.090	0.066	0.065	0.085	0.055
156	0.259	0.418	0.515	0.485	0.275	0.162	0.136	0.097	0.100	0.185	0.105	0.167	0.135	0.110	0.105
158	0.230	0.134	0.182	0.225	0.350	0.293	0.298	0.281	0.355	0.146	0.265	0.333	0.365	0.300	0.315
160	0.034	0.041	0.015	0.030	0.015	0.030	0.025	0.041	0.015	0.031	0.030	0.010	0.025	0.035	0.025

Appendix A continued.

	FDI	TCI	TAZ	OUT	SBL	CHI	KR	LKR	KLSB	PRC	CC	HPB	LLCB	LTLK	UTLK
Omy325 continued															
162	0.023	0.046	0.010	0.015	0.020	0.015	0.015	0.005	0.005	-	0.015	-	-	0.005	-
164	0.029	0.005	0.010	0.005	-	-	-	0.005	-	0.023	-	0.005	0.020	0.005	-
168	0.011	0.005	0.005	0.005	0.035	0.020	0.010	0.015	0.020	0.046	-	0.020	0.030	0.005	0.015
170	0.006	-	-	0.005	-	-	-	-	0.005	-	-	-	-	-	-
172	-	0.005	-	0.010	-	-	0.005	-	-	-	-	-	-	-	-
174	0.006	-	0.010	-	-	-	-	-	-	-	-	-	-	-	-
uSat60															
N	87	97	99	100	100	98	99	98	100	65	100	98	100	100	100
112	-	-	0.010	0.010	-	-	-	-	-	0.038	-	-	0.005	-	-
114	0.011	-	0.010	0.010	-	0.041	0.035	0.046	0.035	0.023	0.035	0.041	0.020	0.025	0.090
116	0.011	-	0.005	0.005	-	0.005	0.005	-	-	-	-	0.005	-	-	-
118	0.603	0.608	0.773	0.735	0.475	0.745	0.717	0.811	0.770	0.677	0.750	0.668	0.650	0.770	0.690
120	0.006	0.005	0.005	0.005	-	0.005	-	-	0.010	-	-	-	-	-	-
122	0.017	0.026	0.056	0.045	-	0.010	-	-	-	-	0.035	0.005	0.005	-	-
124	0.006	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix A continued.

	FDI	TCI	TAZ	OUT	SBL	CHI	KR	LKR	KLSB	PRC	CC	HPB	LLCB	LTLK	UTLK
uSat60 continued															
126	-	-	-	-	0.020	-	-	-	-	0.015	0.005	0.005	-	-	-
128	0.006	-	-	-	-	-	-	-	-	-	-	-	-	-	-
130	0.333	0.345	0.131	0.175	0.505	0.189	0.237	0.138	0.180	0.246	0.175	0.265	0.320	0.200	0.220
134	0.006	0.010	0.010	0.015	-	0.005	0.005	0.005	0.005	-	-	0.010	-	0.005	-
136	-	0.005	-	-	-	-	-	-	-	-	-	-	-	-	-
One21															
N	87	97	99	100	99	99	99	98	100	65	100	99	100	100	100
110	-	-	-	-	-	-	-	-	-	-	-	-	0.005	-	-
124	0.011	-	-	-	0.010	-	-	-	-	-	-	0.005	-	-	-
126	0.017	0.015	-	-	-	0.005	0.035	0.005	0.005	0.023	0.005	0.005	-	0.005	-
128	-	0.010	0.005	-	-	0.010	0.005	-	-	0.008	0.015	-	0.005	0.005	0.005
130	0.247	0.165	0.268	0.330	0.066	0.131	0.116	0.163	0.080	0.069	0.145	0.167	0.170	0.105	0.150
132	0.023	0.031	0.040	0.045	-	0.010	0.005	-	0.010	-	-	-	-	-	0.005
134	0.011	-	0.172	0.135	0.035	0.020	0.005	-	0.010	-	0.005	0.020	0.010	0.005	0.010
136	0.063	0.067	0.056	0.035	-	0.086	0.066	0.010	0.015	0.046	0.020	0.076	0.035	0.065	0.065

Appendix A continued.

	FDI	TCI	TAZ	OUT	SBL	CHI	KR	LKR	KLSB	PRC	CC	HPB	LLCB	LTLK	UTLK
One21 continued															
138	0.034	0.036	0.030	0.020	-	0.010	-	0.015	0.010	0.038	0.030	0.005	0.025	0.010	0.005
140	0.345	0.371	0.313	0.240	0.768	0.475	0.515	0.582	0.625	0.600	0.535	0.444	0.500	0.600	0.495
142	0.172	0.165	0.091	0.140	0.101	0.172	0.197	0.133	0.105	0.131	0.145	0.197	0.130	0.115	0.140
144	0.052	0.108	0.015	0.040	0.020	0.051	0.040	0.087	0.120	0.077	0.090	0.061	0.080	0.090	0.120
146	0.011	0.015	0.005	0.005	-	0.020	0.015	0.005	-	0.008	0.010	0.015	0.040	-	0.005
148	0.006	0.010	-	0.005	-	0.005	-	-	0.020	-	-	0.005	-	-	-
150	0.006	-	0.005	0.005	-	0.005	-	-	-	-	-	-	-	-	-
152	-	0.005	-	-	-	-	-	-	-	-	-	-	-	-	-
Ots3															
N	87	97	99	100	100	98	99	96	100	65	100	99	100	100	100
74	-	0.005	-	0.005	-	-	-	-	-	-	-	-	-	-	-
78	-	-	-	-	-	-	-	-	-	-	0.020	-	0.005	-	0.005
82	-	-	0.005	-	-	-	-	-	-	-	-	-	-	-	-
84	-	-	0.005	-	-	-	-	-	-	-	-	-	-	-	-
86	0.011	-	-	-	-	-	0.015	-	0.005	-	-	0.005	-	-	-

Appendix A continued.

	FDI	TCI	TAZ	OUT	SBL	CHI	KR	LKR	KLSB	PRC	CC	HPB	LLCB	LTLK	UTLK
Ots3 continued															
88	-	-	-	-	-	-	-	0.010	-	-	-	-	-	-	-
90	0.891	0.881	0.763	0.715	0.785	0.939	0.929	0.880	0.940	0.923	0.915	0.904	0.940	0.900	0.945
92	-	0.010	-	-	-	-	-	0.016	-	-	0.010	-	-	0.005	-
94	0.052	0.067	0.136	0.150	0.200	0.061	0.056	0.094	0.055	0.054	0.055	0.081	0.050	0.090	0.045
96	0.040	0.031	0.091	0.125	0.015	-	-	-	-	0.023	-	0.010	0.005	0.005	0.005
98	-	0.005	-	0.005	-	-	-	-	-	-	-	-	-	-	-
100	0.006	-	-	-	-	-	-	-	-	-	-	-	-	-	-
One18															
N	87	97	99	100	100	99	99	97	100	65	100	99	100	100	100
163	-	0.005	-	-	-	-	-	-	-	-	-	-	-	-	-
171	0.316	0.412	0.338	0.315	0.205	0.207	0.177	0.170	0.135	0.177	0.140	0.177	0.220	0.165	0.150
173	0.017	0.005	0.010	-	-	-	-	0.005	-	-	-	-	-	-	-
175	-	-	0.061	0.020	0.090	0.116	0.126	0.072	0.075	0.046	0.095	0.076	0.110	0.075	0.115
177	-	-	-	-	-	-	0.005	-	-	-	-	0.005	-	-	0.005
179	-	-	-	0.005	0.160	0.005	0.020	-	-	0.008	0.030	-	0.005	0.035	0.030

Appendix A continued.

	FDI	TCI	TAZ	OUT	SBL	CHI	KR	LKR	KLSB	PRC	CC	HPB	LLCB	LTLK	UTLK
One18 continued															
181	0.218	0.304	0.328	0.395	0.405	0.520	0.530	0.469	0.530	0.585	0.565	0.510	0.505	0.570	0.550
183	-	-	-	-	-	-	-	-	-	-	0.010	0.005	-	-	0.005
185	0.431	0.258	0.258	0.265	0.140	0.146	0.141	0.278	0.255	0.177	0.160	0.222	0.140	0.150	0.130
187	0.011	-	0.005	-	-	-	-	-	-	-	-	0.005	0.005	-	-
189	0.006	0.015	-	-	-	0.005	-	0.005	0.005	0.008	-	-	0.015	0.005	0.015
One13															
N	87	97	99	100	100	99	99	97	100	65	100	99	100	99	100
154	0.006	0.021	-	-	-	-	-	-	-	-	-	-	-	-	-
156	0.006	-	-	-	-	-	-	-	-	-	-	-	-	-	-
158	0.006	-	0.010	-	-	0.025	0.025	-	0.005	0.031	-	0.025	0.005	-	-
160	0.236	0.397	0.510	0.380	0.415	0.444	0.429	0.340	0.420	0.377	0.485	0.449	0.550	0.545	0.510
162	0.017	0.015	0.051	0.050	0.030	0.020	0.020	0.041	0.005	0.077	0.035	0.030	0.040	0.025	0.045
164	0.006	0.026	0.005	0.020	-	-	-	0.005	-	-	-	-	0.005	0.005	-
166	-	-	0.005	-	-	-	-	-	-	-	-	-	-	-	-
168	0.695	0.495	0.303	0.475	0.545	0.490	0.505	0.577	0.550	0.500	0.470	0.480	0.390	0.399	0.435

Appendix A continued.

	FDI	TCI	TAZ	OUT	SBL	CHI	KR	LKR	KLSB	PRC	CC	HPB	LLCB	LTLK	UTLK
One13 continued															
170	0.006	-	0.111	0.070	-	0.015	0.010	0.021	0.005	-	-	0.010	-	-	-
172	0.017	0.041	0.005	0.005	0.010	0.005	0.010	0.015	0.015	0.015	0.010	0.005	0.010	0.025	0.010
174	0.006	0.005	-	-	-	-	-	-	-	-	-	-	-	-	-
One105															
N	87	97	99	100	98	99	99	98	100	65	100	99	100	100	100
124	-	-	-	-	-	0.010	0.005	-	-	-	0.005	-	-	-	-
128	-	-	0.005	0.005	0.020	0.005	-	0.010	-	-	0.005	0.005	0.005	-	0.005
132	0.822	0.784	0.773	0.775	0.699	0.763	0.803	0.832	0.835	0.723	0.800	0.793	0.845	0.795	0.780
136	0.126	0.113	0.101	0.110	0.133	0.172	0.162	0.092	0.105	0.177	0.145	0.141	0.120	0.170	0.145
140	0.046	0.103	0.061	0.060	0.107	0.051	0.020	0.066	0.060	0.100	0.040	0.051	0.025	0.030	0.040
144	0.006	-	0.061	0.050	0.041	-	0.010	-	-	-	0.005	0.010	0.005	0.005	0.030
Ots107															
N	86	97	99	100	100	99	99	98	100	65	100	99	100	100	100
90	-	-	0.005	-	-	-	-	-	-	-	-	-	-	-	-
102	-	-	0.015	0.010	-	-	-	-	-	-	-	-	-	-	-

Appendix A continued.

	FDI	TCI	TAZ	OUT	SBL	CHI	KR	LKR	KLSB	PRC	CC	HPB	LLCB	LTLK	UTLK
Ots107 continued															
106	-	-	-	0.005	0.080	-	-	-	-	0.008	0.005	-	-	-	-
110	-	-	-	-	-	-	-	-	0.005	-	-	-	0.005	0.005	-
114	0.035	0.021	0.005	0.015	-	0.005	-	0.005	-	-	0.005	-	-	-	-
118	0.895	0.835	0.823	0.825	0.840	0.753	0.793	0.730	0.755	0.762	0.750	0.682	0.730	0.705	0.710
122	0.058	0.113	0.152	0.145	0.080	0.232	0.187	0.219	0.215	0.208	0.235	0.293	0.230	0.270	0.260
126	0.012	0.010	-	-	-	0.010	0.020	0.046	0.025	0.023	0.005	0.025	0.035	0.020	0.030
130	-	0.021	-	-	-	-	-	-	-	-	-	-	-	-	-
Omy77															
N	87	97	99	100	100	98	99	97	98	65	100	99	100	100	100
85	-	-	-	-	-	-	-	-	-	-	-	-	-	0.005	-
89	-	-	-	-	-	-	-	-	-	-	0.005	-	-	0.010	0.005
101	-	-	-	-	-	-	-	-	-	-	-	0.005	-	0.005	-
103	0.006	-	-	-	-	-	-	-	-	-	-	-	-	-	-
105	0.431	0.325	0.455	0.440	0.330	0.750	0.621	0.768	0.719	0.692	0.685	0.753	0.765	0.745	0.765
107	0.006	0.010	-	-	-	0.005	-	-	-	-	-	0.005	-	0.005	-

Appendix A continued.

	FDI	TCI	TAZ	OUT	SBL	CHI	KR	LKR	KLSB	PRC	CC	HPB	LLCB	LTLK	UTLK
Omy77 continued															
109	0.500	0.577	0.505	0.500	0.560	0.209	0.273	0.170	0.189	0.238	0.260	0.172	0.180	0.150	0.175
111	0.006	0.062	0.010	0.010	0.010	0.015	0.005	0.005	0.010	0.023	-	-	0.015	0.015	0.005
113	0.023	0.005	0.015	0.020	0.100	0.015	0.101	0.057	0.082	0.046	0.050	0.066	0.040	0.065	0.050
115	0.023	0.021	0.015	0.030	-	0.005	-	-	-	-	-	-	-	-	-
119	0.006	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Bibliography

Adkison, M. D. 1995. Population differentiation in pacific salmon: local adaptation, genetic drift, or the environment? Can. J. Fish. Aquat. Sci. 52:2762-2776

Alaska Department of Fish and Game (ADFG). 2002. Alaska Department of Fish and Game Division of Commercial Fisheries annual management report 2001. 2001 Bristol Bay area region information report No. 2A02-18.

Allendorf, F. W. 1983. Isolation, gene flow, and genetic differentiation among populations. Pp. 51-65 *in* C. Schonewald-Cox, S. Chambers, B. MacBryde, and L. Thomas, eds. Genetics and conservation. Benjamin/Cummings.

Allendorf, F. W. 1986. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biol. 5:181-190.

Allendorf, F. W., D. Bayles, D. L. Bottom, K. P. Currens. C. A. Frissell, D. Hankin, J. A. Lichatawich, W. Nehlesen, P. C. Trotter, and T. H. Williams. 1997. Prioritizing Pacific salmon stocks for conservation. Cons. Biol. 11:140-152.

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

Allendorf, F. W., and G.H. Thorgaard. 1984. Tetraploidy and the evolution of salmonid fishes. Pages 1-53 *in* B. J. Turner, editor. Evolutionary genetics of fishes. Plenum Press, New York.

Altukhov, Y. P. 1981. The stock concept from the viewpoint of population genetics. Can. J. Fish. Aquat. Sci. 38:1523-1538.

American Public Health Association (APHA), American Water Works Association, and Water Pollution Control Federation. 1980. Standard methods for the examination of water and wastewater, 15th ed. APHA, Washington, D.C.

Anaya, J. 1996. Indigenous peoples in international law. Oxford University Press, New York.

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

Atkinson, I. A. E., and E. K. Cameron. 1993. Human influence on the terrestrial biota and biotic communities of New Zealand. Trends Ecol. Evol. 8:447-451.

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

Bacon, E. S., and G. M. Burghardt. 1976. Learning and color discrimination in the American black bear. Int. Conf. Bear Res. Manage. 3:27-36.

Baldwin, O. 1983. Story of New Zealand's French Pass and D'Urville Island. Book 3. Fields Publishing, Plimmerton, New Zealand.

Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in Chinook salmon (*Oncorhynchus tschawytscha*). J. Hered. 90:281-288.

Barrett, J. C., G. D. Grossman, and J. Rosenfeld. 1992. Turbidity-induced changes in reactive distance of rainbow trout. Trans. Am. Fish. Soc. 121:437-443.

Barrow, T. 1984. An illustrated guide to Māori art. Reed, Auckland, New Zealand.

Beattie, H. 1920. Nature-lore of the southern Māori. Trans. New Zeal. Inst. 52:53-77.

Beattie, J. H. 1994. Traditional lifeways of the southern Māori: the Otago University Museum Ethnological Project, 1920. A. Anderson, editor. University of Otago Press, Dunedin, New Zealand.

Beatty, D.D. 1966. A study of the succession of visual pigments in Pacific salmon (*Oncorhynchus*). Can. J. Zool. 44:429-455.

Beaumont, M. A., and R. A. Nichols. 1996. Evaluating loci for use in the genetic analysis of population structure. Proc. R. Soc. Lond. B Biol. Sci. 263:1619-1626.

Berejikian, B. A., E. P. Tezak, and A. L. LaRue. 2000. Female mate choice and spawning behaviour of Chinook salmon under experimental conditions J. Fish Biol. 57:647-661.

Berkes, F. 1998. Learning to design resilient resource management: indigenous systems in the Canadian subarctic. Pages 98-128 *in* F. Berkes and C. Folke, editors. Linking social and ecological systems: management practices and social mechanisms for building resilience. Cambridge University Press, Cambridge.

Berkes, F., J. Colding, and C. Folke. 2000. Rediscovery of traditional ecological knowledge as adaptive management. Ecol. Appl. 10:1251-1262.

Best, E. 1924. Māori religion and mythology, part 1. Dominion Museum Bulletin Number 10.

Birky, C. W. 1991. Evolution and population genetics of organelle genes: mechanisms and models. Pages 112-134 *in* R. K. Selander, A. G. Clark, and T. S. Whittam, editors. Evolution at the molecular level. Sinauer, Sunderland, MA.

Birky, C. W., P. Fuerst, and T. Maruyama. 1989. Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. Genetics 121:613-627.

Birky, C. W., T. Maruyama, and P. Fuerst. 1983. An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. Genetics 103:513-527.

Blair, G. R., D. E. Rogers, and T. P. Quinn. 1993. Variation in life history characteristics and morphology of sockeye salmon in the Kvichak River system, Bristol Bay, Alaska. Trans. Am. Fish. Soc. 122:550-559.

Blount, J. D., D. C. Houston, and A. P. Moller. 2000. Why egg yolk is yellow. Trends Ecol. Evol. 15:47-49.

Blount, J. D., P. F. Surai, R. G. Nager, and D. C. Houston. 2002. Carotenoids and egg quality in the lesser black-backed gull *Larus fuscus*: a supplemental feeding study of maternal effects. Proc. R. Soc. Lond. B Biol. Sci. 269:26-36.

Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411:944-948.

Brabets, T. 2002. Water quality of the Tlikakila River and five major tributaries to Lake Clark, Lake Clark National Park and Preserve, Alaska, 1999-2001. U.S. Geological Survey, Water-Resources Investigations Report 02-4127.

Brannon, E. L. 1987. Mechanisms stabilizing salmonid fry emergence timing. Pp. 120-124 *in* H. D. Smith, L. Margolis, and C. C. Wood, ed. Sockeye salmon (*Onchorynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96.

Briskie, J. V., and M. Mackintosh. 2004. Hatching failure increases with severity of population bottlenecks in birds. Proc. Natl. Acad. Sci.USA. 101:558-561.

Buck, P. 1949. The coming of the Māori. Whitcombe and Tombs, Christchurch, New Zealand.

Buick, T. L. 1972. The Treaty of Waitangi; how New Zealand became a British Colony. Books for Libraries, Freeport, New York.

Burgner, R. L. 1980. Some features of ocean migration and timing of pacific salmon. Pp. 153-164 *in* W. J. McNeil and D.C. Himsworth, eds. Salmonid ecosystems of the north pacific. Oregon State Univ. Press, Corvallis.

Burgner, R. L. 1991. Life history of sockeye salmon (*Oncorhynchus nerka*). Pp. 1-117 *in* C. Groot and L. Margolis, eds. Pacific salmon life histories. Univ. of British Columbia Press, Vancouver.

Carney, B. L., A. K. Gray, and A. J. Gharrett. 1997. Mitochondrial DNA restriction site variation within and among five populations of Alaskan coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 54:940-949.

Castric, V., L. Bernatchez. 2003. The rise and fall of isolation by distance in the anadromous brook charr (*Salvelinus fontinalis* Mitchill). Genetics 163:983-996.

Chakraborty, R., P. A. Fuerst, and M. Nei. 1980. Statistical studies on protein polymorphism in natural populations III: Distribution of allele frequencies and the number of alleles per locus. Genetics 94:1039-1063.

Chapman, D. W. 1988. Critical review of variables used to define effects of fines in redds of large salmonids. Trans. Am. Fish. Soc. 117:1-21.

Cockerham, C. C. 1973. Analyses of gene frequencies. Genetics 74:679-700.

Congdon, B. C., J. F. Piatt, K. Martin, and V. L. Friesen. 2000. Mechanisms of population differentiation in marbled murreletes: historical versus contemporary processes. Evolution 54:974-986.

Churikov, D., M. Matsuoka, X. Luan, A. K. Gray, V. L. A. Brykov, and A. J. Gharrett. 2001. Assessment of concordance among genealogical reconstructions from various mtDNA segments in three species of Pacific salmon (genus *Oncorhynchus*). Mol. Ecol.10:2329-2339.

Cooper, R. A., and P. R. Millener. 1993. The New Zealand biota: historical background and new research. Trends Ecol. Evol. 8:429-433.

Cornuet, J. M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001-2014.

Costello, A. B., T. E. Down, S. M. Pollard, C. J. Pacas, E. B. Taylor. 2003. The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: an examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus* (Pisces: Salmonidae). Evolution 57: 328-344.

Cox, P. A. 1997. Indigenous peoples and conservation. Pages 207-220 *in* F. Grifo and J. Rosenthal, editors. Biodiversity and human heath. Island Press, Covelo, California.

Craig, J. K., and C. J. Foote. 2001. Countergradient variation and secondary sexual color: phenotypic convergence promotes genetic divergence in carotenoid use between sympatric anadromous and nonanadromous morphs of sockeye salmon (*Oncorhynchus nerka*). Evolution 55:380-391.

Cree, A. 1993. Tuatara recovery plan, *Sphenodon punctatus*. Threatened species recovery plan series Number 9, Department of Conservation, Wellington, New Zealand.

Cree, A., C. H. Daugherty, and J. M. Hay. 1995. Reproduction of a rare reptile, the tuatara *Sphenodon punctatus*, on rat-free and rat-inhabited islands in New Zeal. Cons. Biol. 9:373-383.

Crook, I. G. 1973. The tuatara, *Sphenodon punctatus* Gray, on islands with and without populations of the Polynesian rat, *Rattus exulans* (Peale). Proc. New Zeal. Ecol. Soc. 20:115-120.

Crook, I. G. 1975. The tuatara. Pages 331-352 *in* G. Kuschel, editor. Biogeography and ecology in New Zealand. Dr. W. Junk b. v., the Hague.

Crow, J. F., and K. Aoki. 1984. Group selection for a polygenic behavioral trait: estimating the degree of population subdivision. Proc. Natl. Acad. Sci.USA 81:6073-6077.

Crow, J. F., and M. Kimura. 1970. An introduction to population genetics theory. Harpher and Row, New York

Crozier, G. F. 1970. Tissue carotenoids in prespawning and spawning sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 27:973-975.

Daugherty, C. H., and A. Cree. 1990. Tuatara: a survivor from the dinosaur age. New Zeal. Geogr. 6:66-86.

Daugherty, C. H., A. Cree, J. M. Hay, and M. B. Thompson. 1990. Neglected taxonomy and continuing extinctions of tuatara (*Sphenodon*). Nature 347:177-179.

Daugherty, C. H., G. W. Gibbs, and R. A. Hitchmough. 1993. Mega-island or micro-continent? New Zealand and its fauna. Trends Ecol. Evol. 8:437-442.

Darwin, C. 1859. On the origin of species by means of natural selection, or, the preservation of favoured races in the struggle for life. J. Murray, London.

Darwin, C. 1871. The descent of man and selection in relation to sex. J. Murray, London.

Dawbin, W. H. 1982. The tuatara *Sphenodon punctatus*: aspects of life history, growth and longevity. Pages 237-250 *in* D. G. Newman, editor. New Zealand herpetology. New Zealand Wildlife Service Occasional Publication No. 2.

Demory, R. L., R. F. Orrell, and D. R. Heinle. 1964. Spawning ground catalog of the Kvichak River system, Bristol Bay, Alaska. U.S. Fish and Wildlife Service Special Scientific Report No. 488.

Deur, D. 2002. Rethinking precolonial plant cultivation on the northwest coast of North America. Prof. Geog. 54:140-157.

Doiron, S., L. Bernatchez, and P. U. Blier. 2002. A comparative mitogenomic analysis of the potential adaptive value of Artic Charr mtDNA introgression in Brook Charr populations (*Salvelinus fontinalis* Mitchill). Mol. Biol. Evol. 19:1902-1909.

Douglas, M. S. V., J. P. Smol, J. M. Savelle, and J. M. Blais. 2004. Prehistoric Inuit whalers affected Arctic freshwater ecosystems. Proc. Natl. Acad. Sci.USA 101:1613-1617.

Downes, T. W. 1937. Māori mentality regarding the lizard and taniwha in the Whanganui river area. J. Polyn. Soc. 46:206-224.

Drew, J. A. 2005. Use of traditional ecological knowledge in marine conservation. Cons. Biol. 19:1286-1293.

Duff, R. 1956. The moa-hunter period of Māori culture. R. E. Owen, Government Printer, Wellington, New Zealand.

Eiler, J. H., B. D. Nelson, and R. F. Bradshaw. 1992. Riverine spawning by sockeye salmon in the Taku river, Alaska and British Columbia. Trans. Am. Fish. Soc. 121:701-708.

Ellegren, H. 2000. Microsatellite mutations in the germline: implications for evolutionary inference. Trends Genet. 16:551-558.

El Mousadik, A., and R. J. Petit. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinos* (L.) Skeels] endemic to Morocco. Theor. Appl. Genet. 92:832-839.

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

Endler, J. 1995. Multiple trait coevolution and environmental gradients in guppies. Trends Ecol. Evol. 10:22-29.

Endler, J. A., and A. E. Houde. 1995. Geographic variation in female preference for male traits in *Poecilia reticulata*. Evolution 49:456-458.

England, P. R., A. V. Usher, R. J. Whelan, and D. J. Ayre. 2002. Microsatellite diversity and genetic structure of fragmented populations of the rare, fire-dependent shrub *Grevillea macleayana*. Mol. Ecol. 11:967-977.

Estoup, A., P. Presa, F. Krieg, D. Vaiman, and R. Guyomard. 1993. $(Ct)_n$ and $(Gt)_n$ microsatellites: a new class of genetic markers for *Salmo trutta* L. (brown trout). J. Hered. 71:488-496.

Faire, L. 2000. Report to the Alaska Board of Fisheries on spawning escapement goal evaluations for Bristol Bay salmon. Alaska Department of Fish and Game Regional Information Report No. 2A00-38, Anchorage.

Fair, L. F. 2003. Critical elements of Kvichak River sockeye salmon management. Alaska Fish. Res. Bull. 10:95-103.

Fleming, I. A., and M. R. Gross. 1989. Evolution of adult female life history and morphology in a Pacific salmon (Coho: *Oncorhynchus kisutch*). Evolution 43:141-157.

Fleming, I. A., and M. R. Gross. 1994. Breeding competition in a Pacific salmon (Coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. Evolution 48:637-657.

Fleming, I. A., and S. Ng. 1987. Evaluation of techniques for fixing, preserving, and measuring salmon eggs. Can. J. Fish. Aquat. Sci. 44:1957-1962.

Foote, C. J. 1988. Male mate choice dependent on male size in salmon. Behaviour 106:63-80.

Foote, C. J. 1990. An experimental comparison of male and female spawning territoriality in a Pacific salmon. Behaviour 115:283-314.

Foote, C. J., and P. A. Larkin. 1988. The role of male choice in the assortative mating of anadromous and non-anadromous sockeye salmon (*Oncorhynchus nerka*). Behaviour 106:43-62.

Foote, C. J., G. S. Brown, and C. W. Hawryshyn. 2004. Female colour and male choice in sockeye salmon: implications for the phenotypic convergence of anadromous and nonanadromous morphs. Anim. Behav. 67:69-83.

Ford, J. and D. Martinez. 2000. Traditional ecological knowledge, ecosystem science and environmental management. Ecol. Appl. 10:1249-1250.

French, R., H. Bilton, M. Osako, and A. Hartt. 1976. Distribution and origin of sockeye salmon (*Oncorhynchus nerka*) in offshore waters of the north Pacific ocean. International North Pacific Fisheries Commission Bulletin 34.

Fuller, R. C. 2002. Lighting environment predicts the relative abundance of male colour morphs in bluefin killifish (*Lucania goodei*) populations. Proc. R. Soc. Lond. B Biol. Sci. 269:1457-1465.

Fuller, R. C., K. L. Carleton, J. M. Fadool, T. C. Spady, and J. Travis. 2005. Genetic and environmental variation in the visual properties of blue killifish, *Lucania goodei*. J. Evol. Biol. 18:516-523.

Funk, W. C., J. A. Tyburczy, K. L. Knudsen, K. R. Lindner, and F. W. Allendorf. 2005. Genetic basis of variation in morphological and life-history traits of a wild population of pink salmon. J. Hered. 96:24-31.

Gadgil, M., P. R. Seshagiri Rao, G. Utkarsh, P. Pramod, A. Chhatre, and members of the People's Biodiversity Initiative. 2000. New meanings for old knowledge: the People's Biodiversity Registers Program. Ecol. Appl. 10:1307-1317.

Gall, G. A. E., and R. Neira. 2004. Genetic analysis of female reproductive traits of farmed coho salmon (*Oncorhynchus kisutch*). Aquaculture 234:143-154.

Gall, G. A. E., J. Baltodano, and N. Huang. 1988. Heritability of age at spawning for rainbow trout. Aquaculture 68:93-102.

Garza, J.C., and E. G. Williamson. 2001. Detection of reduction in population size using data from microsatellite loci. Mol. Ecol.10:305-318.

Gaze, P. 2001. Tuatara recovery plan 2001-2011. Threatened species recovery plan Number 47, Department of Conservation, Nelson, New Zealand.

Gende, S. M., T. P. Quinn, and M. F. Willson. 2001. Consumption choice by bears feeding on salmon. Oecologia 127:372-382.

Gharrett, A. J., A. K. Gray, and V. Brykov. 2001. Phylogeographic analysis of mitochondrial DNA variation in Alaskan coho salmon, *Oncorhynchus kisutch*. Fish. Bull. 99:528-544.

Gibbons, A. 2001. The peopling of the Pacific. Science 291:1735-1737.

Giesel, J.T. 1974. Fitness and polymorphism for net fecundity distribution in iteroparous populations. Am. Nat. 103:321-331.

Goodnight, C. J. 1987. On the effect of founder events on epistatic genetic variance. Evolution 41:80-91.

Goodwin, T. W. 1984. The biochemistry of carotenoids, vol. II, animals. Chapman and Hall, New York.

Goudet, J. 1995. Fstat version 1.2: A computer program to calculate F-statistics. J. Hered. 86:485-486.

Gould, S. J., and R. C. Lewontin. 1979. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. Proc. R. Soc. Lond. B Biol. Sci. 205: 581-598.

Grant, V., and K. Grant. 1965. Behavior of hawkmoths on flowers of *Datura metaloides*. Bot. Gaz. 144:280-284.

Green, J. 1965. Chemical embryology of the Crustacea. Biol. Rev. 40:580-600.

Gross, M. 1985. Disruptive selection for alternative life history strategies in salmon. Nature 313:47-48.

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

Habicht, C., J. B. Olsen, L. Fair, and J. E. Seeb. 2004. Smaller effective population sizes evidenced by loss of microsatellite alleles in tributary-spawning populations of sockeye salmon from the Kvichak River, Alaska drainage. Envir.Biol. Fish. 69:51-62.

Hairston, N. G. 1979. The relationship between pigmentation and reproduction in two species of *Diaptomu*s (Copepoda). Limnol. Oceanogr. 24:38-44.

Hamon, T.R., and C. J. Foote. 2000. Changes in midorbital to hypural length and morphology in maturing sockeye salmon. N. Am. J. Fish. Manage. 20:245-249.

Hamon, T.R., and C. J. Foote. 2005. Concurrent natural and sexual selection in wild male sockeye salmon (*Oncorhynchus nerka*). Evolution 59:1104-1118.

Hamon, T. R., C. J. Foote, R. Hilborn, and D. E. Rogers. 2000. Selection on morphology of spawning wild sockeye salmon by a gill-net fishery. Trans. Am. Fish. Soc. 129:1300-1315.

Hankin, D. G., J. W. Nicholas, and T.W. Downey. 1993. Evidence for inheritance of age at maturity in chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 50:347-358.

Hartley, R. C., and M. W. Kennedy. 2004. Are carotenoids a red herring in sexual display? Trends Ecol. Evol. 19:353-354.

Hausle, D. A., and D. W. Coble. 1976. Influence of sand in redds on survival and emergence of Brook trout (*Salvelinus fontinalis*). Trans. Am. Fish. Soc. 105:57-63.

Hay, J. M., C. H. Daugherty, A. Cree, and L. R. Maxson. 2003. Low genetic divergence obscures phylogeny among populations of *Sphenodon*, remnant of an ancient reptile lineage. Mol. Phyl. Evol. 29:1-19.

Hedrick, P.W. 1999. Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution 53:313-318.

Hendry, A. P., and T. Day. 2005. Population structure attributable to reproductive time: isolation by time and adaptation by time. Mol. Ecol. 14:901-916.

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

Hendry, A. P., A. H. Dittman, and R. W. Hardy. 2000a. Proximate composition, reproductive development, and a test for trade-offs in captive sockeye salmon. Trans. Am. Fish. Soc. 129:1082-1095.

Hendry, A. P., J. K. Wenburg, P. Bentzen, E. C. Volk, and T. P. Quinn. 2000b. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science 290:516-518.

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

Hilborn, R., T. P. Quinn, D. E. Schindler, and D. E. Rogers. 2003. Biocomplexity and fisheries sustainability. Proc. Natl. Acad. Sci. U S A 100:6564-6568.

Hitchmough, R. A. 2002. New Zealand threat classification system lists - 2002. Threatened species occasional publication 23. Department of Conservation, Wellington, New Zealand.

Holdaway, R. N. 1989. New Zealand's pre-human avifauna and its vulnerability. New Zeal. J. Ecol. 12:11-25

Holdaway, R. 1996. Arrival of rats in New Zealand. Nature 384:225-384.

Hongi, H. 1922. The tuatara; why the Māori dreads it. Evening Post (New Zealand), variae 34:15-17.

Huntington, H. P. 2000. Using traditional ecological knowledge in science: methods and applications. Ecol. Appl. 10:1270-1274.

Hurles, M. E., E. Matisoo-Smith, R. D. Gray, and D. Penny. 2003. Untangling oceanic settlement: the edge of the knowable. Trends Ecol. Evol. 18:531-540.

Hurtado, L. A., T. Erez, S. Castrezana, and T. A. Markow. 2004. Contrasting population genetic patterns and histories among sympatric Sonoran Desert cactophilic *Drosophila*. Mol. Ecol.13:1365-1375.

Hutchings, J. A. 1991. Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. Evolution 45:1162-1168.

Hutchison, D.W., and A. R. Templeton. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53:1898-1914.

Jensen, K. A., and O. A. Mathisen. 1987. Migratory structure of the Kvichak River sockeye salmon (*Oncorhynchus nerka*) escapement, 1983. Pp. 101-109 *in* H. D. Smith, L. Margolis, and C. C. Wood, eds. Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96.

Johannes, R. E., and B. Yeeting. 2001. I-Kiribati knowledge and management of Tarawa's lagoon resources. Atoll Res. Bull. 489:1-24.

Johnson, D. E. 1998. Applied multivariate methods for data analysis. Brooks/Cole Publishing, Pacific Grove, California.

Jollivet, D., P. Chevaldonné, and B. Planque. 1999. Hydrothermal-vent alvinellid polychaete dispersal in the Eastern Pacific. 2. A metapopulation model based on habitat shifts. Evolution 53:1128-1142.

Karl, S. A., and J. C. Avise. 1992. Balancing selection at allozyme loci in oysters: implications for nuclear RFLPs. Science 256:100-102.

Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends Ecol. Evol. 17:230-241.

Kimmerer, R. W. 2002. Weaving traditional ecological knowledge into biological education: a call to action. BioScience 52:432-438.

Kinnison, M., M. Unwin, N. Boustead, and T. Quinn. 1998. Population specific variation in body dimensions of adult Chinook salmon (*Oncorhynchus tshawytscha*) from New Zealand and their source population, 90 years after introduction. Can. J. Fish. Aquat. Sci. 55:554-563.

Kinnison, M. T., P. Bentzen, M. J. Unwin, and T. P. Quinn. 2002. Reconstructing recent divergence: evaluating nonequilibrium population structure in New Zealand chinook salmon. Mol. Ecol. 11:739-754.

Kittles, R. A., A. W. Bergen, M. Urbanek, M. Virkkunen, M. Linnoila, D. Goldman, and J. C. Long. 1999. Autosomal, mitochondrial, and Y chromosome DNA variation in Finland: evidence for a male-specific bottleneck. Am. J. Phys. Anth. 108:381-399.

Kline, T. C., J. J. Goering, O. A. Mathisen, P. H. Poe, P. L. Parker, and R. S. Scalan. 1993. Recycling of elements transported upstream by runs of Pacific salmon: evidence in the Kvichak River watershed, Bristol Bay, southwestern Alaska. Can. J. Fish. Aquat. Sci. 50:2350-2365.

Koenings, J. P., R. D. Burkett, G. B. Kyle, J. A. Edmundson, and J. M. Edmundson. 1986. Trophic level responses to glacial meltwater intrusion in Alaskan lakes. Pp. 179-194 *in* D. L. Kane, ed. Proceedings: cold regions hydrology symposium. Amer. Water Resources Assoc., Bethesda, Maryland.

Koenings, J. P., R. D. Burkett, and J. M. Edmundson. 1990. The exclusion of limnetic cladocera from turbid glacier-meltwater lakes. Ecology 7:57-67.

Kondolf, G. M., and S. Li. 1992. The pebble count technique for quantifying surface bed material size in instream flow studies. Rivers 3:80-87.

Lande, R. 1980. Genetic variation and phenotypic evolution during allopatric speciation. Am. Nat. 116:463-479.

Landry, P. A., M. T. Koskinen, and C. R. Primmer. 2002. Deriving evolutionary relationships among populations using microsatellites and $(\delta-\mu)^2$: all loci are equal, but some are more equal than others. Genetics 162:2025-2035.

Larson, S., R. Jameson, M. Etnier, M. Fleming, and P. Bentzen. 2002. Loss of genetic diversity in sea otters (*Enhydra lutris*) associated with the fur trade of the 18th and 19th centuries. Mol. Ecol. 11:1899-1903.

Leberg, P. L. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. Evolution 46:477-494.

Lesica, P., and F. W. Allendorf. 1995. When are peripheral populations valuable for conservation? Cons. Biol. 9:753-760.

Li, W. H. 1978. Maintenance of genetic variability under the joint effect of mutation, selection, and random drift. Genetics 90:349-382.

Link, M. R., M. L. Hartley, S. A. Miller, B. Waldrop, J. Wilen, and J. Barnett. 2003. An analysis of options to restructure the Bristol Bay salmon fishery. Bristol Bay Economic Development Corporation, Dillingham, Alaska

Lorenz, J. M., and J. H. Eiler. 1989. Spawning habitat and redd characteristics of sockeye salmon in the glacial Taku River, British Columbia and Alaska. Trans. Am. Fish. Soc. 118:495-502.

Loyd, D. S., J. P. Koenings, and J. D. LaPerriere. 1987. Effects of turbidity in fresh waters of Alaska. N. Am. J. Fish. Manag. 7:18-33.

Luikart G., F. W. Allendorf, J. M. Cornuet, and W. B. Sherwin. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. J. Hered. 89:238-247.

Luikart, G., and J. M. Cornuet. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Cons. Biol. 12:228-233.

Luikart, G., P. R. England, D. Tallmon, S. Jordan, P. Taberlet. 2003. The power and promise of population genomics: from genotyping to genome typing. Nat. Rev. Genet. 4:981-994.

Maffi, L. 1998. Language: a resource for nature. Nat. Resources 34:12-21.

Manly, B. F. J. 1991. Randomization and Monte Carlo methods in biology. Chapman and Hall, London.

Maruyama, T., and P. A. Fuerst. 1985. Population bottlenecks and non-equilibrium models in population genetics II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics 111:675-689.

Mathiesen, O. A. 1962. The effect of altered sex ratios on the spawning of red salmon. Pp. 137-245 *in* T.S.Y. Koo, ed. Studies of Alaska red salmon. University of Washington Press, Seattle.

Matisoo-Smith, E., R. M. Roberts, G. J. Irwin, J. S. Allen, D. Penny, and D. M. Lambert. 1998. Patterns of prehistoric human mobility in Polynesia indicated by mtDNA from the Pacific rat. Proc. Natl. Acad. Sci.USA 95:15145-15150.

Mauro, F. and P. D. Hardison. 2000. Traditional knowledge of indigenous and local communities: international debate and policy initiatives. Ecol. Appl. 10:1263-1269.

McCauley, D. E. 1993. Genetic consequences of extinction and recolonization in fragmented habitats. Pages 217-233 *in* P. M. Kareiva, J. G. Kinsolver, and R. B. Huey, editors. Biotic interactions and global change Sinauer, Sunderland, MA.

McDonald, J. H. 1994. Detecting natural selection by comparing geographic variation in protein and DNA polymorphisms. Pages 88-100 *in* B. Golding, editor. Non-neutral evolution: theories and molecular data. Chapman and Hall, New York.

McKay, J. K., and R. G. Latta. 2002. Adaptive population divergence: markers, QTL, and traits. Trends Ecol. Evol. 17:285-291.

Mead, H. M., and N. Grove. 2001. Ngā pēpeha a ngā tīpuna; the sayings of the ancestors. Victoria University Press, Wellington, New Zealand.

Merilä, J., and P. Crnokrak. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. J. Evol. Biol. 14:892-903.

Miller, J. L., T. Hamon, and T. Jones. *In press*. Piscivorous kokanee: costs and benefits of exploiting large prey. *In* C. A. Woody, ed. Sockeye salmon evolution, ecology, and management. American Fisheries Society, Special Sockeye Salmon Symposium, Bethesda, MD.

Mills, L. S., and F. W. Allendorf. 1996. The one-migrant-per-generation rule in conservation and management. Cons. Biol. 10:1509-1518.

Milner, A. M. 1987. Colonization and ecological development of new streams in Glacier Bay National Park, Alaska. Freshwater Biol. 18:53-70.

Milner, A. M., and R. G. Bailey. 1989. Salmonid colonization of new streams in Glacier Bay National Park, Alaska. Aquac. Fish. Manage. 20:179-192.

Milner, A. M., E. E. Knudsen, C. Soiseth, A. L. Robertson, D. Schell, I. T. Phillips, and K. Magnusson. 2000. Colonization and development of stream communities across a 200 year gradient in Glacier Bay National Park, Alaska, USA. Can. J. Fish. Aquat. Sci. 57:2319-2335.

Moore, J. H. 1986. The ornithology of Cheyenne religionists. Plains Anthr. 31:177-192.

Morris, D.B., K. R. Richard, and J. M. Wright. 1996. Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study of salmonids. Can. J. Fish. Aquat. Sci. 53:120-126.

Murphy, M. L., K. V. Koski, J. Mitchel Lorenz, and J. F. Thedinga. 1997. Downstream migrations of juvenile Pacific salmon (*Oncorhychus* spp.) in a glacial transboundary river. Can. J. Fish. Aquat. Sci. 54:2837-2846.

Nabhan, G. P. 1992. Threatened native American plants. Endangered Species Update 9:1-4.

Nabhan, G. P. 2000. Interspecific relationships affecting endangered species recognized by O'odham and Comcáac cultures. Ecol. Appl. 10:1288-1295.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nei, M., R. Chakraborty, and P. A. Fuerst. 1976. Infinite allele model with varying mutation rate. Proc. Natl. Acad. Sci.USA 73:4164-4168.

Nei, M., and F. Tajima. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 105:207-217.

Nelson, N. J, A. Cree, M. B. Thompson, S. N. Keall, C. H. Daugherty. 2004. Temperature-dependent sex determination in tuatara. Pages 53 -58 *in* N. Valenzuela and V. Lance, editors. Temperature-dependent sex determination in vertebrates. Smithsonian Books, Washington, DC.

Nelson, R. J., and T. D. Beacham. 1999. Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. Anim. Genet. 30:225-244.

Novales-Flamarique, I. 2000. The ontogeny of ultraviolet sensitivity, cone disappearance, and regeneration in the sockeye salmon, *Oncorhynchus nerka*. J. Exp. Biol. 203:1161-1172.

O'Connell, M., R. G. Danzmann, J. Cornuet, J. M. Wright, and M. M. Ferguson. 1997. Differentiation of rainbow trout (*Oncorhynchus mykiss*) populations in Lake Ontario and the evaluation of the stepwise mutation and infinite allele mutation models using microsatellite variability. Can. J. Fish. Aquat. Sci. 54:1391-1399.

Olsen, J. B., S. L. Wilson, E. J. Kretschmer, K. C. Jones, and J. E. Seeb. 2000. Characterization of 14 tetranucleotide microsatellite loci derived from sockeye salmon. Mol. Ecol. 9:2185-2187.

Olson, V. A., and I. P. F. Owens. 1998. Costly sexual signals: are carotenoids rare, risky, or required? Trends Ecol. Evol. 13:510-514.

Ombler, K. 2004. A turnaround for tuatara. Forest and Bird 313:28-31.

Orbell, M. 1995. The illustrated encyclopedia of Māori myth and legend. Canterbury University Press, Christchurch, New Zealand.

Orbell, M., and G. Moon. 1985. The natural world of the Māori. William Collins, Auckland.

Orrell, R. F. 1963. Abundance and age of Lake Clark red salmon smolts, 1962. University of Washington, Fisheries Research Institute Circular 186.

Palumbi, S. R., and C. S. Baker. 1994. Contrasting population structure from nuclear intron sequences and mtDNA of humpback whales. Mol. Biol. Evol. 11:426-435.

Phillips, R. W., R. L. Lantz, E. W. Claire, and J. R. Moring. 1975. Some effects of gravel mixtures on emergence of coho salmon and steelhead trout fry. Trans. Am. Fish. Soc. 104:461-466.

Phillips, W. J. 1955. Māori carving illustrated. Reed, Auckland.

Pierotti, R., and D. Wildcat. 2000. Traditional ecological knowledge: the third alternative (commentary). Ecol. Appl. 10:1333-1340.

Pinkerton, E. 1998. Integrated management of a temperate montane forest ecosystem through wholistic forestry: a British Columbia example. Pages 363-389 *in* F. Berkes and C. Folke, editors. Linking social and ecological systems: management practices and social mechanisms for building resilience. Cambridge University Press, Cambridge.

Poe, P. H., and O. A. Mathisen. 1981. Enumeration of sockeye salmon escapements to the Newhalen River – Lake Clark system in 1979 and 1980. University of Washington, Fisheries Research Institute manuscript report, January 28, 1981, Seattle.

Poe, P. H., and O. A. Mathisen. 1982. 1981 Newhalen river sockeye escapement studies. University of Washington, Fisheries Research Institute Report FRI-UW-8211, Seattle.

Pogson, G.H., K. A. Mesa, and R. G. Boutilier. 1995. Genetic population structure and gene flow in the Atlantic Cod, *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. Genetics 139:375-385.

Pope, L. C., A. Estoup, and C. Moritz. 2000. Phylogeography and population structure of an ecotonal marsupial, *Bettongia tropica*, determined using mtDNA and microsatellites. Mol. Ecol. 9:2041-2053.

Posey, D. A., and G. Dutfield. 1996. Beyond intellectual property rights: towards traditional resource rights for indigenous peoples and local communities. International Development Research Centre, Ottowa, Canada.

Quinn, T. P. 1984. Homing and straying in pacific salmon. Pp. 357-362 *in* J. D. McCleave, G. P. Arnold, J. J. Dodson, and W. H. Neill, eds. Mechanisms of migration in fishes. Plenum Press, New York.

Quinn, T. P. 1985. Homing and the evolution of sockeye salmon (*Oncorhynchus nerka*). Pp. 353-366 *in* M. A. Rankin, ed. Migration: mechanisms and adaptive significance. Contrib. Mar. Sci. 27(Suppl.)

Quinn, T. P., and A. H. Dittman. 1990. Pacific salmon migrations and homing: mechanisms and adaptive significance. Trends Ecol. Evol. 5:174-177.

Quinn, T. P. and C. J. Foote. 1994. The effects of body size and sexual dimorphism on the reproductive behaviour of sockeye salmon (*Oncorhynchus nerka*). Anim. Behav. 48:751-761.

Quinn, T. P., and M. T. Kinnison. 1999. Size-selective and sex-selective predation by brown bears on sockeye salmon. Oecologia 121:273-282.

Quinn, T. P., A. P. Hendry, and L. A. Wetzel. 1995. The influence of life history trade-offs and the size of incubation gravels on egg size variation in sockeye salmon (*Oncorhynchus nerka*). Oikos 74:425-438.

Quinn, T. P., J. L. Nielsen, C. Gan, M. J. Unwin, R. Wilmot, C. Guthrie, and F. M. Utter. 1996. Origin and genetic structure of Chinook salmon, *Oncorhynchus tshawytscha*, transplanted from California to New Zealand: allozyme and mtDNA evidence. Fish. Bull. 94:506-521.

Quinn, T. P., M. J. Unwin, and M. T. Kinnison. 2000. Evolution of temporal isolation in the wild: genetic divergence in timing of migration and breeding by introduced chinook salmon populations. Evolution 54:1372-1385.

Quinn, T. P., L. Wetzel, S. Bishop, K. Overberg, and D. E. Rogers. 2001. Influence of breeding habitat on bear predation and age at maturity and sexual dimorphism of sockeye salmon populations. Can. J. Zool. 79:1782-1793.

Ramstad, K. M., C. J. Foote, J. Olsen, and D.E. Rogers. 2003. Genetic and phenotypic evidence of reproductive isolation between seasonal runs of sockeye salmon in Bear Lake, Alaska. Trans. Am. Fish. Soc. 132:997-1013.

Ramstad, K. M., C. A. Woody, G. K. Sage, and F.W. Allendorf. 2004. Founding events influence genetic population structure of sockeye salmon (*Oncorhynchus nerka*) in Lake Clark, Alaska. Mol. Ecol. 13:277-290.

Raymond, M., and F. Rousset. 1995a. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86:248-249.

Raymond, M., and F. Rousset. 1995b. An exact test for population differentiation. Evolution 49:1280-1283.

Reed, A. W. 1999. Māori myths and legendary tales. New Holland Publishers, Auckland, New Zealand.

Regnart, J. R. 1998. Kvichak River sockeye salmon spawning ground surveys, 1955-1998. Alaska Department of Fish and Game, Regional Information Report No. 2A98-38, Anchorage.

Reiman, B.E., and F. W. Allendorf. 2001. Effective population size and genetic conservation criteria for bull trout. N. Am. J. Fish. Manage. 21:756-764.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Ricker, W. E. 1972. Hereditary and environmental factors affecting certain salmonid populations. Pp. 19-160 *in* R. C. Simon and P. A. Larkin, eds. The stock concept in pacific salmon. Univ. of British Columbia Press, Vancouver.

Roberts, M., W. Norman, N. Minhinnick, D. Wihongi, and C. Kirkwood. 1995. Kaitiakitanga: Māori perspectives on conservation. Pac. Cons. Biol. 2:7-20.

Rogers, D. E. 1987. The regulation of age at maturity in Wood river sockeye salmon (*Oncorhynchus nerka*). Pp. 78-89 *in* H. D. Smith, L. Margolis, and C. C. Wood, eds. Sockeye salmon (*Onchorynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96.

Rogers, D. E., T. Quinn, and B. Rogers. 1999. Alaska salmon research, annual report to Bristol Bay processors. University of Washington, No. FRI-UW-9904.

Rousset. F. 1996. Equilibrium values of measures of population subdivision for stepwise mutation processes. Genetics 142:1357-1362.

Royle, N. J., R. F. Surai, and I. R. Hartley. 2003. The effect of variation in dietary intake on maternal deposition of antioxidants in zebra finch eggs. Func. Ecol. 17:472-481.

Ruggerone, G. T., R. Hanson, and D. E. Rogers. 2000. Selective predation by brown bears (*Ursus arctos*) foraging on spawning sockeye salmon (*Oncorhynchus nerka*). Can. J. Zool. 78:974-981.

Saint-Laurent, R., M. Legault, and L. Bernatchez. 2003. Divergent selection maintains adaptive differentiation despite high gene flow between sympatric rainbow smelt ecotypes (*Osmerus mordax* Mitchill). Mol. Ecol. 12:315-330.

Sandström, P., T. Granqvist Pahlén, L. Edenius, H. Tømmervik, O. Hagner, L. Hemberg, H. Olsson, K. Baer, T. Stenlund, L. Göran Brandt, and M. Egberth. 2003. Conflict resolution by participatory management: remote sensing and GIS as tools for communicating land-use needs for reindeer herding in Northern Sweden. Ambio 32:557-567.

Schlenger, P. T. 1996. Distributions and potential for competition between juvenile sockeye salmon (*Oncorhynchus nerka*) and least cisco (*Coregonus sardinella*) in Lake Clark, Alaska. M.Sc. thesis, University of Washington, Seattle, WA.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin ver 2.000: A software for populations genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Scribner, K.T., J. R. Gust, and R. L. Fields. 1996. Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. Can. J. Fish. Aquat. Sci. 53:833-841.

Seeb, L. W., C. Habicht, W. D. Templin, K. E. Tarbox, R. Z. Davis, L. K. Brannian, and J. E. Seeb. 2000. Genetic diversity of sockeye salmon of Cook Inlet, Alaska, and its application to management of populations affected by the *Exxon Valdez* oil spill. Trans. Am. Fish. Soc. 129:1223-1249.

Seehausen, O., J. J. M. van Alphen, and F. Witte. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. Science 277:1808-1811.

Shaklee, J. B., F. W. Allendorf, D. C. Morizot, and G. S. Whitt. 1990. Gene nomenclature for protein-coding loci in fish. Trans. Am. Fish. Soc. 119:2-15.

Shriver, M. D., L. Jin, R. Chakraborty, and E. Boerwinkle. 1993. VNTR allele frequency distributions under the stepwise mutation model: a computer simulation approach. Genetics 134:983-993.

Siitonen, L., and G. A. E. Gall. 1989. Response to selection for early spawn date in rainbow trout, *Salmo gairdneri*. Aquaculture 78:153-161.

Singh, J. S. 2002. The biodiversity crisis: a multifaceted review. Curr. Sci. 82:638-647.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264-279.

Slatkin, M. 1994. Gene flow and population structure. Pages 3-17 *in* L. A. Real, editor. Ecological genetics. Princeton University Press, Princeton.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457-462.

Slatkin, M. 2002. A vectorized method of importance sampling with applications to models of mutation and migration. Theor. Pop. Biol. 62:339-348.

Slatkin, M., and C. A. Muirhead. 1999. Overdominant alleles in a population of variable size. Genetics 152:775-781.

Smith, C. T., B. F. Koop, and R. J. Nelson. 1998. Isolation and characterization of coho salmon (*Oncorhynchus kisutch*) microsatellites and their use in other salmonids. Mol. Ecol. 7:1614-1616.

Smith, B. E., R. W. Hardy, and O. J. Torrissen. 1992. Synthetic astaxanthin deposition in pan-sized coho salmon (*Oncorhynchus kisutch*). Aquaculture 104:105-119.

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

Sokal, R. R., and F. J. Rohlf. 1981. Biometry: the principals and practice of statistics in biological research, 2nd edition. WH Freeman and Company, San Fransisco.

Soulé, M. E., and L. S. Mills. 1998. No need to isolate genetics. Science. 282:1658-1659.

Spitze, K. 1993. Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. Genetics 135:367-374.

Spong, G., and L. Hellborg. 2002. A near-extinction in lynx: do microsatellite data tell the tale? *Conservation Ecology* 6,15, [online] URL: http://www.consecol.org/vol6/iss1/art15.

Spruell, P., B. E. Rieman, K. L. Knudsen, F. M. Utter, and F. W. Allendorf. 1999. Genetic population structure within streams: microsatellite analysis of bull trout populations. Ecol. Fresh. Fish. 8:114-121.

Stanford, J. A., and J. V. Ward. 1992. Management of aquatic resources in large catchments: reorganizing interactions between ecosystem connectivity and environmental disturbance. Pp. 91-124 *in* R. J. Naiman, ed. Watershed management: balancing sustainability and environmental change. Springer, New York.

Stearns, S. C. 1992. The evolution of life histories. Oxford Univ. Press, New York.

Stickman, K., A. Balutta, M. McBurney, and D. Young. 2003. K'ezghlegh: Nondalton traditional ecological knowledge of freshwater fish. Final fisheries project report 01-075. U. S. Fish and Wildlife Service, Anchorage, AK.

Stilwell, K. B, and D. S. Kaufman. 1996. Late Wisconsin glacial history of the northern Alaska Peninsula, southwestern Alaska, USA. Arct. Alp. Res. 28:475-487.

Stockwell, C. A., A. P. Hendry, and M. Kinnison. 2003. Contemporary evolution meets conservation biology. Trends Ecol. Evol. 18:94-101.

Stork, N. E. 1999. The magnitude of global biodiversity and its decline. Pages 3-32 *in* J. Cracraft and F. T. Grifo, editors. The living planet in crisis: biodiversity science and policy. Columbia University Press, New York.

Storz, J. F., U. Ramakrishnan, and S. C. Alberts. 2002. Genetic effective size of a wild primate population: influence of current and historical demography. Evolution 56:817-829.

Sutherland, W. J. 2003. Parallel extinction risk and global distribution of languages and people. Nature 423:276-279.

Tardiff, S. E., and J. A. Stanford. 1998. Grizzly bear digging: effects on subalpine meadow plants in relation to mineral nitrogen availability. Ecology 79:2219-2228.

Tarr, C.L., S. Conant, and R. C. Fleischer. 1998. Founder events and variation at microsatellite loci in an insular passerine bird, the Laysan finch (*Telespiza cantans*). Mol. Ecol. 7:719-731.

Taylor, E. B. 1991. A review of local adaption in Salmonidae, with particular reference to Pacific and Atlantic salmon. Aquaculture 98:185-207.

Taylor, E. B., S. Harvey, S. Pollard, and J. Volpe. 1997. Postglacial genetic differentiation of reproductive ecotypes of kokanee *Oncorhynchus nerka* in Okanagan Lake, British Columbia. Mol. Ecol. 6:503-517.

Taylor, E. B., and J. D. McPhail. 1985. Variation in body morphology among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. Can. J. Fish. Aquat. Sci. 42:2020-2028.

Thompson, M. B., C. H. Daugherty, A. Cree, D. C. French, J. C. Gillingham, and R. E. Barwick. 1992. Status and longevity of the tuatara, *Sphenodon guntheri*, and Duvaucel's gecko, *Hoplodactylus duvaucelii*, on North Brother Island, New Zealand. J. Roy. Soc. New Zeal. 22: 123-130.

Thompson, M. B., G. C. Packard, M. J. Packard, and B. Rose. 1996. Analysis of the nest environment of tuatara, *Sphenodon punctatus*. J. Zool. 238:239-251.

Tiedemann, R., K. B. Paulus, M. Scheer, K. G. Von Kistowsk, K. Skirnisson, D. Bloch, and M. Dam. 2004. Mitochondrial DNA and microsatellite variation in the eider duck (*Somateria mollissima*) indicate stepwise postglacial colonization of Europe and limited current long-distance dispersal. Mol. Ecol. 13:1481-1494.

Turesson, G. 1922. The genotypic response of the plant species to the habitat. Hereditas 3: 211–350.

Turgeon, J., and L. Bernatchez. 2001. Clinal variation at microsatellite loci reveals historical secondary intergradation between glacial races of *Coregonus artedi* (Teleostei: Coregoninae). Evolution 55:2274-2286.

Turner, N. J., M. B. Ignace, and R. Ignace. 2000. Traditional ecological knowledge and wisdom of aboriginal peoples in British Columbia. Ecol. Appl. 10:1275-1287.

van den Berghe, E. P., and M. R. Gross. 1989. Natural selection resulting from female breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*). Evolution 43:125-140.

Varnavskaya, N. V., C. C. Wood, and R. J. Everett. 1994a. Genetic variation in sockeye salmon (*Oncorhynchus nerka*) populations of Asia and North America. Can. J. Fish. Aquat. Sci. 51:132-146.

Varnavskaya, N. V., C. C. Wood, R. J. Everett, R. L. Wilmot, V. S. Varnavsky, V. V. Midanaya, and T. P. Quinn. 1994b. Genetic differentiation of subpopulations of sockeye salmon (*Oncorhynchus nerka*) within lakes of Alaska, British Columbia, and Kamchatka, Russia. Can. J. Fish. Aquat. Sci. 51:147-157.

Volpe, E. P. 1985. Understanding evolution, 5th ed. McGraw Hill, Boston.

Waits, L., P. Taberlet, J. E. Swenson, F. Sandegren, and R. Franzen. 2000. Nuclear DNA microsatellite analysis of genetic diversity and gene flow in the Scandinavian brown bear (*Ursus arctos*). Mol. Ecol. 9:421-431.

Waples, R. S. 1988. Estimation of allele frequencies at isoloci. Genetics 118:371-384.

Waples, R. S. 1991. Genetic methods for estimating the effective size of cetacean populations. Report of the International Whaling Commission (special issue) 13, 279-300.

Waples, R.S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. J. Hered. 89:438-450.

Waples, R. S. 2002. Definition and estimation of effective population size in the conservation of endangered species. In: *Population Viability Analysis* (eds. Beissinger SR, McCullough DR), pp. 147-168. University of Chicago Press, Chicago.

Weber, J. L., and C. Wong. 1993. Mutation of short tandem repeats. Human Molecular Genetics 2:1123-1128.

Weir, B. S. 1996. Genetic data analysis II. Sinauer,Sunderland, MA.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370

Wenburg, J. K., P. Bentzen, and C. J. Foote. 1998. Microsatellite analysis of genetic population structure in an endangered salmonid: the coastal cutthroat trout (*Oncorhynchus clarki clarki*). Mol. Ecol. 7:733-749.

Westing, C., S. Morstad, K. A. Weiland, T. Sands, L. Fair, F. West, and C. Brazil. 2005. Annual management report 2004 Bristol Bay Area. Alaska Department of Fish and Game, Fishery Management Report No. 05-41, Anchorage.

Whitaker, T. 1992. Was the kawekaweau the world's largest gecko? Forest and Bird 23:44-46.

Wilding, C. S., R. K. Butlin, and J. Grahame. 2001. Differential gene exchange between parapatric morphs of *Littorina saxatilis* detected using AFLP markers. J. Evol. Biol. 14:611-619.

Willis, J. H., and H. A. Orr. 1993. Increased heritable variation following population bottlenecks: the role of dominance. Evolution 47:949-957.

Willson, M. F., and K. C. Halupka. 1995. Anadromous fish as keystone species in vertebrate communities. Cons. Biol. 9:489-497.

Wilmot, R. L., and C. V. Burger. 1985. Genetic differences among populations of Alaskan sockeye salmon. Trans. Am. Fish. Soc.114:236-243.

Wilson, E. O. 1965. The challenge from related species. Pp. 7-24 *in* H. G. Baker and G. L. Stebbins, eds. The genetics of colonizing species. Academic Press, New York.

Wilson, A. C., R. L. Cann, S. M. Carr, M. George, U. B. Gyllensten, K. M. Helm-Bychowski, R. G. Higuchi, S. R. Palumbi, E. M. Prager, R. D. Sage, and M. Stoneking. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linn. Soc. 26:357-400.

Withler, R. E. 1986. Genetic variation in carotenoid pigment deposition in the red-fleshed and white-fleshed Chinook salmon (*Oncorhynchus tshawytscha*) of Quesnel River, British Columbia. Can. J. Genet. Cytol. 28:587-594.

Withler, R. E., K. D. Le, J. Nelson, K. Miller, and T. D. Beacham. 2000. Intact genetic structure and high levels of genetic diversity in bottlenecked sockeye salmon (*Oncorhynchus nerka*) populations of the Fraser River, British Columbia, Canada. Can. J. Fish. Aquat. Sci. 57:1985-1998.

Wood, C. C. 1995. Life history variation and population structure in sockeye salmon. Am. Fish. Soc. Symp. 17:195-216.

Wood, C. C., B. E. Riddell, D. T. Rutherford, and R. E. Withler. 1994. Biochemical genetic survey of sockeye salmon (*Oncorhynchus nerka*) in Canada. Can. J. Fish. Aquat. Sci. 51:114-131.

Woody, C. A. 2004. Population monitoring of Lake Clark and Tazimina River sockeye salmon, Kvichak River drainage, Bristol Bay, Alaska, 2000-2003. U.S. Fish and Wildlife Service, Office of Subsistence Management, Fisheries Resource Monitoring Program Final Report 01-095, Anchorage.

Woody, C. A., J. Olsen, J. Reynolds, and P. Bentzen. 2000. Temporal variation in phenotypic and genotypic traits in two sockeye salmon populations, Tustemena Lake, Alaska. Trans. Am. Fish. Soc. 129:1031-1043.

Woolington, J. D., B. A. Cross, B. L. Stratton, and B. G. Bue. 1990. Bristol Bay sockeye smolt studies for 1988. Alaska Department of Fish and Game, Technical Report 90-16.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97-159.

Wright, S. 1951. The genetical structure of populations. Ann. Eugen.15:323-354.

Wright, S. 1969. Evolution and the genetics of populations, vol. 2, the theory of gene frequencies. Univ. of Chicago Press, Chicago.

Young, D. B. 2004. The migration and spawning distribution of sockeye salmon within Lake Clark, Alaska. M.Sc. thesis, University of Alaska, Fairbanks, AK.

Zar, J. H. 1984. Biostatistical analysis, 2nd ed. Prentice Hall, NJ.