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Bimodal Return Distribution in a Northern Population of Salmon: Genetic, Life History, and Habitat Analysis of Adult and Juvenile Sockeye Salmon (*Oncorhynchus nerka*)

by

Elizabeth Kathleen Fillatre

A Thesis Submitted to the Faculty of Graduate Studies and Research through Biological Sciences in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor

Windsor, Ontario, Canada

2002

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Abstract

Bimodal return migration patterns are evident in many populations of North American salmonids. This thesis analyzes how bimodal return distribution affects genetic structure, life history, and habitat partitioning in a Northern population of sockeye salmon (Oncorhynchus nerka). This thesis is divided into two sections: 1) adult population genetics and 2) juvenile habitat distribution. Genetic analysis of adult spawners using eight microsatellite loci indicates that the early and late runs are genetically divergent in all seven years analyzed (1994-2000), and that differentiation is greater between the runs than among the seven years analyzed. Life history analysis (age at maturation, sex ratio, fork length) of the early and late run fish showed contradictory results: while fork length, sex ratio, and age at maturation did differ significantly between early and late runs in some years, no consistent pattern emerged. Using the same genetic markers as for the adult analysis, habitat ecology was addressed in early and late run juvenile Kluksu sockeye salmon. Specifically, I tested whether early and late runs offspring are utilizing similar rearing habitat and intermixing. Extensive sampling effort at many sites allowed the determination of juvenile rearing habitat for this stock, and the primary rearing habitat (Kluksu Lake) was intensively sampled at seven sites over two consecutive years (2000-2001) for fin clips for genetic analysis. An assignment test based on a maximum likelihood function (WHICHRUN 4.1) was utilized to assign captured juveniles to the early or late run category. The allele frequencies from early and late run adults (1999 and 2000) were utilized to characterize the source populations and pooled juvenile individuals (2000 and 2001) were assigned to the two source populations.
based on multilocus genotyping. Both early and late run juveniles were found in Kluksu Lake, intermixing at most sites, however the runs were found to show divergent distribution within the lake. The relative contribution (productivity) of each subpopulation was calculated based on the ratio of early returning adults in the parent year to early run recruits, and similarly for the late run. Relative productivity ratios were similar for the early and late runs in 2000, however in 2001 significant differences were found. This study showed that the sympatric early and late run sockeye salmon are genetically differentiated, and are likely undergoing processes similar to sympatric speciation and must be administered as two management units. In addition, juvenile Kluksu sockeye salmon inhabit one freshwater lake and exhibit divergent distribution in one of the sampling years (2000). This further reinforces the need for run-specific management, conservation and research initiatives.
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GENERAL INTRODUCTION

Sockeye Salmon and Life History

Pacific salmon (*Oncorhynchus sp.*) are particularly interesting scientifically due to their genetic (ancestral tetraploids) and migratory nature (anadromy), and are widely studied due to a 2.5 billion dollar salmon industry (Groot and Margolis 1991). Pacific salmon spend the majority of their adult life in temperate and sub-arctic waters of the North Pacific and the adjacent Bering Sea and Sea of Okhotsk (Burgner 1991), between 41-61°N latitude (Wood 1995). After spending approximately 1-7 years within the Pacific Ocean, mature salmon move inshore and migrate to natal freshwater tributaries to spawn and die (See Fig. i.1; Wright and Bernhard 1972; Godfrey 1965; Beacham 1984; Healey 1987; Groot and Margolis 1991).

![Diagram of Sockeye Salmon Life Cycle](image)

Figure i.1. Sockeye salmon (*Oncorhynchus nerka*) life cycle
Migrations to natal streams may last from days to weeks, depending on the environmental conditions and geographic location. Run timing has been shown to be heritable (Smoker et al. 1998; Quinn et al. 2000), and shows great precision year-to-year (Brannon 1987). Multiple runs of salmon may return to one stream at different times in the year. run timing has been shown to vary among and within tributaries (Banks et al. 2000; Wood 1995) or populations (Ricker 1972; Quinn et al. 2000). Multiple runs have been reported in many species of Pacific salmon, including pink (O. gorbuscha), steelhead (O. mykiss), chum (O. keta); chinook (O. tshawytscha) and sockeye salmon (O. nerka) (Burger et al. 1985; Tallman and Healey 1994: Adams et al. 1994: Smoker et al. 1994: Varnavskaya et al. 1994; Olsen et al. 2000a: Woodey et al. 2000; Hendry et al. 2002).

Upon arrival on the spawning grounds, salmon utilize streams, creeks, rivers and lakes for spawning habitat (Wood 1995; Burger et al. 1985). Females typically dig redd depositions and wait for a suitable male to fertilize the eggs. Fecundity is known to vary within and among stocks of salmon, primarily due to the varying age and size of salmon (Burgner 1991). The females deposit between 1.200 and 10,000 eggs, ranging considerably among species (1.200-1.900 pink; 2.018-3.077 chum; 2.000-10.000 chinook; 1.724-6906 coho; and 2.000-5,000 sockeye salmon) (McGregor 1923; Semko 1954; Foerster 1968; Kulikova 1972; Drucker 1972: Healey and Heard 1984). However, it is unknown to what extent fecundity and relative productivity vary among closely related populations, particularly amongst run times within one population.

Within the freshwater habitat, eggs will remain in the gravel redds as alevin, and emerge in early spring as free swimming fry (Burgner 1991). Depending on the species
of salmon. fry may migrate directly to ocean habitat (e.g., typically pink and chum salmon; Neave 1955; Hoar 1956; Groot and Margolis 1991). or remain in freshwater stream, tributary and lake environments. (e.g., typically coho, chinook and sockeye salmon) (Drucker 1970; Godfrey 1965; Bill 1984; Healey 1983; Groot and Margolis 1991). However, it is unknown what specific habitat is utilized for rearing nor the extent of juvenile interaction between closely related populations. such as early and late runs.

Sockeye salmon are typical of Pacific salmon, spending 2-5 years in the Pacific Ocean before returning to natal freshwater environments to spawn (See Fig. i.1: Healey 1987). However, sockeye salmon are distinctive due to their slow marine growth and small size at maturity. as well as their dramatic maturation coloration (Burgner 1991). Sockeye salmon spawn in creek, river and lake environments from summer to late fall (e.g., Foerster 1968; Burgner et al. 1969; Brannon 1987; Rogers 1987). Sockeye salmon differ from other species due to their use of lake rearing habitats. in fact most sockeye salmon juveniles utilize freshwater lake habitats for 1-3 years (See Fig. 1: Drucker 1970; Bill 1984).

Site Information, History and Significance

Kluksu River is a tributary of the Alsek drainage basin, flowing into Tatshenshini River in the Southwest corner of Yukon Territory, Canada. Known for popular rafting excursions, the watershed includes many natural barriers, including fast moving water and steep slopes, as is evident from the mountainous areas of the St. Elias and Coast Mountains (Petkovich 1999). Three Oncorhynchus species spawn and utilize Kluksu River for freshwater residency; coho (O. kisutch), chinook (O. tshawytscha) and
sockeye salmon (*O. nerka*). Average returns (1989-1999) of each species are 2,600 coho, 3,900 chinook and 17,000 sockeye salmon (Department of Fisheries and Oceans 2000).

This river is unique for its northern location and cultural and economic value. Klukshu River is a transboundary river jointly managed by the Department of Fisheries and Oceans, the Champagne and Aishihik First Nations and the United States Fish and Wildlife Service. Klukshu River supports the only source of sockeye salmon in the Yukon Territory available for both sport and aboriginal fishers, and thus represents significant economic value. Klukshu River sockeye salmon are particularly important to the Champagne and Aishihik First Nations, who rely heavily on this stock for subsistence. In addition, this northern stock of sockeye salmon is important due to its unique genetic makeup. A study completed by Wood *et al.* (1994), comparing all major sources of sockeye salmon in North America using allozyme electrophoresis, found that the Klukshu River population differed greatly from those in other Northern geographic areas, clustering weakly with southern geographic areas.

The number of returning Klukshu River salmon has been monitored by the Department of Fisheries and Oceans since 1976, and in recent years, the number has decreased significantly. The decline has led to closures of sport and subsistence fisheries, as well as low numbers of sockeye salmon entering spawning grounds. Current management practices disallow sport fishery retention prior to August 15th; following this date, provided a minimum number of sockeye salmon have reached the spawning grounds, each sport fisher may retain two sockeye salmon, while aboriginal fishers may obtain sockeye salmon for subsistence purposes throughout the season. To promote better conservation and management practices, fisheries managers need more information.
on Kluksu River sockeye salmon to determine, for example, if the runs are genetically distinct groups (e.g., Heath et al. 1995; Burger 1997; Waples et al. 2001). In addition, information regarding juvenile rearing habitat and relative productivity of the early and late runs is important for general ecology, management and conservation (e.g., Werner et al. 1977; Schaller et al. 1999). This study provides an excellent opportunity to examine the sympatric population of sockeye salmon and determine evolutionary processes that may be occurring.

Genetics

With the advent of modern genetic technologies, such as polymerase chain reaction (PCR), scientists are able to amplify degraded and/or small amounts of DNA for genetic analysis. Fish DNA is comprised of approximately 3 billion nucleotides (Moyle and Cech 1996), much of which is repetitive DNA sequences (Barnum 1998). Microsatellites are short DNA repeat motifs comprised of di, tri or tetra-nucleotide repeats, and are located throughout the nuclear genome (Jarne and Lagoda 1996). Microsatellites can be amplified using PCR and primers designed to target the flanking areas of non-repetitive DNA. Microsatellite repeats are evaluated based on the length of repeated sequences, which can be compared among and within closely related species (Scribner et al. 1994).

Microsatellite markers are useful because they are thought to be neutral markers, are abundant, codominant, and typically show high levels of variation (Wright and Bentzen 1994; Garcia de Leon et al. 1997). Also, analysis of microsatellites requires only minute amounts of DNA, hence tissue may be collected non-destructively (Wright and Bentzen 1994; Garcia de Leon et al. 1997).
Traditionally, allozyme electrophoresis and more recently, mtDNA were the most common techniques to analyze genetic differentiation at the population level (Allendorf and Phelps 1981; Adams et al. 1994; Varnavskaya et al. 1994; Burger 1997; Estoup et al. 1998). Allozyme markers are codominant, coding markers, exhibiting low amounts of variation (Jarne and Lagoda 1996; Hartl and Clark 1997). Mitochondrial DNA (mtDNA) is maternally inherited, and non-recombinant DNA; however it cannot reveal measures of heterozygosity, and may not reveal variation among populations (e.g., Lansmann et al. 1981; Wilson et al. 1985).

In recent years, the importance of microsatellite markers for population genetics has increased significantly (Poetsch et al. 2001). Microsatellite markers are widely used to compare and contrast closely related individuals and populations in a wide variety of species including plants (Lagercrantz et al. 1993), insects (Lehmann et al. 1998), mammals (Poetsch et al. 2001; Paetkau et al. 1995; Rosenbaum et al. 2000), and fish (Beacham et al. 2000; Heath et al. 2001; Hendry et al. 2002). In addition, microsatellites have been utilized for a variety of purposes, such as pedigree analysis (Estoup et al. 1998), gene mapping (Lindner et al. 2000), and population assignment (Olsen et al. 2000a), as well as for conservation and management of endangered or threatened species (e.g., Rosenbaum et al. 2000).

Project Goals

Spawning Klukshu sockeye salmon return to natal streams at two distinct times in a given year: the “early” run returns in the early summer months, the “late” run returns in the late summer and early fall. The main goal of this project is to genetically characterize
the bimodal distribution of the sockeye salmon population and examine the impact of bimodality on juvenile rearing ecology. I addressed the goals of this project using a combination of molecular genetic techniques, advanced genetic analysis, as well fish biology and aquatic ecology. The results represent a significant contribution to the general understanding of sympatric population interaction, habitat use, evolutionary ecology, and provide significant and timely management and conservation information.
CHAPTER 1.

Bimodal Run Distribution in a Northern Population of Sockeye Salmon

(*Oncorhynchus nerka*): Life History and Genetic Analysis on a Temporal Scale*

* Under review for Molecular Ecology
1.1 ABSTRACT

Life history variation and genetic differentiation were analyzed in two sympatric populations of sockeye salmon inhabiting Kluksu River, Yukon Canada over seven years (1994-2000). Sockeye salmon return to Kluksu River in two distinct runs, with a small "early run" in June-August, and a larger "late run" in August-September. A maximum likelihood test for clusters indicated that the return frequency distribution is bimodal in all years analyzed. Life history differences (fork length, sex ratio, age at maturity, fresh and salt water residency times) were found between the early and late runs; however, no consistent patterns were observed, making adaptive divergence unlikely. Analysis of variation at eight microsatellite loci showed the early and late runs are genetically differentiated in all years examined (F\textsubscript{ST} and Exact test). The variance between early and late run (2.27%) was twice the variance among years (1.16%) based on AMOVA. The Neighbor-Joining tree showed early and late runs generally cluster separately from one another, indicating higher gene flow among the early or late run fish across years relative to between-run gene flow. Two years did not fit the general clustering pattern: although the early and late runs in 1995 and 2000 were genetically differentiated, they clustered separately from the rest of the groups. No explanation for the anomalies is suggested; however, the two years are separated by the mean generation time for this stock (5 years), and thus parent/offspring similarity may be affecting the clustering. The data indicate that the runs are at least partially reproductively isolated due to temporal and/or spatial isolating mechanisms. Such reproductive isolation has important implications for conservation and management of the Kluksu sockeye salmon, and make them an evolutionarily interesting
group due to possible incipient sympatric speciation.
1.2 INTRODUCTION

Speciation is fundamental to our understanding of evolution, however the process is not well understood (Lynch 1989; Schluter 2001). Speciation may occur in allopatry, sympatry or parapatry (e.g., Mayr 1963; Bush 1994; Schluter 2000; Via 2001). Speciation in allopatry, where gene flow is prevented due to geographic isolation, is supported by a number of empirical studies in a range of organisms, including plants, amphibians, birds, and fish (e.g., Hillis et al. 1983; Johnson and Kurzweil 1998; Grant et al. 2000; Waters et al. 2001). Parapatric speciation is a special case of allopatry, since no range overlap exists between adjacent populations (Turelli et al. 2001). Empirical evidence for sympatric speciation, where no geographical barriers to gene flow exist, is less common, although some examples have been published (e.g., Bush 1994; Via 2001; Schliwein et al. 1994). An important factor distinguishing sympatry from allopatry is the lack of barriers restricting gene flow, so populations breeding in different locations may still be in sympatry. Examples of sympatric speciation are uniquely valuable for studying speciation mechanisms, since it provides information on the role of ecological and temporal reproductive isolation (Taylor and Bentzen 1993; Schluter 2001).

Pacific salmon (genus Oncorhynchus) are particularly useful in the study of evolutionary processes in nature since many salmon populations, or "stocks", are genetically differentiated (e.g., Wilmot and Burger 1985; Varnavskaya et al. 1994), and thus may enable processes similar to those in the early phases of speciation. The ability of Pacific salmon to home to their natal streams with low straying rates (Forester 1936; Quinn 1993) re-enforces the geographic separation of populations and thus facilitates divergence due to
both selection pressures and genetic drift (Taylor 1991; Gustafson and Winans 1999). However, the mechanism of divergence among groups in sympatry is perhaps less straightforward. While variations in environmental conditions are likely, no external barriers to gene flow exist.

Genetic differentiation, as well as temporal, spatial, behavioral, and selective isolating mechanisms, has been observed between groups living in sympatry (e.g., Bernatchez et al. 1996; Taylor et al. 1996; Schluter 2001). Genetically differentiated sympatric ecotypes have been documented in Arctic charr (Salvelinus alpinus), rainbow trout (Oncorhynchus mykiss), lake whitefish (Coregonus clupeaformis), Atlantic salmon (Salmo salar), and sockeye salmon (Oncorhynchus nerka) (Verspoor and Cole 1989; Taylor 1995; Bernatchez et al. 1996; Taylor et al. 1996; Jonsson and Jonsson 2001). For example, when 24 co-occurring sockeye salmon and kokanee (lake resident nonanadromous sockeye salmon) populations were examined using minisatellite and restriction fragment length polymorphism (RFLP) data, genetic differentiation was identified in all cases (Taylor et al. 1996). The isolating mechanism driving such genetic divergence has rarely been conclusively identified, although temporal reproductive isolation is evident in some sympatric stocks (e.g., sockeye salmon and steelhead, Oncorhynchus mykiss). In such cases, different ecotypes return to spawn in their natal habitat at discrete periods with little overlap (e.g., Brykov et al. 1999; however see Wood and Foote 1996). Spatial or microhabitat differences may also be evident between sympatric ecotypes, separating groups based on water depth or habitat type, such as main stem versus tributary-spawning chinook (Burger et al. 1985), and coastal versus inland charr (Taylor et al. 2001). Behavioral traits may isolate ecotypes through both assortative mate and size selection (Foote and Larkin 1988;
Finally, natural selection may act to isolate populations (Schluter 2000; Hendry et al. 2000). Individuals may be isolated due to selection in three ways: First, selection may act against individuals that travel between multiple habitats. Second, selection may act against hybrids due to decreased fitness. Finally, selection may cause morphological differences leading to decreased mate attraction (Schluter 2000; Hendry et al. 2000; Hendry 2001).

Return time into natal spawning habitats varies among some populations of chinook. pink (O. gorbuscha), steelhead and sockeye salmon (Burger et al. 1985: Olsen et al. 2000a: Hendry et al. 2002: Woody et al. 2000), and such variation in return time may be heritable (e.g.. Smoker et al. 1998: Quinn et al. 2000). In addition to spawning time differences, life history trait differences (age and size at maturation) have also been reported (e.g., Woody et al. 2000: Burger et al. 1985: Smoker et al. 1994). For example, Smoker et al. (1994) found that pink salmon in Auke Creek, Alaska, were larger in the late run, relative to the early run in the same creek. Conversely, Woody et al. (2000) found the early run sockeye salmon from Nicola Creek, Alaska, were larger than the late run from the same creek. Such body size differences between runs may reflect adaptation to their natal or salt water environments (Taylor 1991), and/or the location and time that they spawn (e.g., Ricker 1972), and may explain the general lack of success of sockeye salmon introductions outside of their native environment (Helle 1981).

Sockeye salmon are native to the northwestern and northeastern shores of the Pacific Ocean (Burgner 1991). After spending 1-3 years in fresh water and 2-4 years in salt water, sockeye salmon return to natal habitats to spawn in one or multiple runs. Sockeye salmon from the Kluksu River, Yukon, Canada are known to return in two runs: an early
run in July-August and a second, larger, late run in August-September. Enumerated annually since 1976 by the Canadian Department of Fisheries and Oceans (DFO), life history data and tissue samples are available for mature fish from both runs. Recent declines in the number of sockeye salmon returning to Kluksu River has led to serious concern from managers and the many stakeholders.

This study was designed to examine sympatric groups of sockeye salmon in the Kluksu River for genetic and life history differences. The first goal was to determine if the sympatric early and late runs of sockeye salmon are genetically differentiated. The second goal was to determine if life history characteristics differ between the early and late runs. Finally, to ascertain whether temporal patterns (1994-2000) of genetic structure and life history differences are consistent across time. because temporal analysis is critical to ensure that the observed differences are not due to sporadic, or short-lived events. The results of this study have significance for both the study of sympatric reproductive isolation, as well as for the management and conservation of the Kluksu River sockeye salmon, and by implication, other populations with bimodal return distributions.
1.3 MATERIALS AND METHODS

1.3.1 Sampling

Kluhshu River is a tributary of the Alsek drainage basin, located approximately 80 kilometers southwest of Haines Junction, Yukon Territory (Fig. 1.1). Kluhshu River sockeye salmon were sampled at a weir located at the mouth of Kluhshu River (Fig. 1.1). Tissue samples (scales or adipose clips), as well as life history data were collected for Kluhshu River sockeye salmon from June until the middle of October over a seven-year period: 1994, 1995, 1996, 1997 (scales), 1999 and 2000 (adipose clips). No samples were taken in 1998. All scale samples were air-dried and stored in scale books while adipose fin clips were preserved in 95% ethanol. Samples utilized for genetic analysis were chosen from within the peak of the early run (July 1-August 1) and the peak of the late run (September 1-October 1) to ensure minimal potential overlap and thus accurately characterize the runs (Phelps et al. 1994). Sex, based on secondary sexual characteristics (see Burgner 1991), and fork length, measured from snout to fork of caudal peduncle, were recorded for each sampled fish. In addition, total age of mature fish and age at salt water migration was determined by scale analysis (Department of Fisheries and Oceans aging lab: Nanaimo, B.C., Canada).

1.3.2 DNA extraction and microsatellite analysis

A modified proteinase K and phenol: chloroform: isoamyl alcohol (PCI) DNA extraction protocol was utilized (Devlin et al. 1991). Briefly, scale and adipose fin tissue were digested in 475μl of proteinase K buffer (10mM Tris-HCl (pH 8.0). 15mM
ethylenediaminetetraacetic acid (EDTA) (pH 8.0), and 0.5% sodium dodecylsulfate) with 25ul of proteinase K (10ug/uL), and incubated at 37°C with gentle mixing for 8 hours. The samples were extracted (twice for tissue samples) with PCI and precipitated with 0.1 volumes of sodium acetate (3.0M), and 0.6 volumes of isopropanol. The DNA pellet was
washed in 70% ethanol, dried and resuspended in 50ul of TE buffer (1M Tris-HCl (pH 8.0), 0.5M EDTA (pH 8.0)).

Allele variation at eight highly variable microsatellite loci was examined: Oneu2, Oneu8, Oneu18, One108, One115, Ots3, Ssa85, and usat60 (Estoup et al. 1993; O’Reilly et al. 1996; Scribner et al. 1996; Banks et al. 1999; Olsen et al. 2000b). Microsatellite polymerase chain reactions (PCR) were performed in 15.0 uL volumes: 0.8uL of template DNA, 10.95uL ddH2O, 0.9uL 25mM MgCl2, 1.5uL PCR buffer (0.1M Tris-HCl, 0.5uL KCl, 0.025M MgCl2, 0.5ug/uL bovine serum albumin), 0.3uL of dNTP (10mM each dNTP), 0.2uL dye-labeled primer (15mM), 0.3uL non dye-labeled primer (15mM), and 0.25 U of Taq polymerase (Gibco- BRL). A standard thermocycler protocol on a PTC100 thermocycler (MJ Research, Massachusetts) was followed: 2 minute denaturation cycle (94°C), followed by 34 cycles of: 1 minute denaturation (94°C), 1 minute annealing and a 1.5 minute extension (72°C); and a final 5 minute extension (72°C). Annealing temperatures were optimized for use with Kluksu River sockeye salmon DNA: Oneu2, Ssa85, One108, & One115 (56°C); Oneu8 (58°C); Oneu18 & usat60 (53°C), and Ots3 (50°C).

PCR products were loaded onto a 6% polymerized acrylamide gel and analyzed for fragment size (+/- 0.5 bp) using an automated DNA sequencer (Visible Genetics, Toronto). Approximately 5% of all PCR reactions were replicated to determine repeatability.

1.3.3 Run timing bimodality

Bimodality was tested for using a maximum likelihood test for clusters (Engleman and Hartigan 1969), based on the complete sockeye salmon enumeration data for all years sampled. The null hypothesis is that the population returns in a normal distribution across
return dates, while the alternative hypothesis partitions the population into a bimodal return pattern.

1.3.4 Life history differences

Life history attributes (size and age at maturity, sex ratio) were compared between the early and late runs. A one-way ANOVA was used to test for differences in fork length between the runs for all years. Sex ratios were compared using a two-way crosstab chi-square analyses for all years. Age at maturity and fresh and salt water residency times were compared using two-way crosstab chi-square analyses for all years.

1.3.5 Population structure

All genetic analyses were performed using ARLEQUIN version 2.0 (Schneider et al. 1997) unless otherwise stated. An exact test for goodness of fit to Hardy-Weinberg equilibrium was conducted for all loci within each group, and among all years using the Monte Carlo method (total 20,000 permutations). The results of the Hardy-Weinberg test were adjusted for significance using the Bonferroni correction factor (Rice 1989), to account for multiple, simultaneous tests.

The degree of population substructure was estimated by calculating pairwise $F_{ST}$ (Weir and Cockerham 1984). An exact test for differences in allele frequencies among groups was completed (20,000 permutations; Raymond and Rousset 1995) using Tools for Population Genetic Analyses (TFPGA 1.3) (M. Miller, Department of Fisheries and Wildlife: Utah State University; 5210 Old Main Hill, Logan Utah, 84322-5210, USA). Significance levels were subsequently adjusted using the Bonferroni correction method.
(Rice 1989). Analysis of molecular variance (AMOVA) was performed to partition observed variance into run timing (early versus late) component, sample year component, and among individuals component, as described in Excoffier et al. (1992). An unrooted Neighbor-Joining cluster analysis was performed with Cavalli-Sforza and Edwards (1987) chord distance using Populations Version 1.2.14 (O. Langella. Centre National de la Recherche Scientifique, Laboratoire Populations. Génétique et Evolution. Gif sur Yvette: www.cnrs-gif.fr/puc/biinfo/populations) and Treeview (Page 1996). The resulting tree was bootstrapped among loci, with replacement, for 1000 permutations.
1.4 RESULTS

1.4.1 Return distribution

Cumulative counts of returning Klukshu River sockeye salmon (1994-2000) exhibit considerable variation, with total returns of approximately 20,000 in 1995 and 5,000 in 1999 and 2000 (Fig. 1.2). Return frequency distributions were significantly bimodal (p<0.001) indicating the presence of an early and late run in all years examined. The maximum between to within sum of squares ratio, which indicates the most likely division date between the two modes (i.e. early versus late run), ranges from August 11th in 1995 to Aug 28th in 2000 (Fig. 1.2).

1.4.2 Life History

The fork lengths of the early and late run sockeye salmon differ significantly in all years except 1995 and 1999 (Table 1.1). The late run had a larger mean fork length in 1994, 1996, and 2000; however, in 1997 the early run was significantly longer than the late run (Table 1.1). Sex ratios differed significantly between the early and late run in 1994, 1996 and 1999. The early run contained a larger proportion of males, likely due to the fact that males may return earlier than females (Morbey 2000: Table 1.1). However, no significant differences were observed in 1995, 1997 and 2000.

The age at maturity (1994-2000) ranged from three to six years of age, with significant differences in age distribution between the early and late runs in all years except 1995 and 2000 (Table 1.2). Overall, the late runs of 1994 and 1996 contained a higher proportion of age 5 mature fish relative to the early run, while in 1997 and 1999 the early
Figure 1.2. Cumulative numbers of returning Kluksu River sockeye salmon shown for each year sampled (1994-2000). Early and late run return distributions were defined using Engleman and Hartigan’s (1975) test for clusters, and the date of maximum likelihood separation of the two modes is identified.
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### Size at First Maturity (mm) and Sex Ratio

Table 1.1. Sex Ratio (percent) and fork length (mm) of early and late run sockeye salmon returning to Kluxshu River (1994-2000). Significant differences between early and late runs are denoted by p-value and non-significant values are denoted with NS.
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With NS salmon (1994—2000), significant relationships are denoted with a p-value, while non-significant values are denoted.

Table 1.2: Age at maternity, years in freshwater, and years in saltwater for early and late run Kuskus River sockeye.
runs contain a larger percentage of 5 year olds than the late runs (Table 1.2). The
distribution of the fresh water residency times in the early and late run fish included mainly
two years, and with the exception of 1994, did not differ between the early and late runs
(Table 1.2). The elevated proportion of one-year fresh water residency times in the 1994
early run may have been due to unusual environmental conditions in that year; interestingly,
the 1999 early and late runs (mostly offspring of the 1994 spawners) experienced a large
reduction in population numbers (see Fig. 1.2). The distribution of the salt water residency
times in the early and late run fish showed significant differences in 1994, 1996, 1997 and
1999 (Table 1.2). Elevated proportions of three year salt water residency times were found
in the early run of 1994, 1997, and 1999, while in 1996 the late run exhibited the higher
proportion of three year residency times (Table 1.2), suggesting possibly divergent habitat
utilization. No significant differences were observed in the salt water residency time
distributions for 1995 and 2000 (Table 1.2).

1.4.3 Population Structure

The eight microsatellite loci used for population structure analysis (1994- 2000)
showed high levels of variation (13-39 alleles: Table 1.3), and 15 out of the 96 tests showed
significant deviation from Hardy-Weinberg equilibrium after Bonferroni correction; all due
to a deficiency in heterozygotes (Table 1.3). This may be attributed to null alleles, Wahlund
effects among age classes (see Waples and Teel 1990), selection, or possibly assortative
mating. However, there was no apparent pattern to the deviation from H-W equilibrium
among loci. run. or year. nor was there any association between departure from Hardy-
Weinberg equilibrium and the number of individuals that failed to amplify, i.e. putative
Table 1.3. Number of alleles (A), sample sizes (N), and observed and expected heterozygosity (H₀ and Hₑ) at eight microsatellite loci for early and late run Kluksu River sockeye salmon (1994–2000). Significant deviations from Hardy-Weinberg equilibrium are in bold.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Early</th>
<th>Late</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oneu2</td>
<td>20</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Ho</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>0.7</td>
</tr>
<tr>
<td>Oneu8</td>
<td>22</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Ho</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>0.81</td>
</tr>
<tr>
<td>Ots3</td>
<td>16</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Ho</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>0.6</td>
</tr>
<tr>
<td>Oneu18</td>
<td>13</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>A</td>
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<tr>
<td></td>
<td>Ho</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>0.66</td>
</tr>
<tr>
<td>usat50</td>
<td>13</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Ho</td>
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</tr>
<tr>
<td></td>
<td>HE</td>
<td>0.75</td>
</tr>
<tr>
<td>One108</td>
<td>27</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Ho</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>HE</td>
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</tr>
<tr>
<td>Ssa85</td>
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<tr>
<td></td>
<td>A</td>
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<td>One115</td>
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<tr>
<td></td>
<td>A</td>
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<tr>
<td></td>
<td>Ho</td>
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</tr>
<tr>
<td></td>
<td>HE</td>
<td>0.95</td>
</tr>
</tbody>
</table>
null allele homozygotes (Table 1.3).

Significant $F_{ST}$ for all pairwise comparisons of early and late runs in 1994-2000 were found, with the exception of 1995 (Table 1.4), after correction for multiple simultaneous comparisons (as above; Rice 1989). The $F_{ST}$ values, reflecting gene flow among runs and years, generally increase as time interval increases (Table 1.4). Exact tests for differences in allele frequency between groups were significant for all groups and years after Bonferroni correction factor ($p < 0.001$; Table 1.4). Analysis of molecular variance (AMOVA) between early and late runs, years, and among individuals, showed greater variance explained by run ($2.27\%$; $p < 0.001$; Table 1.5) than by year ($1.16\%$; $p < 0.015$; Table 1.5). The Neighbor-Joining tree shows divergence among the early and late runs as well as among years (Fig. 1.3), with low bootstrap values. The early and late runs for 1995, 1996, 1997, and 1998 cluster by early versus late, rather than by year; however, the 1995 and 2000 years cluster together (although the early & late runs are still divergent; Fig. 1.3).
<table>
<thead>
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<tbody>
<tr>
<td>Lane</td>
<td>Lane</td>
<td>Lane</td>
<td>Lane</td>
<td>Lane</td>
<td>Lane</td>
<td>Lane</td>
<td>Lane</td>
</tr>
<tr>
<td>Early</td>
<td>Early</td>
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<td>Early</td>
<td>Early</td>
<td>Early</td>
<td>Early</td>
<td>Early</td>
</tr>
</tbody>
</table>

Values are significant according to the F test (F > 0.05). Excluded those denoted in bold form (p = 0.05).
Table 1.5. Analysis of molecular variance (AMOVA) for microsatellite allele frequencies among years (1994-2000), between early and late runs, and among individuals in Kluksu River sockeye salmon.

<table>
<thead>
<tr>
<th>Variance Component</th>
<th>d.f</th>
<th>Variance Component</th>
<th>% Total variance</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among years</td>
<td>5</td>
<td>0.037</td>
<td>1.16%</td>
<td>P &lt; 0.015</td>
</tr>
<tr>
<td>Between early vs. late</td>
<td>6</td>
<td>0.072</td>
<td>2.27%</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Within samples</td>
<td>1160</td>
<td>3.089</td>
<td>96.57%</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Total</td>
<td>1171</td>
<td>3.198</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1.3. Un-rooted Neighbor-Joining tree for early and late run Kluksu River sockeye salmon (1994-2000) based on Cavalli-Sforza and Edwards (1987) chord distance, calculated using eight microsatellite loci. The data were bootstrapped 1000 times with replacement over loci.
Kluksu River sockeye salmon migrate to natal habitats in a bimodal pattern that is consistent over time (1994-2000). Furthermore, genetic analysis of the two modes (i.e. early and late runs) indicates significant genetic differentiation in all years sampled (1994-2000). Similar genetic divergence has been observed in other run timing genetic analyses (e.g., Nielsen and Fountain 1999; Wilmot and Burger 1985; Varnavskaya et al. 1994; Hendry et al. 2000; Hendry et al. 2002); however, few of those included temporal sampling over one generation or more (Lessios et al. 1994). AMOVA results support the genetic differentiation of the two runs. and, in fact, show that variance between runs was double that observed over time. This indicates that, while changes in allele frequency occur over time, a larger degree of differentiation exists between the early and late run fish. Thus, the results contrast with other studies that found more variation over time than among runs (e.g., Nielsen and Fountain 1999). The Neighbor-Joining tree, while generally supporting genetic divergence of the runs, had low bootstrap values, and the 1995 and 2000 sample years were identified as anomalies. In those years, the early and late runs are differentiated but do not cluster with other early and late run fish (see Fig. 1.3). Since the mean generation time is approximately five years, the two anomalous years (1995 and 2000) likely include parents and offspring. Although the data cannot be used to determine why these two years cluster separately from the rest of the samples, it is likely that the anomaly is propagated from generation to generation. Thus, it is predicted that the 2005 spawning generation will also exhibit a divergent allele frequency distribution. Nevertheless, the early and late run sockeye salmon in the Kluksu River are differentiated genetically in all years sampled and
may be reproductively isolated in ways that characterize the early stages of speciation, despite the lack of obvious geographic or physical barriers (Tessier and Bernatchez 2000; Vamosi et al. 2000).

Speciation is a process whereby populations become reproductively isolated and consequently diverge genetically due to evolutionary processes. Genetic differences between the early and late run Kluksu sockeye salmon indicate the two are at least partially reproductively isolated through temporal, spatial, and/or behavioral mechanisms, as well as selection against migrant and hybrid individuals. Temporal isolation between the runs is a likely mechanism, since the peak of the early and late runs are separated by 30-40 days (although some overlap does occur). Sockeye salmon returning early in the season simply may not have the opportunity to interbreed with the late run and vice-versa. Given the heritable nature of return timing (Quinn et al. 2000; Smoker et al. 1998), reproductive barriers could thus evolve (Quinn et al. 2000). Pacific salmon are known to be reproductively isolated among rivers due to accurate and precise natal migrations (e.g., Quinn et al. 1999) and, not surprisingly, geographically separated tributaries are often genetically differentiated. Spatial isolating mechanisms may also play a role in the reproductive isolation of the Kluksu early and late run sockeye salmon at the microgeographic scale. A radio-telemetry study of the early and late run sockeye salmon entering Kluksu River indicated that they may use different spawning habitat, with the early run fish spawning in stream/river habitat and the late run fish spawning in lake habitat (Petkovich 1999). Behavioral isolation between the runs, while possible, was not addressed in this study.
Once reproductively isolated, populations may be affected differentially by evolutionary processes such as genetic drift and selection, leading to genetic differentiation (e.g., Wright 1951; Slatkin 1981). Divergent selection is expected to primarily affect populations inhabiting clearly different environments, and hence geographically separated areas. It is thus unclear what role selection plays in the divergence of populations located in close geographic proximity or in sympathy (such as in the Kluksu system). Reproductively isolated populations will also genetically diverge by drift (Woody et al. 2000). The early run Kluksu sockeye salmon are typically at low population numbers, and undergo periodic extreme population bottlenecks (Fig. 1.4), factors that should accelerate drift. Thus, the potential exists for rapid genetic (microsatellite) divergence between the early and late run Kluksu sockeye salmon through genetic drift, as well as possibly selection.

![Graph](image)

Figure 1.4. Numbers of returning early and late run Kluksu River sockeye salmon from 1976-2000, based on counting weir enumeration by DFO, Canada.
One expected consequence of reproductive isolation is life history divergence accompanying genetic divergence. In salmon, divergent life history characteristics among populations may be due to adaptation for long and/or difficult migrations (Kinnison et al. 2001), as well as spawning habitat differences (Blair et al. 1993; Quinn et al. 2001). Variation in fresh and salt water residence times may be due to different fresh and salt water habitat usage patterns, genetic factors, or both (Ricker 1972; Peterman 1985; Bradford and Peterman 1987). However, adaptive arguments are not supported unless the life history differences are consistent over time. Life history characteristics (body size, sex ratio, fresh and salt water residence, and age at maturity) in the Kluksu sockeye salmon differed between the early and late runs, but not consistently among the sample years. Thus there is, as yet, no evidence for adaptive divergence for any of the life history traits examined in the Kluksu early and late run sockeye salmon. Woody et al. (2000) examined genetic and life history variation in sockeye salmon, reported body size differences between runs, and suggested the difference was adaptive; however, they based their conclusion on data from one sample year. If the 1996 samples had been analyzed, for example, the runs would have been divergent in sex ratio, fork length, age at maturity and salt water residency time. Clearly, analyses of life history differences among runs within a population should include temporal data, whenever possible, to minimize potential artifacts (e.g. demographic stochasticity and nonrandom sampling) and to ensure capture of dynamic population effects (Wood et al. 1989; Tessier and Bernatchez 1999).

Determining population genetic structure in fish is generally logistically difficult using traditional non-genetic approaches: however such information is critical for the effective management and conservation of fish stocks. Analysis of the Kluksu early and
late run sockeye salmon provide the evidence necessary for fisheries managers to justify implementing separate conservation strategies for the two runs. A cyclical pattern of population abundance is apparent in both the early and late runs. This is further complicated by a significant decreasing trend in return numbers in the late run (regression analysis; P<0.02; Fig. 1.4), while the early run maintains a very low population size with frequent severe population bottlenecks (Fig. 1.4). Although it is important that both the early and late runs be conserved, conservation efforts for the early run must be implemented aggressively. It is recommended to not only limit harvests, but also to conserve and preserve rearing habitat.

Kluksu River early and late run sockeye salmon were genetically differentiated in all years sampled, suggesting consistently low gene flow as well as the possibility that sympatric populations are evolving reproductive isolation similar to processes that promote speciation. Although the data show that life history characteristics such as age and size at maturity and fresh and saltwater residence times differed between runs; these differences were not consistent among sample years. Thus, no phenotypic evidence is reported for adaptive divergence or character displacement. Clearly, temporal data are recommended for the analysis of population structure using genetic or life history markers, especially when microevolutionary hypotheses are to be tested, or management recommendations are to be made.
CHAPTER 2.

Juvenile Rearing, Habitat Partitioning and Relative Productivity Ratio of Early and Late Run Sockeye Salmon (*Oncorhynchus nerka*) in Kluksu Lake
2.1 ABSTRACT

Kluksu River sockeye salmon return in a bimodal distribution in “early” and “late” runs that are genetically distinct, however it is unknown where sockeye salmon juveniles spend their freshwater residency and how the reproductive isolation may affect habitat partitioning. All major lake and river habitats in and around Kluksu River and Kluksu Lake, Yukon, Canada were extensively sampled for juvenile sockeye salmon to assess freshwater residency use. No sockeye salmon juveniles were collected outside of Kluksu Lake indicating that the lake is likely the sole rearing habitat. Juvenile sockeye salmon were captured at seven Kluksu Lake sites in two consecutive years, 2000 and 2001, while one river site was sampled in 2001. Fin clips were collected from the juvenile sockeye salmon and DNA was extracted for genetic analysis using eight microsatellite markers. An assignment test (WHICHRUN 4.1), based on a maximum likelihood function was utilized to designate juveniles as early or late run progeny. Adult early and late run microsatellite allele frequencies (1999 and 2000) were utilized to characterize the source populations, and juvenile individuals (2000 and 2001) were allocated, based on their allele profile, to the correct run. Both early and late run juveniles were present within Kluksu Lake, and while intermixing did occur, differing habitat preferences were exhibited in one sampling year. Late run juveniles predominate in Kluksu Lake at all sites, with the exception of Van Creek (site 8) in 2001, while early run juveniles were most numerous in the north and south (outlet) of Kluksu Lake. Using the number of early and late run juveniles and the number of early and late run parental fish, a relative productivity ratio was calculated, and tested for significant differences between runs for both years. Productivity ratios did not differ
significantly between early and late runs in 2000; however, a significant difference was found in 2001. This study was successful in determining juvenile habitat use using genetic techniques, the first such analysis to my knowledge.
Habitat partitioning (division of habitat), and consequently habitat utilization, has important consequences for ecology, management, conservation and evolutionary processes (e.g., Werner et al. 1977; Benson and Magnuson 1992; Tessier and Bernatchez 1999; Nielsen et al. 1999; Rosenfeld et al. 2000). Generally, systems that maintain a greater variety of habitats, such as diverse spawning habitat, cover, gradient, water depth, as well as variety and abundance of prey types, exhibit greater species richness (e.g., Bensen and Magnuson 1992). Complex ecosystems enable one or multiple species to occupy similar but various roles within one ecosystem. Partitioning of habitat use is one of the main reproductive isolating mechanisms in both terrestrial and aquatic ecosystems (Schoener 1974; Werner et al. 1977). Examples of habitat partitioning have been shown among species and populations within a species (e.g., Werner 1977). Bluegill sunfish (Lepomis macrochirus), largemouth bass (Micropterus salmoides), and black crappie (Pomoxis nigromaculatus) inhabiting Lawrence Lake, Michigan State, utilize similar freshwater habitat; however, they are partitioned due to differences in prey size between bluegill sunfish and largemouth bass, while the black crappie exhibits nocturnal foraging behavior (Seaburg and Moyle 1964). When populations of green sunfish (Lepomis cyanellus) and warmouth (L. gulosus) co-occur, their habitats are partitioned mainly due to depth preference (Werner et al. 1977).

Published studies have provided information on food and habitat partitioning in sympatric populations of Arctic charr, rainbow smelt, and sticklebacks as well as sockeye and kokanee salmon (Taylor and Bentzen 1993; Wood and Foote 1996; Vamosi and
Schluter 1999; Jonsson and Jonsson 2001). Sympatric but genetically differentiated populations of Arctic charr may coexist in freshwater due to partitioning of food resources, as well as differences in the location and time of prey capture (Schoener 1974; Keast 1978; Witte 1984). Sympatric benthic and limnetic sticklebacks are separated due to different preferred prey, depths, and spawning site preferences (McPhail 1993; Hatfield and Schluter 1996; Vamosi and Schluter 1999). Habitat partitioning also occurs between sympatric anadromous and nonanadromous ecotypes such as in rainbow smelt and sockeye salmon (Taylor and Bentzen 1993; Wood and Foote 1996). Rainbow smelt (Osmerus mordax) exhibit anadromous and nonanadromous forms within one watershed (Taylor and Bentzen 1993), utilizing saltwater versus freshwater habitat for the adult life stage. Within the nonanadromous smelt, dwarf and normal ecotypes are further separated based on their morphology, prey (planktivorous versus piscivorous); (Kendall 1927; Bajkov 1936), and spawning habitat and time (Lanteigne and McAllister 1983; Taylor and Bentzen 1993). Sympatric populations of sockeye salmon (Oncorhynchus nerka) and kokanee (nonanadromous sockeye salmon) are separated due to saltwater versus freshwater adult life stages, as well as spawning site preferences, and differences in peak spawning time within freshwater environments (Foote 1989; Wood and Foote 1996).

Pacific salmon (Oncorhynchus sp.) are typically anadromous and migratory, exhibiting complex community interactions and habitat partitioning in both freshwater and saltwater environments (e.g., Neave 1955; Godfrey 1965; French et al. 1976; Groot and Margolis 1991; Wood 1995). Within saltwater environments, geographically isolated populations utilize partially overlapping, but differentiated habitats. For example, central Alaska sockeye salmon stocks migrate further west than stocks from southeastern Alaska.
(French et al. 1976). The return of salmon populations to natal spawning grounds may include behavioral habitat partitioning; individual populations or stocks select different rivers or various sites within one river system, such as stream, tributary or lake spawning environments (Burger et al. 1985; Quinn 1999; Blair et al. 1993; Petkovich 1999). Run timing, stocks returning to natal streams in one or multiple time intervals, is also a form of habitat partitioning, and is exhibited in many Pacific salmon species, including pink (*O. gorbuscha*), steelhead (*O. mykiss*), chum (*O. keta*), coho (*O. kisutch*), chinook (*O. tshawytscha*) and sockeye salmon (Gribanov 1948; Burger et al. 1985; Tallman and Healey 1994; Olsen et al. 2000a; Woodey et al. 2000; Hendry et al. 2002; See Chapter 1). Fry (underyearling) habitat partitioning may be due to many factors including utilization of lake (e.g., sockeye salmon) versus river habitat (e.g., chinook salmon) as well as duration of freshwater residency among and between stocks (e.g., Burgner et al. 1991; Wood 1995). While data on habitat partitioning is available for adult fish in some sympatric but differentiated populations, little is known regarding the habitat use of the juvenile fish in such systems.

Habitat partitioning during juvenile freshwater residency is important to ensure access to adequate food supplies, temperature regimes, and for predator avoidance (Rosenfeld et al. 2000). This partitioning may occur due to the utilization of different rearing environments as well as varying durations of freshwater residency (Wood 1995). Some juvenile populations have been shown to amalgamate during freshwater residency, such as in rainbow trout (*O. mykiss*), coho (*O. kisutch*), Atlantic (*Salmo salar*), and chinook salmon (Quinn and Busack 1985; Olsen 1989; Brown and Brown 1992; Marshall et al. 2000). It has been shown that individual stocks may benefit from collective rearing.
through increased foraging opportunities (Quinn and Busack 1985: Olsen 1989), and
decreased predation, which is high during the freshwater life stage (Pella 1968). In some
coho salmon populations, related individuals have been shown to display cooperative
preference towards kin (Fontaine and Dodson 1999) and laboratory studies have
demonstrated that some individuals may prefer the chemical emanations (feces) of their
own population over other populations (Selest and Doving 1980: Groot et al. 1986: Folke
et al. 1992). However, in sympatric populations of kokanee and sockeye salmon, which
consume similar prey types and intermix within freshwater habitats, habitat partitioning is
exhibited (Wood et al. 1999). It is critical to understand juvenile habitat utilization and
partitioning to identify the extent of subpopulation habitat utilization, and to ensure rational
conservation via habitat protection in freshwater environments.

Estimating population fitness is important for conservation; however, it is
technically difficult to do. Fitness components such as growth rate, developmental
stability, viability, fecundity, and resistance to environmental stress (e.g., Mitton and Grant
1984: Allendorf and Leary 1986) have all been used to infer the contribution of a
population. An alternative approach is to examine the relationship between the numbers of
adults versus one-year-old juveniles (recruits). This study refers to the number of early and
late run adults versus recruits as the relative productivity, or contribution, of each run.
Typically, recruitment estimates are compared between years, or between various
geographic locales (e.g., Fogarty et al. 2001). However, recruitment estimation becomes
increasing difficult when multiple stocks or runs inhabit one river and no morphological
characteristics can be utilized to differentiate between the populations. This study was able
to compare the relative productivity of two sympatric sockeye salmon populations using molecular genetic markers and population demographic data.

This study was designed to address three main questions. First, to determine critical rearing habitats for Klukshu River sockeye salmon juveniles. Second, to ascertain if the early and late run juveniles are intermixed, and if so, whether their distribution is random, or does one type predominate at specific sites. Finally, to determine the relative productivity of the early versus late run. These questions were addressed using a novel application of molecular population genetics based on microsatellite genotypes of parent and offspring generations, combined with a maximum likelihood function analysis.
2.3 METHODS AND MATERIALS

2.3.1 Study System

Kluksu River and Kluksu Lake are located approximately 400 km southwest of Whitehorse, Yukon (60°N and 137°W; Fig. 2.1). Kluksu River supports sockeye salmon returns ranging from 5,000-30,000 fish annually (Department of Fisheries and Oceans 2000). Sockeye salmon migrating to Kluksu River return at two times, prior to and after August 15th, termed early and late runs (See Chapter 1). Early and late run spawning occurs mainly in the upper tributary of Kluksu River and within Kluksu Lake respectively (Petkovitch 1999). The Kluksu system also supports populations of chinook (O. tshawytscha), coho (O. kisutch), whitefish (Coregonus clupeaformis), and sculpins (Cottus sp.)

Figure 2.1 A map depicting the extensive sampling locations (A-H), and juvenile DNA collection sites (1-8), in and around Kluksu River and Kluksu Lake, Yukon, Canada.
2.3.2 Nursery Habitat

In order to identify sockeye salmon rearing habitats, eight lake and river sites were sampled during July-August 2000 and in June-July 2001 (Fig. 2.1), using two capture methods. Minnow traps were set in slow to medium moving river habitats, and in lake habitat ranging in depth from 30 cm to 1.5 meters. Minnow traps were collected and checked for the presence of sockeye salmon after a period of 12-24 hours. A seine-net was also used at the same site within shallow pools and slow to medium moving water, and all sockeye salmon fry captured were noted. All sites sampled in 2000 field season were resampled in summer 2001 (Fig. 2.1).

2.3.3 DNA Collection

Kluksu Lake sampling sites were chosen based primarily on the presence of intermittent or year-round water inflow. Seine-net collection was most successful, and approximately 50 juvenile sockeye were sampled per site (range 28-56), at seven sites, in two consecutive years (2000 and 2001). A small amount of caudal fin was removed from each age one sockeye fry (Fig. 2.1). The possibility of misidentifying two-year-old fish was minimal, as two-year-old fish were much larger. Following capture, juvenile sockeye were released unharmed and all fin samples preserved in 95% ethanol for subsequent DNA extraction.

At the most northern sampling site (Fig. 2.1; site 1), bottom substrate is very soft and muddy, with medium year-round inflow from Little Kluksu River. Rocky shores are further south, and no rearing habitat was present until the next sampling site (Fig. 2.1; site 2). In the northwest, sites (Fig. 2.1; site 2 and 3) are characterized by intermittent water
runoff and medium to large gravel substrate. In the northeast, (Fig. 2.1; site 4) the site is characterized by intermittent flow, shallow habitat and small and fine gravel substrate. The southwest site (Fig. 2.1; site 5) contained intermittent flow, possibly significant ground water runoff, small to medium gravel substrate, and narrow littoral zones prior to rapid increases in depth. The southern sampling locations (site 6) is shallow, contained grassy vegetation, and soft substrate, while the mouth of Kluksu Lake (site 7) had small pebble substrate and is the barrier to fast moving water (Fig. 2.1).

Adult tissue samples were obtained from early and late run Kluksu River sockeye salmon in 1999 and 2000 and tissue samples used for genetic analysis were chosen from within the peak of the early run and peak of the late run (See Chapter 1). The adults sampled in 1999 and 2000 are the parent generation of fry sampled in 2000 and 2001.

2.3.4 DNA Extraction and Microsatellite Analysis

Juvenile sockeye salmon DNA was isolated using WIZARD genomic purification kits (Madison, WI). The adult DNA extraction protocol was a modified protease K and phenol: chloroform: isoamyl alcohol (PCI) technique, as described in chapter 1. Briefly, adipose fin tissue was digested in 475ul proteinase K buffer (10mM Tris-HCl (pH 8.0), 15mM ethylenediaminetetraacetic acid (EDTA) (pH 8.0), and 0.5% sodium dodecylsulfate) with 25ul proteinase K enzyme (10ug/uL), and incubated at 37°C with gentle mixing for 8 hours. The samples were extracted twice with PCI and precipitated with 0.1 volumes sodium acetate (3.0M), and 0.6 volumes isopropanol. The DNA pellet was washed in 70% ethanol, dried and rehydrated in 50ul of TE buffer (1M Tris-HCl (pH 8.0), 0.5M EDTA (pH 8.0)).
Juvenile and adult allele variation was examined at eight highly variable microsatellite loci. Microsatellite polymerase chain reactions (PCR) were performed as described in Chapter 1, and the same microsatellite loci were used. PCR products from the juvenile and parent individuals were loaded onto a 6% polymerized acrylamide gel and analyzed for molecular size (+/- 0.5 bp) using an automated DNA sequencer (Visible Genetics, Toronto). Approximately 5% of all PCR reactions were replicated to ensure repeatability.

2.3.5 Analysis

An exact test for goodness of fit to Hardy-Weinberg equilibrium was conducted for adult early and late run individuals in 1999 and 2000, using ARLEQUIN version 2.0 (Schneider et al. 1997), and adjusted for significance using the Bonferroni correction factor (See Chapter 1: Rice 1989). The degree of population substructure was evaluated for the 1999 and 2000 adults, by calculating pairwise $F_{st}$ using TFPGA (See Chapter 1). An exact test for goodness of fit to Hardy-Weinberg equilibrium was conducted at all loci, for the juvenile collections in both years (2000 and 2001) using the Monte Carlo method (total 20,000 permutations). The results of the Hardy-Weinberg test were adjusted for significance using the Bonferroni correction (Rice 1989).

An assignment test based on multilocus genotyping (WHICHRUN 4.1) was used to assign juvenile sockeye salmon to their early and late run source populations (Banks and Eichert 2000). The parent early and late run allele frequencies (1999 and 2000) were used to characterize the source populations, and a likelihood estimate calculated based on the probability the juvenile alleles originated from the source population. Likelihood estimates
were multiplied locus by locus to give a multilocus likelihood function. or probability that all 16 alleles occurred within the source population. Using a maximum likelihood format (P(n)/ P(max)), a value is assigned between 0 (least likely) and 1 (most likely). that the juvenile belongs to the source population (Banks and Eichert 2000). To ensure correct population assignment, parent allele frequencies were reevaluated using the jackknife option (WHICHRUN 4.1; Banks and Eichert 2000): adult individuals were removed from the baseline file one at a time and the allele frequencies recalculated in the absence of each genotype. Acceptable maximum likelihood values were chosen based on the stringency of parent reassignment to their original population, with less than 2% error of reassignment. WHICHRUN has previously been used to discriminate between populations of southern flounder along the Atlantic coast and Gulf of Mexico as well as between populations of chinook salmon in California’s Central Valley (Banks et al. 2000; Blandon et al. 2001).

To test for differences in juvenile distribution, the number of early and late run juveniles was compared among all seven sampling sites (site 1-7), using a two-way crosstab chi-square analysis in 2000, and in 2001. In addition, a crosstab chi-square analysis was used to test for significant year-to-year (2000 to 2001) change in the proportion of early and late run juveniles at each site.

The relative productivity of each run was determined by comparing the proportion of adults to the proportion of recruits in both the early and late run. To test for differences in the relative productivity between runs, the numbers of juveniles versus adults in each run were compared using a crosstab chi-square analysis in 2000, and 2001.
RESULTS

2.4.1 Genetic Variation

The eight microsatellites used for population structure analysis showed high levels of variation in the adults (13-39 alleles; See Chapter 1), and juveniles (9-45 alleles; Table 2.1). In 9 out of 240 juvenile tests, significant deviations from Hardy-Weinberg equilibrium after Bonferroni correction were observed (Table 2.1). All deviations were due to a deficiency in heterozygotes, with the exception of site 5 in 2000 at locus Usat60. In 15 of 96 adult tests, significant deviations from Hardy-Weinberg equilibrium after Bonferroni correction were observed: all due to a deficiency in heterozygotes (See Chapter 1). This experiment does not allow the determination of which is the likely mechanism, but deficiencies in heterozygotes may be attributed to null alleles, Wahlund effects among age classes (see Waples 1990), selection, or possibly assortative mating. We found significant differences in all pairwise Fst comparisons of adult early and late runs, evaluated in 1999-2000, after correction for multiple simultaneous tests (Table 2.2).

2.4.2 Juvenile Distribution

Percent juvenile assignment at the seven sites ranged from 50-77% success in 2000 (Avg. 65%) and 43-73% success in 2001 (Avg. 56%). The distribution of assignment is illustrated with pie charts for each of the seven lake and one river sampling sites, indicating the number of early, late and unidentified fry (Figure 2.2). Both the early and late runs utilize Kluksu Lake for rearing habitat, and are intermixed at
Table 2.1. Number of alleles (A), sample sizes (N), and observed and expected heterozygosity (H₀ and Hₑ) at eight microsatellite loci for juvenile Klukshu River sockeye salmon (2000 and 2001). Significant deviations from Hardy-Weinberg equilibrium are in bold.

<table>
<thead>
<tr>
<th>Locus</th>
<th>2000</th>
<th>2001</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Site 1</td>
<td>Site 2</td>
</tr>
<tr>
<td>Oneu2</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ho</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>Oneu8</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ho</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>Ots3</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ho</td>
<td></td>
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<tr>
<td></td>
<td>HE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>46</td>
</tr>
<tr>
<td>Oneu18</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td></td>
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<tr>
<td></td>
<td>Ho</td>
<td></td>
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<tr>
<td></td>
<td>HE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>50</td>
</tr>
<tr>
<td>Usat60</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ho</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>50</td>
</tr>
<tr>
<td>One108</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ho</td>
<td></td>
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<tr>
<td></td>
<td>HE</td>
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<td></td>
<td>52</td>
<td>49</td>
</tr>
<tr>
<td>Ssa85</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td></td>
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<tr>
<td></td>
<td>Ho</td>
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<td></td>
<td>HE</td>
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<td></td>
<td>52</td>
<td>50</td>
</tr>
<tr>
<td>One115</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ho</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>50</td>
</tr>
</tbody>
</table>
Table 2.2. $F_{ST}$ and Cavalli-Sforza Edwards ($D_e$) genetic distance between adult early and late run Klukshu River sockeye salmon (1999 and 2000), all values are significant ($p < 0.001$).

<table>
<thead>
<tr>
<th></th>
<th>Year</th>
<th>$F_{ST}$</th>
<th>$D_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early vs. Late</td>
<td>1999</td>
<td>0.021</td>
<td>0.313</td>
</tr>
<tr>
<td>Early vs. Late</td>
<td>2000</td>
<td>0.011</td>
<td>0.280</td>
</tr>
</tbody>
</table>

Figure 2.2. Map of the seven Klukshu Lake and one river sampling sites depicting the numbers of early run, late run, and unknown juveniles at each site (sites 1-8) using multilocus genotyping and a maximum likelihood function.
most sites, however frequency distribution differs significantly within the early and late runs within the seven sites (p<0.01) in 2000 and but not in 2001 (p = 0.160).

When tested for year-to-year differences at each site, little significant variation was found; however, site 2 showed significant year-to-year variation (p = 0.020) in the number early and late run juveniles, and at the river site (site 8), no juveniles were found in 2000.

2.4.3 Adult Relative Productivity

In total, 213 juveniles were successfully identified in 2000, (20 early and 193 late run), and 185 juveniles were identified in 2001 (26 early and 159 late run) (Table 2.3). Relative productivity estimates (proportion of parents versus juveniles) did not vary significantly between the early or late run in 2000 (p = 0.633), but in 2001 productivity estimates did differ (p < 0.001) (Table 2.3).

Table 2.3. Number and percent total escapement of early and late run Klukshu River spawners and the number and percent successfully identified early and late run fry (2000 and 2001). No significant differences between early and late run relative productivity was observed in 1999-2000. However a significant differences was noted in 2000-2000 (p=0.022).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early Run</td>
<td>Late Run</td>
</tr>
<tr>
<td>Adults</td>
<td>371 (7.5%)</td>
<td>4557 (92.5%)</td>
</tr>
<tr>
<td>Fry</td>
<td>20 (9.4%)</td>
<td>193 (90.6%)</td>
</tr>
</tbody>
</table>
2.5 DISCUSSION

Early and late Kluksu River sockeye salmon are genetically differentiated (see Chapter 1), similar to the divergence shown in sympatric runs of steelhead, chinook, pink and sockeye salmon (Burger et al. 1985; Gharett and Smoker 1991; Varavskaya et al. 1994; Nielsen et al. 1999; Woody et al. 2000; Hendry et al. 2002). However, relatively little is known about the ecology or population genetics of the juveniles in such systems. One of this project's goals was to determine juvenile rearing habitat of Kluksu River sockeye salmon. Consequently an important result was that both the early and late run juveniles utilize Kluksu Lake as their primary rearing habitat. Sockeye salmon juveniles typically inhabit lake environments for 1-3 years (Burgner 1991; Pella 1968; Blair et al. 1993), however variations to this rule exist, such as tributary freshwater residency or decreased freshwater residency times (Burgner 1962; Bugaev 1983). Kluksu River sockeye salmon remain in freshwater for 1-3 years (Table 2.4). No juvenile sockeye salmon were found in tributary environments, with the exception of site 8 in 2001. It is not surprising that the early and late run juveniles utilize the same freshwater lake; other studies have shown multiple populations of beach and stream spawning sockeye salmon juveniles within the same lake, as well as sympatric populations of anadromous and non-anadromous sockeye salmon juveniles in the same rearing lake (Blair et al. 1993; Wood et al. 1999). Kluksu River early and late run adults are genetically differentiated, as well as temporally and spatially isolated. However early and late run juveniles appear to utilize the same rearing habitat. On the other hand, it is unknown if the early and late run juveniles are randomly distributed or segregate to some degree within the lake environment.
Table 2.4. Freshwater age distribution of early and late run spawning sockeye salmon 
(*Oncorhynchus nerka*) returning to Kluksu River (1999 and 2000).

<table>
<thead>
<tr>
<th>Year</th>
<th>Run</th>
<th>N</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>Early</td>
<td>140</td>
<td>0</td>
<td>140 (100.0)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>65</td>
<td>1 (1.5)</td>
<td>63 (97.0)</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>2000</td>
<td>Early</td>
<td>61</td>
<td>4 (6.6)</td>
<td>57 (93.4)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Late</td>
<td>91</td>
<td>0</td>
<td>91 (100.0)</td>
<td>0</td>
</tr>
</tbody>
</table>

The partitioning of habitat by adult Kluksu River sockeye salmon is well characterized (Chapter 1): early and late sockeye salmon are temporally and spatially reproductively isolated and experience limited gene flow. Juvenile Kluksu Lake sockeye salmon exhibited habitat partitioning in July-August (2000), but not in June-July (2001). These inconsistent results may be due to differences in the statistical power of juvenile identification between years, temporally transient juvenile habitat preferences, and changes in spawning site preferences of adults. Independent of the inconsistency, the 2000 data represent clear evidence that habitat partitioning in Kluksu Lake early and late run juvenile occurs in some years. Different species living in sympatry have been shown to have overlapping, but differentiated, habitat use, such as in populations of juvenile dolly varden and bull trout in Thutade Lake, BC; juvenile chinook and steelhead salmon in Bridge River, BC; and juvenile sockeye and kokanee salmon in Takla Lake, BC (Hagen
and Taylor 2001; Bradford and Higgins 2001; Wood et al. 1999). A number of mechanisms for juvenile fish habitat partitioning in freshwater ecosystems has been proposed: a) habitat preferences by the juveniles and/or spawning adults. b) competition among individual fry. and c) increased survival and growth (Pella 1968; Burgner and Rogers 1963; Seppä et al. 2001). Juvenile habitat partitioning may be correlated to where the parents spawn, suggesting that juveniles may remain in close proximity to their emergence habitat (Burgner and Rogers 1963; Wood et al. 1999). However, because the majority of early run adults utilize tributary environments for spawning (Petkovich 1999), the migration of early run juveniles from the tributary spawning grounds into Klukshu Lake complicates such a model. Sockeye salmon juveniles inhabiting or entering Klukshu Lake may actively select rearing sites, such as sockeye salmon populations in Lake Aleknagik, Alaska (Pella 1968). In addition, habitat partitioning in Klukshu Lake may result from fitness related choice; populations composed of familiar (individuals using similar environments during embryonic or early life development), versus unfamiliar Arctic Charr showed increased survival, nutritional status, body size and experienced less aggression, than individuals in unfamiliar groups (Seppä et al. 2001). Therefore, juvenile fish may tend to associate with more familiar individuals to maximize their fitness (Magurran et al. 1994; O’Connor et al. 2000). Competition between the runs may also influence Klukshu Lake early and late run habitat partitioning; inter- and intra-specific competition related to changes in population density has been shown to influence habitat partitioning in other systems (Pella 1968; Wood et al. 1999; Essington et al. 2000). Juvenile early and late run Klukshu River sockeye salmon appear to utilize different habitats. however the relative contribution of the early and late runs to the cohort is not known.
Populations inhabiting different environments may exhibit different levels of survival or fitness. For example, fry to adult sockeye salmon survival in Shuswap Lake and Quesnel Lake was 5.6% and 14.8% respectively (Hume et al. 1996; Beacham and Murray 1990). Recruitment estimates vary over time, among species and stocks (e.g., Rothschild 2000). The combined factors of fecundity and offspring survival suggest that populations may contribute varying numbers of offspring to the population. We developed a non-destructive genetic technique to estimate the relative juvenile contribution of the early and late run Kluksu sockeye salmon. These estimates were based on the number of spawning adults and the number of resulting identified early and late run juveniles. The relative error associated with the productivity estimates is difficult to estimate, however they provide an important comparison between the runs. Although the early and late runs migrate at different times and utilize different spawning habitat (Chapter 1), the relative juvenile contribution of the subpopulations were remarkably similar in 2000, but differed significantly in 2001. The difference in 2001 may be due to changes in mean fecundity in the runs, however life history data (fork length) do not support this (See Chapter 1). The early run contributed more offspring relative to 2000, however the late run had a statistically larger fork length. The change in juvenile contribution is likely due to differences in fry survival and viability. Many factors may affect fry survival and viability such as food availability and environmental conditions within the emergence habitat (Brannon 1987; Beacham and Murray 1987; West and Larkin 1987). It is interesting to note that the relative productivity of the runs appears to change in relation to the number of spawners entering the spawning grounds. When the number of late run spawners increased from 2000 to 2001, the relative productivity of that population decreased. In the early run
when the number of spawners decreased in 2000 to 2001, the relative productivity increased. This suggests the possibility of a density-related limiting factor in the Kluksu River system, perhaps optimal spawning-or rearing-habitat limitations.

Many studies have stressed the importance of protecting runs that utilize different spawning habitats or exhibit unique characteristics (Brannon 1987; Wood 1995; Spruell et al. 1999; Waples et al. 2001). Kluksu Lake is unique and must be preserved due to its reproductively isolated sockeye salmon runs, differences in relative productivity between the runs, and due to the partitioning of early and late run juveniles. Furthermore, it is important to preserve Kluksu Lake sockeye due to their cultural, economic and recreational value to the many stakeholders involved. Since Kluksu River early and late run juveniles utilize Kluksu Lake as their primary rearing habitat, the protection and management of rearing habitat can be focused on Kluksu Lake. Managers can target the early run, which are more susceptible to extinction (Wilmot and Burger 1985), for special conservation action because they exhibit distinct habitat preferences for spawning and, at least partially, for juvenile rearing. The Canadian Department of Fisheries and Oceans is considering enhancement of the Kluksu sockeye salmon population through adding juveniles to the lake system. The results of this study can be used to improve such stocking initiatives, by characterizing subpopulation habitat utilization, and thus identifying optimal sites for introduction. Thus, the results of this study may serve to increase freshwater juvenile survival and population recruitment (Koenings and Burkett 1987; Kocik and Ferreri 1998). Furthermore, this study’s methodology has applications for other tributaries where sympatric populations cannot be distinguished morphologically (e.g., Wilmot and Burger 1985; Rosenfeld et al. 2000). This study describes a novel molecular genetic
approach for habitat determination and relative population productivity estimation, and thus provides valuable data on the ecology, evolution, conservation, and management of Kluksu River salmon populations.
CONCLUDING REMARKS AND RECOMMENDATIONS

Previous researchers have observed that Kluksu River sockeye salmon return at two different times (Department of Fisheries and Oceans 2000), yet it was unknown how the two runs interacted, nor the impact divergent run timing may have had on the juvenile life stages. Adult sockeye salmon from the two runs were shown to be genetically differentiated; so different that the juveniles within one lake environment were assigned to their source early and late run population with 57% assignment success. While Kluksu Lake is the primary rearing habitat for Kluksu sockeye salmon fry, and both the early and late run juveniles were intermixed, their distribution was differentiated in one year.

The two chapters of this thesis can be linked in several ways, however both individually contribute to our knowledge of sockeye salmon biology, genetics and ecology. The first chapter examined sympatric speciation, particularly genetic and life history characteristic differences between adult early and late run sockeye salmon. The second chapter examined concepts in juvenile habitat use and partitioning between early and late run fry. While the chapters examine different life stages, both chapters focus on sympatric speciation, the mechanisms of genetic differentiation and both contribute to the overall understanding of sockeye salmon life history and consequently to the management of this system.

Sympatric, reproductively isolated populations are thought to be in the early phases of speciation, and are likely undergoing processes similar to sympatric speciation (Hendry et al. 2000: 2002). However, no evidence was available on the genetic, life
history or habitat utilization of the sympatric early and late Kluksu River sockeye salmon. This thesis provides evidence for limited gene flow between the Kluksu River sockeye salmon runs, and shows that the genetic separation was consistent over time. Genetic variance observed between the early and late runs was greater than the variance observed over time. While the sympatric populations are isolated during the adult life stage, published data suggested that juvenile sympatric sockeye salmon runs may intermix while in freshwater rearing environments (Varnavskaya et al. 1994: Quinn et al. 1999). This thesis provides evidence that isolation not only occurs during the adult life stage, but during the juvenile life stage as well, despite physical intermixing in the lake rearing habitat.

Habitat partitioning exists between the sympatric populations of Kluksu River sockeye salmon. Early and late run adult Kluksu River sockeye salmon are spatially and temporally separated (with some overlap). However, little information is available on habitat partitioning in early and late juvenile sockeye salmon. Genetic techniques were helpful to determine differences in habitat use and while the early and late run juveniles intermix, habitat partitioning was observed in one of the sampling years. The inconsistencies between years may be due to statistical limitations, or possibly changes in spawning and rearing sites over time. Therefore, the results of this study emphasize the need for temporal studies in population genetics and for management and conservation. Habitat utilization, whether different or not, provides important information for understanding the ecology and evolution of this system, and for effective management and future stock enhancement.
Management of Kluksu River sockeye salmon is complex with multiple organizations involved throughout the life stages, including Canadian Department of Fisheries and Oceans, Champagne and Aishihik First Nations, and Alaska Department of Fish and Game. In addition, multiple stakeholders such as commercial, aboriginal and sport fishers in the United States as well as sport and aboriginal fishers in Canada depend on this resource. This study provides a step forward in the understanding of Kluksu River sockeye salmon, specifically that the population is not composed of one run, but two genetically differentiated runs. This stresses the need for separate management units with stock allocation and escapement quotas set independently for each run.

Contributions to Science

1) Sockeye salmon life history

Kluksu River sockeye salmon exhibit bimodal return times and are partially isolated due to spawning time and location. Early and late run juvenile sockeye salmon inhabit the same rearing habitat, however habitat partitioning is observed.

2) Sympatric speciation

Sympatric early and late sockeye salmon are genetically differentiated in all years examined; however they do not exhibit consistent differences in life history.

3) Temporal studies

Ecological life history data (fork length, age at maturity etc..) provide conflicting life history data over a temporal scale, thereby stressing the need for temporal studies to ensure trends in data are consistent.
4) Juvenile rearing habitat usage

Both early and late run Kluksu sockeye salmon utilize the same freshwater rearing lake, enabling more focused conservation efforts.

5) Habitat partitioning

Habitat partitioning is exhibited within a sympatric population of juvenile sockeye salmon, thus allowing the possible targeting of run-specific juveniles for conservation and management.

6) Relative productivity of sympatric populations

The relative productivity of the early and late runs was significantly different in one of the two sampling years. In addition, productivity estimates suggest a possible density-dependent limiting factor within the Kluksu system.

Future research on Kluksu sockeye salmon

Recommendations for future research include examining the key issues of reproductive isolation between early and late run Kluksu River sockeye salmon. The first recommendation would be to examine the degree of temporal overlap. Adults from the early and late run overlap could be sampled and using genetic techniques assigned to a source population. The results could be used to determine the degree of early and late run temporal overlap. Second, to examine behavioral differences between the runs, adults could be sampled during the peak of each run, as well as from the overlap between the runs. Artificial spawning channels could be used to examine sexual selection behavior and determine differences between the two. Finally, to gain additional data on the nature of reproductive isolation between runs, a radio-tagging study could be
completed on a large number of sockeye salmon. from the peak of each run and in the
temporal overlap. The individuals could be tracked for the entire season and thereby
determine complete spawning habits of each run.

Recommendations for management and conservation of Kluksu sockeye salmon

1) Manage Kluksu sockeye salmon as two separate units: the early and late runs are
   clearly reproductively isolated over time and must be managed as such.

2) Continue monitoring the population size; Kluksu River sockeye salmon populations
   are small and stochastic. especially the early run. The need for continued population
   monitoring by the DFO counting weir is important for the small early run as well as
   the declining late run to ensure minimum escapement levels and to close sport and/or
   aboriginal fisheries when minimum levels have not been met.

3) Conserve Kluksu Lake rearing habitat; Kluksu Lake is the primary rearing habitat
   for this system, while the chance of targeting each of the runs for habitat protection is
   high. conservation of the entire lake system is important for both spawning and
   rearing habitat.

4) Determine Kluksu Lake site-specific habitat characteristics: Conduct detailed habitat
   surveys of the lake and river sampling sites to determine exact habitat properties.
   Such data can contribute to the understanding of required and preferred juvenile
   habitats as well as for potential habitat regeneration projects.

5) Effective population size (Ne); Using genetic methods determine the effective
   population size (the number of individuals contributing to the gene pool) to better
   understand spawning interactions and to establish minimum population escapements.
References Cited


Bugaev VF (1983) Spatial structure of the sockeye salmon *Oncorhynchus nerka* population in the basin of Kamchatka River. Translation from Russian; *Canadian Translation Fisheries and Aquatic Science* 5102.


Quinn TP (1999) Variation in pacific salmon reproductive behavior associated with species, sex and levels of competition. *Behaviour.* 136. 179-204.


Rosenfeld J, Porter M, Parkinson E (2000) Habitat factors affecting the abundance and distribution of juvenile cutthroat trout (*Oncorhynchus clarki*) and coho salmon


Seaburg KG, Moyle JB (1964) Feeding habits, digestion rates, and growth of some Minnesota warm water fishes. Transactions of the American Fisheries Society. 93, 269-285


Waters JM, Esa YB, Wallis GP (2001) Genetic and morphological evidence for reproductive isolation between sympatric populations of Galaxias (Teleostei:


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