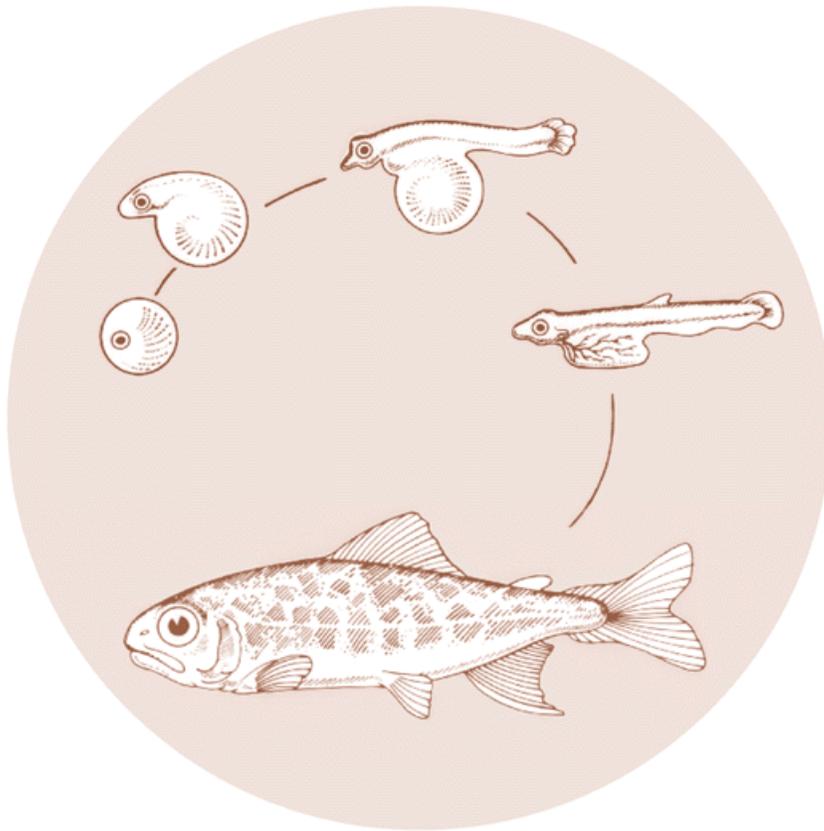


October 1994

**GENETIC ANALYSIS OF ONCORHYNCHUS NERKA:
LIFE HISTORY AND GENETIC ANALYSIS OF
REDFISH LAKE ONCORHYNCHUS NERKA**

Completion Report



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LAKE *ONCORHYNCHUS* NERKA**

Completion Report

Prepared by:

E. Brannon
A. Setter, T. Welsh, R. Danner
K. Collins, and M. Casten

University of Idaho

G. Thorgaard, K. Adams, S. Cummings

Washington State University

Prepared for:

U. S. Department of Energy
Bonneville Power Administration
Environment, Fish and Wildlife
P. O. Box 3621
Portland, OR 97208-3 62 1

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Abstract

The study has shown through life history examination and DNA analysis that three forms of *O. nerka* are present in Redfish Lake. The three forms are closely related, but may be sufficiently different to be considered three separate stocks. Fishhook Creek kokanee are temporally isolated from the beach spawners, and may represent the gene pool most similar to the historic sockeye population that once spawned there. Fishhook Creek offers the best spawning area available in the lake system, and should be considered for use in reestablishing an anadromous Fishhook Creek sockeye strain. The resident beach spawning strain of *O. nerka* is likewise the most similar genetic form of the companion anadromous beach spawning *O. nerka*, and needs to be considered the most appropriate genetic source to help **minimize** reduced fitness of the sockeye from inbreeding.

Life History and Genetic Analysis of ***Oncorhynchus nerka***

Project Number (90-93)

Introduction:

Redfish Lake sockeye salmon, the remaining anadromous strain of ***Oncorhynchus nerka*** in the Snake River system, reached the point of near extinction with no recorded returns in 1990, four fish in 1991, one male in 1992, eight fish in 1993 and one female in 1994. This remnant stock represents the Evolutionarily Significant Unit (ESU) with regard to the 1991 listing of the Snake River sockeye as endangered. The immediate response for recovery included development of a captive brood stock. Returning sockeye were captured, spawned and the progeny reared to maturity. Outmigrants from the lake were also intercepted and reared to maturity. In 1991 and 1992, outmigrants from Redfish Lake were more numerous than expected when brood year anadromous spawners were either absent or in very low numbers. Outmigrants, therefore, had to originate from other than just anadromous parents. Because of the uncertain origin of these migrants, any fish distinguishable from the ESU needed to be isolated from the sockeye to preserve the anadromous strain.

In Redfish Lake the anadromous sockeye are reported to spawn only along a 400 meter section of shallow beach on the northeast shoreline of the lake (Fig.1) during the months of October and November. Documents from the 1930's to the present have confirmed the same general location and time of spawning for Redfish Lake sockeye. However, Evermann (1895) reported that large redfish (sockeye) spawned in Fishhook Creek, one of the two larger streams flowing into Redfish Lake, in mid-August, 1887-89 and 1893, much earlier than the present population. No beach spawning was observed by Evermann, although he may have been there too early in the season to observe fish at that site. Sockeye have not been observed in Fishhook Creek for several decades, but the most successful kokanee population in Redfish Lake spawns in the stream during August and September, coinciding with the historic spawning time of Fishhook Creek sockeye. The different incubation temperature on the beach and the cooler inlet stream is the reason for the temporal separation between Fishhook Creek kokanee and the beach spawning sockeye.

There has been ample opportunity to establish other strains of sockeye or kokanee in Redfish Lake from past transplants (Howell et al., 1985), but no evidence has been

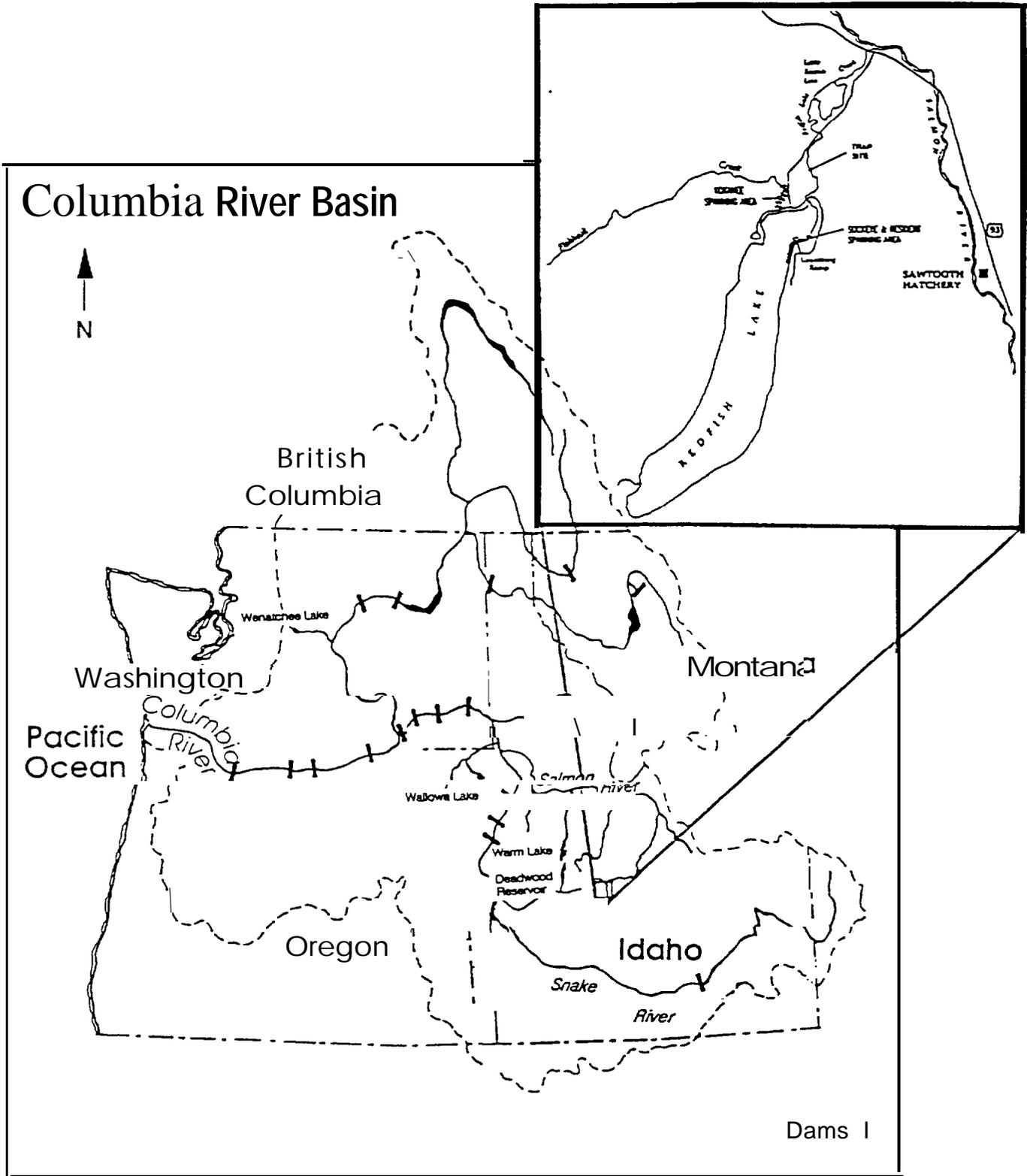


Figure 1. Spawning sites of *O. nerka in* Redfish Lake in the Snake River system of the Columbia River basin.

produced that suggests these attempts were successful. Reports of spawning activity at various locations in the lake other than the two traditional sites could be attributed to transplants. Transplants would tend to distribute randomly with no homing affinity for particular locations in the lake.

The life history of sockeye in Redfish Lake, the kokanee in Fishhook Creek, and transplants that could have contributed to the Redfish Lake population structure, provided the background on which research of relationships between life history forms of *O. nerka* was undertaken. DNA analysis and life history studies were initiated to develop techniques for separating and subsequently determining relationships among populations of *O. nerka* in Redfish Lake. The DNA research concentrated on the development of single locus probes, fingerprint analysis of nuclear DNA, and analyzing genomic DNA for mitochondrial Restriction Fragment Length Polymorphisms (RFLPs) that could be used to separate populations of *O. nerka*, principally in Redfish Lake. Sockeye/kokanee life history studies concentrated on examination of potential spawning sites for spawners in addition to the historic tributary and beach areas in the lake. Spatial/temporal differences between spawning locations, and development rates of eggs and alevins during incubation were examined. Physiological and behavioral studies were also undertaken to characterize differences between anadromous and nonanadromous stocks of *O. nerka*. This report summarizes the annual activities undertaken to isolate differences among subpopulations of *O. nerka* specifically in Redfish Lake, and in the Snake River system in general.

Origin and Genetic Segregation of Kokanee:

The different forms of *O. nerka* that characterize the species consist of the larger (> 1.5 kg) anadromous fish, or sockeye, that emigrate to sea as yearlings and return as adults just before spawning, and the smaller (<1 kg) resident form, or kokanee, that remains and matures in the lake. Offspring of kokanee are primarily resident fish, although they are capable of producing migrants that return as anadromous adults (Foerster, 1947). Kokanee may colonize new areas, but their origin is commonly from the sympatric native anadromous sockeye population. Anadromous sockeye populations spawning in their native stream often produce a small portion of offspring (“residuals”) that remain in lake residence and complete their life cycle entirely in freshwater. Although these residuals are aberrations from the normal pattern of anadromous freshwater residence, some of their progeny would conform to the anadromous life history pattern because the heritability of these traits is not absolute. However, a number of the progeny may also be expected to show their parental residual tendencies, and become the progenitors of resident sockeye or

kokanee. By their recent origin they would doubtless be genetically indistinguishable from their anadromous forebearers. The anadromous (sockeye) and resident (kokanee) beach spawners in Redfish Lake are an example of such a close relationship between sockeye and kokanee. Both of these forms use the same spawning beach, both apparently produce migrants, and they appear indistinguishable from one another genetically. Kokanee, therefore, are a reservoir of much of the investment their ancestral sympatric anadromous strain will have made in genetic uniqueness.

The question of how related are kokanee and the anadromous sockeye within Redfish Lake was initially raised because of concerns in maintaining the integrity of the ESU when capturing brood stock for recovery purposes. Foote et al. (1989) showed that sympatric sockeye and kokanee populations demonstrate a closer relationship genetically than do allopatric populations of sockeye. The degree of similarity, however, will depend on the length of time breeding populations have been separated and the extent of gene flow between them. In sympatric interbreeding populations of anadromous and resident fish, except for anadromy, spawning stocks can be genetically indistinguishable.

In other instances, sympatric stocks of sockeye and kokanee have demonstrated genetic segregation (Brannon et al., 1992; Foote et al., 1989). The question of interest, therefore, is that if gene flow can readily occur between sympatric forms, how can genetic segregation between such forms occur? The first consideration to such a question is that sympatry among stocks does not necessarily mean common ancestry. Most often sockeye are going to be the most abundant form in a lake system, unless free access to and from the marine environment is limited. Fish size, spawning sites, fecundity, and fry size (Williams, 1975; Moyle and Cech, 1988) markedly favor the competitive success of sockeye over kokanee. However, in the absence of sockeye, kokanee will fill the 0. *nerka* niche and can number in the millions within a single lake. Where sockeye and kokanee are found well represented in sympatry, it indicates a situation where the stability of the sockeye population may be tenuous, and they have been unable to usurp dominance. In these situations the original sockeye population from which the kokanee originated may have been very different from the most recent sockeye population inhabiting the area. The degree of similarity, therefore, may not reflect sympatric segregation at all, but rather introgressive gene flow between two previously unrelated sources. Populations in systems where sockeye have reestablished themselves after being nearly extinct may show marked differences from the ancestral stock, as in some of the populations in the Fraser River system in British Columbia.

Kokanee can also originate from sockeye that stray from their home destination. Natural events such as land slides or severe rapids blocking their return migration can result in sockeye selecting new spawning areas. Since the orientation of migrants leaving their ancestral lake is strongly influenced by the genetic predisposition of the stock, offspring of strays could be severely disoriented in new habitats and become behaviorally landlocked. These fish could then interbreed with the native kokanee population. Resident fish originating from such events could be very different from the native sockeye or kokanee stocks, depending on the magnitude of the introgression.

Genetic segregation among sympatric sockeye and kokanee populations, however, is not inconsistent with their life history. Kokanee will evolve characteristics favorable for total freshwater residence to maximize their fitness under selective pressures of their particular environments. Traits such as spawning time will become distinctly different from their anadromous cousins as they colonize new spawning areas. Because of the competitive advantage of sockeye, selection will favor resident forms that use different spawning sites, or develop slightly different spawning times to avoid displacement by their larger progenitors. In sympatry, kokanee spawning times that are slightly later than the sympatric sockeye, for instance, would permit them to utilize spawning substrate prepared by deeper digging sockeye, and still allow them to target optimum emergence timing in the spring. Spawning later would optimize emergence because kokanee eggs are smaller than sockeye eggs, and development requires fewer temperature units. Thus, gene flow between sympatric forms does not necessarily negate directional selection within the mixed population. Gene flow will not prevent kokanee from spawning later than sockeye any more than it prevents the evolution of early and late spawning segments within a given anadromous sockeye population.

Gene flow among sympatric stocks of a common origin, however, will prevent complete genetic isolation, even under directional selection. Matings between kokanee and sockeye, as observed by McCart (1970), will maintain sufficient gene flow to discourage total separation. Furthermore, residualism is not a one time event. Since sockeye and kokanee tend to exhibit assortive mating behavior based on size (Foote and Larkin, 1988), residuals of the anadromous population, or marine migrants of the resident population, would be more apt to spawn with fish of their own size than with their conspecifics. Such circumstances will continually introduce sockeye genes into the resident kokanee population. Finally, since size is a major factor that dictates kokanee spawning age

(Patterson, 1994), asynchrony in year classes between the two forma would also discourage isolation.

Generally, genetic similarity of *O. nerka* stocks within a lake, or between closely positioned lakes, suggests common ancestry. Resident sockeye (kokanee) in Fishhook Creek that rear in Redfish Lake, and are thus sympatric during the rearing phase with anadromous sockeye and resident beach spawners, are genetically distinct from those fish, but still more similar to them than other geographically distant Columbia River anadromous sockeye.

Identification of differences specific enough to separate closely related populations of *O. nerka* in a single lake system was undertaken by the use of DNA techniques which have the potential to demonstrate population specific markers or patterns in the stocks of interest. The ability to identify differences among sympatric populations of *O. nerka* depends on the degree of isolation and thus the opportunity for selective evolution to have moved them apart genetically while exposed to different habitats.

Objectives:

The objectives of the *O. nerka* studies in Redfish Lake were to:

1. Determine if there were different spawning demes present in Redfish Lake.
2. Qualify relationships among life history forms present in the Snake River system, and
3. Characterize differences between anadromous and nonanadromous forms.

Laboratory and Field Activities:

The principle questions examined in the present study were: (1) are the beach spawning sockeye and Fishhook Creek kokanee populations known in Redfish Lake the only populations of *O. nerka* present, and (2) how similar or dissimilar to one another are these various populations. The questions were broken into three tasks with the first to survey the lake for spawning populations previously undetected, then develop DNA analysis techniques that could be used to readily segregate them, and finally assess life history characteristics that may help differentiate relationships among populations.

Task I - Spawning Populations of *O. nerka* in Redfish Lake

The first task to survey Redfish Lake for previously undetected populations of *O. nerka* was undertaken during daylight and darkness at intervals from August to November in 1992. An aluminum barge and outboard motor were used as the observation platform to

move along potential spawning areas, and search lights provided exceptional lighting for observation at night. Also, diving gear was used at night by Bruce Rieman, Scott Spaulding, and Paul Dann in the vicinity of the sockeye beach.

Daylight surveys over the shallow beach areas around the lake generally revealed only an occasional rainbow trout or sucker. Similarly, day and night surveys of the lake bottom with an underwater video camera disclosed only shiners and suckers. Suckers were seen as deep as the lake floor at mid-lake. Night surveys over the sockeye beach, however, showed substantial activity in the shallows by whitefish, trout, suckers, sculpins, and later by resident sockeye.

On the 8th and 9th of October in 1992, night surveys were made over the sockeye spawning beach and other shoreline areas by boat with flood lights, but no resident sockeye or kokanee were observed. On October 16th and 20th, Bruce Rieman made night snorkel surveys and observed what appeared as kokanee on the sockeye beach spawning area. Follow-up observations were made on October 22nd at night using the boat with search lights, and two fish that appeared to be kokanee were observed along with whitefish, suckers and trout. On the 26th of October night observations were undertaken, and again on the 27th. Four kokanee were observed on nest sites, and one partially spawned female was captured by seine for tissue sampling (22:30 hr). A blood sample and adipose fin were taken for DNA analysis (fish # 101), and length was measured (Table 1). Coloration was olive green on the back with white belly and dark gray lateral line. Spots on the back and mottled sides were characteristic of kokanee. The female was held overnight and released the following morning.

On November 4th, one (male) of two fish observed was captured (22:30 hr) and later three (one female and two males) of four fish observed were captured. Lengths were measured (Table 1), and blood and adipose samples were taken (Fish #s 102, 103, 104, & 105). The fish were left in a live-box overnight and transferred to Eagle Laboratory by IDF&G personnel the following morning, for holding. Coloration was from light to medium olive green, with variable spotting patterns on the dorsal area, from a few spots on one fish to dark and larger spots on another. A slight reddish tint was noticed on the males, and the female had the dark gray lateral line. Three other kokanee were seen on the spawning beach, but no attempt was made to capture them.

Table 1. Lengths and meristics of kokanee beach spawners.

Fish No.	Sex	Fork Length cm	Gill Raker No.	Color
101	F	22.4		Mottled olive green, spotted gray lateral line
102	F	21.3	R- 29	Mottled olive green, spotted gray lateral line
103	M	22.4	R- 32	Olive green, w/ml tint, spotted
104	M	21.6	R- 33	Olive green, w/red tint, spotted
105	M	20.6	R- 30	Olive green, w/red tint, spotted

Daylight observations on the sockeye beach were made on the the following day (Nov 5 at 11:00 hr) from the barge moving rapidly over the shallows. One small concentration of about 10 to 15 fish was seen scattering from the approach of the boat. It appears that daylight observations, which were generally unsuccessful, may detect fish at least during the peak spawning time.

Task I Discussion

With respect to the first objective, it was demonstrated that at least one additional form of *O. nerka* is present in Redfish Lake in addition to the anadromous sockeye and Fishhook Creek kokanee. The size of the resident population of beach spawners that was discovered does not appear to be large, but by the number of outmigrants that they apparently generated in 1991 and 1992, their abundance would probably exceed 500 fish.

Task II - Genetic Analysis

The second task was the analysis of DNA from Redfish Lake *O. nerka* populations. This work included assessment of other stocks in the Snake River system, the Upper Columbia River and outside the Columbia River basin to assist in differentiating patterns that would be helpful in separating Redfish Lake stocks and determining population relationships.

Task II (a) Nuclear DNA

We sought to more rapidly and easily identify markers distinguishing the stocks by applying DNA fingerprinting to mixed DNA samples from a number of individuals from each population (Spruell et al., 1994). This allows differences between populations to be

more readily identified DNA from a number of individuals of each population were mixed to provide composite DNA samples. The mixed DNA samples were then digested with a restriction enzyme, and fragments were separated by electrophoresis and blotted onto a nylon membrane. Membranes prepared in this way were then hybridized to DNA probes to reveal DNA fingerprints. It was found that the mixed DNA samples provided good DNA fingerprint patterns. This suggested that some DNA fragments containing repeated sequences are low enough in variability to be present in a majority of individuals. It also indicated that in closely related populations, the mixed DNA fingerprint patterns are nearly identical. However, enough variability was present to indicate that use of this approach provided a method to more efficiently screen for potential DNA markers.

Task II (a) Methods

DNA was extracted from fish blood. The samples were digested for 24 hours in restriction endonuclease, Hae III at 37 °C. In each gel lane, 2.0 ug of digested DNA were electrophoresed in 1.0 % Low EEO agarose gel in 1x TAE at 50 volts for 24 hours. The gel was constantly cooled to 10 °C to insure even electrophoretic conditions. The DNA , was denatured, neutralized and then transferred to Magnagraph nylon membrane by capillary action (Southern blot) for 4 hours with 10 sheets of Quick Draw blotting paper. A record of the gel condition, a photograph of the gel, and a record of each hybridization of the membrane was kept on a experimental worksheet.

The nylon membrane was probed with three hypervariable minisatellite probes (Jeffreys 33.6, Hpa I and Per). The probes are small known sequences of single stranded DNA that attach to similar sequences found on the 0. *nerka* DNA annealed to the nylon membrane. The Jeffreys 33.6 probe is based upon a core fragment found near centromeres (Jeffreys et al., 1985). The Hpa I probe is based upon a salmonid repeated DNA sequence (Kido et al., 1991; Spruell et al., 1994). The Per probe is based on a sequence of *the Drosophila* Per gene (Shin et al., 1985).

The hybridized membranes were exposed to x-ray film for 7 hours. Fingerprint patterns were analyzed for band sharing by image analysis using a Mircotek ScanMaker 600ZS and a Macintosh IICI computer (Spruell et al., 1994). Images were converted to an array of molecular weights and intensities for each band using the NCSA GelReader program (Redman and Jacobs, 1991). The accuracy of the scanner was tested by overlaying the image with an acetate sheet and marking each band with a permanent marker. Both the original image and the marker reproduction were scanned and the results compared.

A presence/absence matrix was constructed from the array of molecular weights utilizing a customized program interfaced with the GelReader program. Output format and allowed error tolerance was selected by the user. Error tolerances were empirically determined by alternating two individuals across one gel and obtaining 8 molecular weight estimates for each band. The error tolerance was defined as the difference between the maximum and minimum values divided by the average value for each band. The deviation increases with increasing molecular weight, To compensate for the weight dependent deviation, the allowed error tolerance was adjusted to reflect the empirically determined value for each molecular weight. An error tolerance of 4% was determined for bands 20 kilobase (kb) in weight, and decreased linearly to 2% for bands 1 kb in weight. The error values for the computer determined molecular weights are in accord with other published error estimates (Haig et al., 1993).

Bands were identified as being identical if their molecular weights were within the error tolerance identified for that molecular weight. In proceeding through the analysis, the program identified the band with the highest molecular weight. If the second largest fragment fell within the error tolerance range it was identified as the same band. The molecular weights of these two bands were then averaged to obtain an adjusted molecular weight value. This new average was then compared to the third largest fragment. Each time a new band was added to a group, a new average was calculated. When a band did not fall within the error tolerance for the molecular weight, it began a new molecular weight size class. Bands assigned to the previous molecular weight group were then reevaluated. If a band was more similar in weight to the average of the next molecular weight group it was reassigned to that group. Each fragment from largest to smallest was evaluated in this manner. A matrix was then constructed identifying the presence or absence of a band for each molecular weight class in each lane.

From the presence/absence matrix a band sharing index was calculated as $BS = 2N_{ab} / (N_a + N_b)$ (Wetton et al., 1987) where N_{ab} is the number of shared bands, N_a is the number of bands in one lane and N_b is the number of bands in the other lane. Fifteen individuals from each population were used to calculate an average band sharing index within and between populations. All pairs of individuals being compared were present on the same membrane. A minimum of 150 comparisons was used to estimate the average within and between population band sharing indices. Band sharing indices were also calculated for DNA mixtures by treating each mix as if it were an individual.

The NCSA GelReader program produced an output that provided the molecular weights of each band on the membrane. The membrane containing DNA comparisons between individual *O. nerka* and a mixed DNA sample was hybridized with oligonucleotide probe, Jeffreys 33.6, scanned, and the molecular weights of each band were calculated by the NCSA GelReader program. The benefit of using the computer to determine band weights and band sharing was its unbiased analysis. Bands must fall within a specific error range to be considered equal; the computer objectively measures the distances between the bands. This unbiased analysis is a significant advantage of computer scoring over hand scoring. From the output, a band sharing matrix was calculated. The band sharing matrix was used to construct an Unweighted Pair Grouped Method Analysis (UPGMA) tree of **genetic** relatedness. The UPGMA tree assumes a constant rate of evolution, this means that the accuracy of the branching is good unless there is a small number of genetic substitutions or the overall rate of genetic substitutions is variable. The UPGMA tree illustrates the usefulness of this technique. The comparison between the individual *O. nerka* DNA and the mixed *O. nerka* DNA revealed that each individual sample was more closely related to the other individuals than it was to the mix. However, the individual samples were more closely related to the mixed DNA sample than to *O. nerka* outside the Stanley Basin.

Task II (a) Results

Several differences were observed in the mixed DNA fingerprints when comparing sockeye and kokanee samples, and later with outmigrants. The differences observed showed that the outmigrants appeared more similar to the sockeye. When DNA was cut with **Dpn II** and probed with 33.6 (a human minisatellite sequence originally identified in 1985 which is widely used for fingerprinting in mammals), for instance, the mixed fingerprint patterns showed a 4 kb fragment in the sockeye and outmigrants that was absent in the kokanee. A second fragment, at 2.2 kb, was present in both the sockeye and outmigrants but was slightly smaller in the kokanee. A second probe, Hpa I-5' is based on the sequence reported by Kido et al. (1991). This probe revealed polymorphisms at 4 and 1.6 kb that were the same in the sockeye and outmigrants but different in the kokanee.

While experiments with mixed DNA continued, experiments were started to determine the best method for examining these potential markers in individuals. Our aim was to determine whether the band differences observed in mixed DNA samples represented diagnostic markers or frequency differences. Initial attempts to score **polymorphic** fragments in individuals were hindered by the large number of bands and the complexity of

the patterns. However, modifications of agarose electrophoresis conditions helped to resolve the bands of interest by increasing electrophoresis time to 40 hours.

Analysis of DNA from individuals using Dpn II cut DNA probed with 33.6 showed that a 15 kb fragment band present only in the Fishhook Creek mixed sample was observed in four of five kokanee, and wasn't present in either the sockeye or beach spawners. A second band at 20 kb was present in all five beach spawners, but didn't appear in either the sockeye or Fishhook Creek kokanee individuals.

Examination of potential methods to develop single locus probes from the polymorphic fragments observed in the mixed DNA patterns was successful. A lambda cloning system was selected and attempts were made to clone an 8 kb Spe I fragment into it. This fragment was chosen because it had shown a sockeye/kokanee difference in DNA fingerprinting trials with the Hpa I-S probe. A sockeye genomic library was prepared from the 8 kb fragment in the Stratagene Lambda Zap phage vector system and the resulting plaques were screened using the Hpa I-5' probe. Several positive lambda clones were picked and purified. Four of these were chosen for initial hybridization trials. The cloned fragments provided multiple band patterns instead of the single band patterns expected. Our interpretation is that the fragments contain mostly tandem repeats. However, there were fewer bands than observed in our standard DNA fingerprinting experiments. The probes showed sockeye/kokanee differences and provided simpler patterns that proved easier to analyze than our previous methods. Similar approaches are also being applied to the library using the 33.6 probe; clones have been isolated and the patterns detected are being analyzed.

A DNA polymorphism obtained using the Hpa I-5' probe hybridized to Spe I cut DNA samples showed positive results. In mixed DNA samples, DNA fingerprints showed an 8 kb fragment in Redfish and Alturas kokanee that was not present in the Redfish sockeye or outmigrants. The Lambda Zap II cloning kit from Stratagene was selected for use and a single locus probe from the polymorphic 8 kb Spe I **DNA** fragment observed in fingerprints using the Hpa I-5' probe was developed. The probe was designated Spe8.1I-W0.8, and tissue from individuals of 12 different groups by brood year or sample site was analyzed (Table 2). Except for the Babine sockeye used as reference, all sample groups were from the Stanley Basin stocks. Results showed strong similarity between Redfish Lake sockeye and resident beach spawners, but a difference between these **two** groups and the kokanee. The A allele was absent in the sockeye and resident beach

Table 2. Distribution of genotypes and allele frequencies for the single locus probe Spe8.1IIP-0.8.

Population # Individuals Scored)	Genotypes						Allele Frequencies		
	AA	BB	CC	AB	AC	BC	A	B	C
Kokanee 1990 (25)	0	1	9	4	6	5	0.20	0.22	0.58
Kokanee 1991 (18)	0	0	8	1	7	2	0.22	0.08	0.69
Kokanee combined (43)	0	1	17	5	13	7	0.21	0.16	0.63
Sockeye 1991 (4)	0	0	3	0	0	1	0.00	0.12	0.88
Sockeye 1992 (1)	0	0	0	0	0	1	0.00	0.00	1.00
Sockeye 1993 (8)	0	0	7	0	0	1	0.00	0.06	0.94
Sockeye combined (13)	0	0	10	0	0	3	0.00	0.12	0.88
Beach Spawn 1992 (5)	0	0	5	0	0	0	0.00	0.00	1.00
Beach Spawn 1993 (18)	0	0	12	0	0	6	0.00	0.17	0.83
Beach Spawn combined(23)	0	0	17	0	0	6	0.00	0.13	0.87
Outmigrants 1992 sample (19)	0	0	17	0	0	2	0.00	0.05	0.95
Outmigrants 1993 sample (313)	0	5	249	0	6	58	0.02	0.18	0.80
Outmigrants combined (332)	0	5	263	0	6	60	0.01	0.09	0.90
Alturas (10)	1	0	7	0	2	0	0.20	0.00	0.80
Babine Sockeye (10)	2	5	0	3	0	0	0.35	0.65	0.00
Stanley (10)	0	2	3	0	1	4	0.05	0.40	0.55

spawners, and present in the kokanee population. Therefore, with regard to the composition of the outmigrants it appears that with the low frequency of the A allele, outmigrants are primarily from the sockeye or beach spawners.

Task II (a) Discussion

The second objective to develop a method sufficient to resolve differences between *O. nerka* sources in Redfish Lake was successful. The single locus probe, Spe8.1HP-0.8, made it possible to isolate three alleles (A, B, & C) in the Redfish Lake populations that proved diagnostic. By virtue of the facts (1) that the A allele was absent in sockeye and beach spawners, and present in kokanee, and (2) that the outmigrants showed very low frequency of the A allele, made it possible to conclude that the kokanee population contributed very few migrants. This is especially significant given the fact that yearling kokanee number in the tens of thousands in the lake.

It is of interest that the A allele present in Redfish kokanee was also present in the other Stanley Basin kokanee populations (Alturas & Stanley). It was also present in Babine sockeye, introduced in the Alturas and Stanley lakes (Hall-Griswold, 1990). However, with the C allele absent in the Babine samples and so prevalent in Redfish Lake sockeye (allele frequency > 88%), and Stanley Basin resident populations in general (allele frequency > 55%), there is no evidence that Babine made a dominant contribution to any of the populations. Moreover, it would appear by the absence of the B allele in Alturas kokanee, and the low A frequency in Stanley kokanee, Babine sockeye transplants may not have been successful at all.

Task II (b) Mitochondrial DNA

Mitochondrial DNA (mtDNA) has been used to geographically differentiate populations which have recently diverged (Hoelzer & Metick, 1994; Williams et al., in press b). Our assumption for investigating this molecule was that the mtDNA would have evolved sufficiently for differentiation of the *O. nerka* populations in Redfish Lake, but not to such an extent that convergent mutations would dominate the findings. The premise for this is the apparent likelihood of a transition (ie. purine for purine) rather than a transversion (ie. purine for pyrimidine) occurring under mutation conditions within the molecule (Templeton, 1983). This molecule's utility in most species is that the control region evolves at a rate approximately two to three times faster than the rest of the molecule. Brown et al. (1979) has estimated that in mammals the entire mtDNA molecule evolves ten times faster than a single copy nuclear gene. The mtDNA molecule is haploid and maternally inherited.

Based on these molecular characteristics, we proposed that mtDNA would successfully differentiate the anadromous from the non-anadromous *O. nerka* of Redfish Lake due to gene flow historically having become restricted between these different life history groups and hence allowing them to diverge evolutionarily. It was assumed that spatial segregation of spawning ground selection provided sufficient evidence to support this hypothesis. Our goal was to identify polymorphisms in mtDNA that would discriminate between the races or sympatric populations within Redfish Lake. Polymorphisms which correlate with habitat preference have been documented in sympatric populations (Bush, 1994).

Task II (b) Methods

1991-1992

Our work originally was focused on probing Southern blots for RFLP's using either mtDNA, or 28s /18s ribosomal DNA. This work has been previously summarized in a short manuscript (Setter and Brannon, 1994). Problems with clean probe isolations and insufficient probe labeling caused time setbacks throughout this phase of the study. The procedure generally was to screen four individuals of sockeye salmon and four individuals of kokanee salmon on each Southern blot. Each blot also had a lane of molecular weight marker labeled with digoxigenin that could be used for fragment size estimation. The procedures for genomic DNA extraction and southern blot preparation are described in Setter and Brannon (1992). Detection of the hybridized probe utilized either colorimetric or chemiluminescent methods (Boehringer Mannheim-Genius kit). Individual fish were examined from the following populations: Redfish Lake - sockeye, kokanee, outmigrants; Babine Lake - sockeye; Lake Wenatchee - sockeye; Okanogan Lake/River - sockeye, kokanee; Alturas Lake - kokanee; and Warm Lake - kokanee. A summary of the DNA sequence recognition sites used for digestion and subsequent hybridization with a probe are shown in Table 3. Our data collection efforts focused only on population level variation that could potentially be diagnostic.

1993-1994

Our progression in methodology from Southern blots (1991-1992) to a Polymerase Chain Reaction (PCR) - based RFLP detection proved beneficial. Amplifying segments of the mitochondrial molecule allows detection of more restriction site changes in a shorter time than Southern blot analysis. PCR-RFLP allows for the inspection of a specific target within a DNA strand rather than the entire molecule generically as with Southern blots. This finer tuning provides information on the conservation of the specific region evolutionarily and can be used for comparison with the entire molecule or nuclear genes.

Table 3. Summary of restriction enzyme sequences surveyed noting the probe used

Sequence	28s	18s	mtDNA
GGGCCC	⊕		
GGWCC	⊕		
GCATGC	⊕	⊕	
AGATCT	⊕	⊕	⊕
TTTAAA	⊕	⊕	⊕
GCGGCCGC	⊕	⊕	
CGATCG	⊕	⊕	⊕
CTGCAG	⊕	⊕	⊕
GGATCC	⊕	⊕	
GAATTC	⊕	⊕	
GTPyPuAC	⊕	⊕	⊕
GGTACC	⊕	⊕	
GAGCTC	⊕		
TCTAGA			⊕
GGTNACC			⊕

Note: N = any base, Pu = purine, Py = pyrimidine, W = A or T

This methodology has been used successfully for intraspecific mtDNA assessments in chinook and chum salmon (Cronin et al., 1993). We utilized this procedure to collect data from four regions (Cyt B, NDI, ND2, ND5/6) of the mitochondrial molecule. The amplifications were performed on genomic DNA extracted from body tissue, usually liver or a fin clip. The amplification reaction conditions followed those generally recommended with the purchase of Taq polymerase from Perkin-Elmer. Primers for Cyt B and ND2 were obtained from LGL Ecological Genetics. Primer sequence information for ND1 were taken from Cronin et al. (1993) and for ND5/6 were obtained from L. Park (NMFS). Amplification reaction products were examined on a 0.8- 1% agarose gel using TAE buffer and pUC19 as the molecular weight marker (Biosynthesis, Inc.).

The initial screen of the Redfish Lake subpopulations included the following restriction endonucleases: Alu I (4), **Hha** I (4), **Dde** I (5), **Hae** III (4), **Hinf** I (5), **Mse** I (4), **Hpa** II (4), **Rsa** I (4), **Sau3a** I (4), **Bfa** I (4), **Taq** I (4), **Bst**U I (4), Mnl I (4), **Pst** I (5), **Eco**R I (6), **Eco**R V (6), **Sph** I (6), **Nco** I (6), **Acc** I (6) and **Bsa** I (6). Digestion reaction products were electrophoresed through a 3% agarose gel, and photographed. For fragment size determinations, TBE buffer with 4% Metaphor agarose gels were run with both pUC19 and Bioladder molecular weight standards.

Fragment patterns were described and transformed into restriction site data. Data were entered into REAP [Restriction Enzyme Analysis Package] (McElroy et al., 1991) to obtain the number of haplotypes and estimates of nucleotide diversity. In addition, frequency data by population for each haplotype were tested within REAP with a chi square heterogeneity program. The haplotype distance data were then run through the PHYLIP [Phylogenetic Inference Package](Felsenstein, 1993) program and site data to PAUP parsimony program to obtain an estimate of phylogenetic relationships among the haplotypes examined.

Populations that were examined in addition to those within Redfish Lake were: Alturas Lake, Stanley Lake, Warm Lake, Wallowa Lake, Pettit Lake and Dworshak Reservoir kokanee and Babine sockeye. Although all of the kokanee populations have had numerous introductions of strains from outside their geographic vicinity, it is unknown which, if any, of these successfully propagated themselves. The data have allowed for an evolutionary relationship dendrogram and cladogram based on maternal inheritance to be constructed.

Task II (b) Results

1991-1992:

The genomic DNA digested with restriction enzyme Bgl II and probed with mtDNA showed the presence of a restriction site change. Data collected to investigate this are shown in Table 4. The absence of a site (3 fragments) was noted in 30% of all sockeye tested and no kokanee. Sockeye from four populations were examined but only some individuals from Lake Wenatchee and Babine Lake showed the banding pattern.

Table 4. BGL-II digests probed with entire molecule, labeled with digoxigenin-dUTP.

<u>Area</u>	<u>Pattern A</u>	<u>Pattern B</u>
Redfish sockeye	4	
Redfish outmigrants	4	
Redfiih kokanee	4	
Lake Wenatchee sockeye	4	4
Babine sockeye	2	2
Altzuras kokanee	4	
Warm Lake kokanee	5	
Okanogan River sockeye	4	
Okanogan kokanee	4	

Because only one enzyme displayed a pattern indicative of a site change in the DNA sequence using blot methodology, this procedure was eliminated and all further work assembled data using PCR. We noted a variant RFLP pattern in each of the four regions of the mtDNA molecule we amplified with PCR. In Cyt B, the restriction endonuclease **Hae** III revealed an apparent site alteration, This same enzyme also delineated two site changes in region ND2. Region ND1 was digested with restriction enzyme **Dpn** II and the ND5/6 region was digested with restriction enzyme **Taq** I to yield fragment patterns which were variable among individuals. The number of fragment patterns was two for each region except ND2 where there was three. The estimated fragment sizes for each pattern are shown in Table 5. The particular combination of the pattern noted for each mitochondrial region investigated defined the number of haplotypes (Table 6). Ten haplotypes were observed among the 0. *nerka* specimens from the kokanee and sockeye populations included in this study (Table 7). Three populations, Pettit, Stanley and Babine, were monomorphic for a particular haplotype. Redish Lake kokanee displayed the greatest diversity of all populations examined with nine haplotypes represented.

The quantification of dissimilarity between individuals or groups is referred to as genetic distance. Estimates are made from measurable characters, in our case the presence/absence of restriction sites derived from haplotype data, to define this lack of identity. This distance information is then drawn into dendrograms which illustrate their probable evolutionary relationship. Distance (percent sequence divergence) estimates generated from our site data are shown in Table 8. In addition, the site data was used for a parsimony analysis for comparative and consensus purposes with the distance data and the resultant clustering of the haplotypes. Analysis by program Monte to test for geographic heterogeneity with a chi square statistic confirmed that the mtDNA frequencies for each group examined were different ($\chi^2=420.5$).

Task II (b) Discussion

Based on our results represented in Table 7, we postulate that Redfish Lake kokanee haplotypes from Fishhook Creek may represent both the original anadromous 0. *nerka* of the region as well as some transplants. Our rationale is based on the extensive variability in mtDNA that the population demonstrates, due to both site mutations which imply divergence and imported stocks. For example, the haplotypes S3, S4, S8, and S9 represented in our data set by only one or two individuals do not appear to overlap with either the current anadromous or other regional kokanee populations. Shared diversity

Table 5. Fragment lengths (base pair) from restriction enzyme digests (enzyme used) with pattern designator for each of the four mitochondrial regions which we have examined, These sizes are estimates taken from photos where digests were run out on agarose gels.

Cyt B (Hae III)		ND5/6(TagI)				
A	B	A	B			
	948	1386				
592		604	604			
325			574			
154	154		517			
105	105	252	252			
52	52					
		<u>2242</u>		1947		
1228	<u>1259</u>					
ND1 (Dpn II)		ND2 (Hae III)				
A	B	A	B	c		
585		1279				
	517		840			
310	310			546		
252	252			400		
216	216	315	315	315		
195	195					
167	167		278			
108	108		208	208		
	88	131	131	131		
88	68	85	85	85		
1921	<u>1921</u>	1810	<u>1857</u>	<u>1685</u>		

Table 6. Breakdown of haplotype designations used in the Redfish Lake 0. *nerka* study, listed by region: Cyt B, ND1, ND2 and ND5/6.

<u>Code</u>	<u>Haplotype</u>	<u>Total</u>
S1	BAAA	65
S2	BABA	77
S3	BBBB	2
S4	BBAB	1
S5	AAAB	2
S6	BAAB	3
s7	AABB	1
S8	BABB	22
s9	BBBA	2
S10	AABA	2
S11	BACA	16

Table 7. Summary of haplotypes found by sampling area.

POPULATION	N	S1	s2	S3	S4	S5	S6	S7	S8	S9	S10	
RFL sockeye	5	3	2	-	-	-	-	-	-	-	-	
RFL beach	18	12	6	-	-	-	-	-	-	-	-	
RFL kokanee	19	1	3	2	1	1	1	6	2	1	-	
RFL oulmigrants	46	23	23	-	-	-	-	-	-	-	-	
Alturas	6	3	3	-	-	-	-	-	-	-	-	
Pettit	12	-	-	-	-	-	-	-	-	-	12	
Stanley	21	21	-	-	-	-	-	-	-	-	-	
Warm take	22	1	13	-	-	-	-	7	-	1	-	
Wallowa	23	-	10	-	-	-	-	9	-	-	4	
Dworshak	4	1	-	-	1	2	-	-	-	-	-	
Babine	17	-	17	-	-	-	-	-	-	-	-	
TOTAL	193	65	77	2	1	2	3	1	22	2	2	16
% of Total	100	33.7	39.9	1.0	0.5	1.0	1.6	0.5	11.4	1.0	0.5	8.3

which appears to be regional can be noted in Warm Lake and Wallowa Lake which shows overlap with S1, S2, S7 and S9. These haplotypes provide possibilities for being descendants of the historical *O. nerka* populations that utilized the Snake River corridor.

Table 8. Percent sequence divergence among *O. nerka* mtDNA haplotypes.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
S1	0										
S2	0.08	0									
S3	0.30	0.23	0								
S4	0.23	0.30	0.08	0							
S5	0.22	0.30	0.22	0.15	0						
S6	0.15	0.23	0.15	0.07	0.07	0					
S7	0.30	0.23	0.15	0.22	0.07	0.15	0				
S8	0.23	0.15	0.08	0.15	0.15	0.07	0.07	0			
S9	0.15	0.08	0.15	0.23	0.38	0.30	0.30	0.23	0		
S10	0.15	0.08	0.30	0.38	0.22	0.30	0.15	0.23	0.15	0	
S11	0.15	0.08	0.30	0.38	0.38	0.30	0.30	0.23	0.15	0.15	0

The S2 haplotype is one of the two most commonly shared among the populations examined (7 out of 11). Babine sockeye, which have been transplanted into the Snake River system, appear monomorphic for S2, based on the present sample size. The Redfish Lake sockeye and kokanee beach spawners, timed similarly to Babine sockeye, show a high representation of the S2 form. In contrast, the Redfish Lake kokanee population from Fishhook Creek doesn't show a high representation of the S2 form, are early spawners and temporally isolated from the beach spawners by the lower incubation temperatures of Fishhook Creek.

Warm Lake and Wallowa Lake kokanee also show strong representation of the S2 haplotype. Indeed, the S2 haplotype could very well be descendant from the historical populations of the Snake River corridor, which is a likely scenario, but sockeye plants from unknown sources were made in Warm Lake as early as 1942, and several introductions are believed to have occurred in Wallowa Lake, which could have been sources of the S2 form and complicate determination of relationships. The S2 form is only a site change different than S1, S7, S9 and S10, and this makes base substitution mutations an equally likely possibility.

Stocking sources were recorded in more recent years. Redfish Lake received kokanee from Anderson Ranch Reservoir, Idaho, in the 1960s and 1970s. Anderson Ranch Reservoir was stocked from British Columbia, directly or via Island Park Reservoir in Southern Idaho, and from Northern Idaho. Fish from B.C. were Aug/Sept spawners, and Northern Idaho were Nov/Dec spawners. Dworshak Reservoir, which shares haplotypes S5 and S6 with Redfish Lake kokanee, originated from transplants of kokanee from Flathead Lake, Montana, via Grandby Lake in Colorado, which are fish of the same origin as the Northern Idaho lakes, and late spawners which may relate them to the Redfish Lake beach spawners, but not Fishhook Creek. The presence of haplotypes S 1 and S2 in Redfish Lake kokanee individuals examined supports the idea that some gene flow may exist between the anadromous and nonanadromous *O. nerka* forms within the lake.

The monomorphic populations representing haplotypes S 1 (Stanley Lake), S2 (Babine Lake) and S 10 (Pettit Lake), and the dimorphic populations representing haplotypes S 1 and **S2** (Redfish and Alturas lakes) all have provided important information critical to our present assessment. Pettit Lake kokanee are monomorphic for S 10, and its relationship with other populations is still undefined. Pettit Lake has been eradicated, restocked and a barrier installed on the outlet to prevent fish entry. The present haplotype should be descended from the transplants. It is informative to look at the haplotypes (S 1-S 10) and their occurrence in populations (columns) and at the populations with respect to haplotype diversity (rows) [Table 7]. These data can be evaluated dimensionally in the bar graphs (Fig. 2a & b) where Fig 2a shows the number of populations in which each specific haplotype occurred and Fig 2b where the number of haplotypes found in each population are shown. Bar graph Fig 2a shows a pattern where two haplotypes, S 1 and S2, occur in most populations examined thus far in our study, but all other haplotypes (S3-S10) occur in 3 or fewer populations. The two widespread haplotypes also occurred in nearly 75% of all specimens examined. This pattern is similar to one observed in mtDNA diversity in bull trout (*Salvelinus confluentus*) in the mid- and upper-Columbia basin (Williams et al., in press a). Bar graph Fig 2b shows the number of haplotypes found in each population. Two very different patterns are observed in this graph.

In general, populations had low mtDNA haplotype diversity. At this point, most of our populations (7 of 11) had 2 or fewer mtDNA haplotypes. This pattern is consistent with most natural fish populations that have not experienced gene flow from other populations (Bermingham and Avise, 1986; Bermingham et al., 1991; Billington and Hebert, 1991;

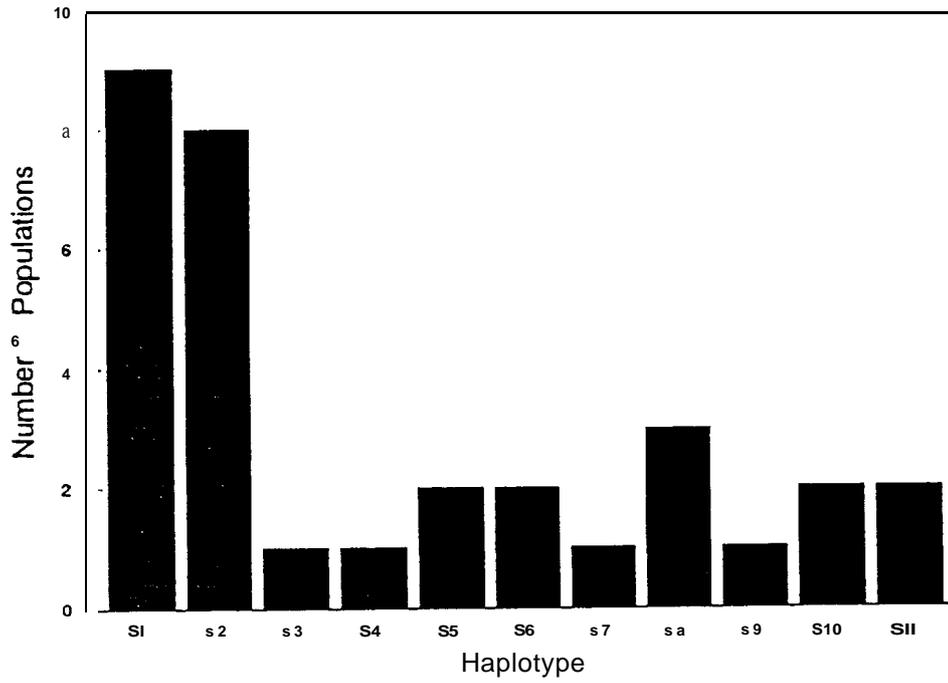


Figure 2a Number of populations in which each haplotype occurred-

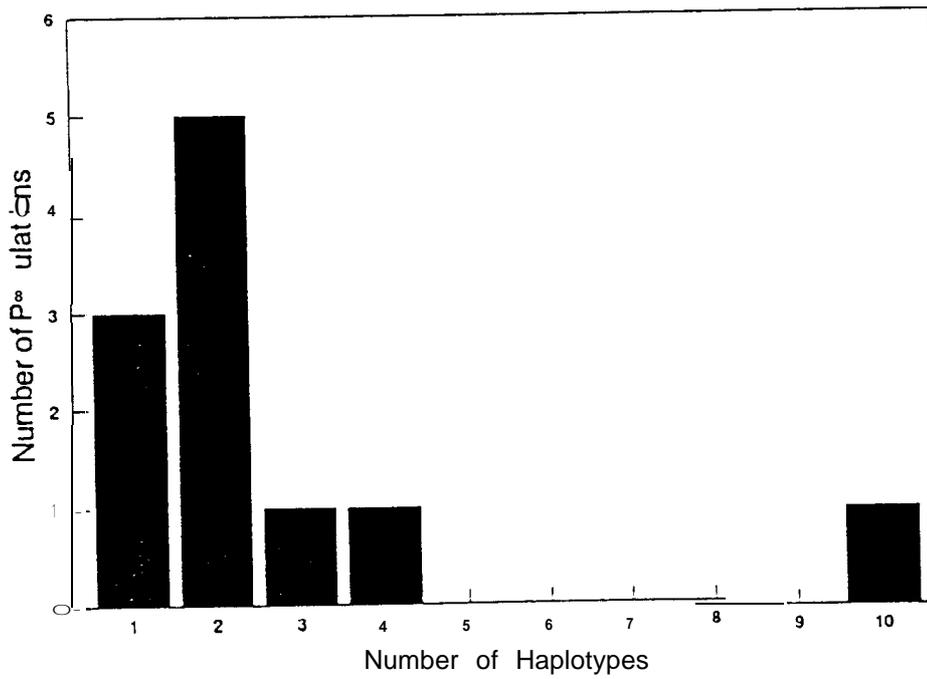


Figure 2b. Number of haplotypes found in each population.

Williams et al., in press a). Interestingly, the Redfish Lake kokanee population from Fishhook Creek (n=19), exhibited 9 of 10 mtDNA haplotypes observed to date in this study, which is a high level of mtDNA haplotype diversity. Most wild fish populations exhibit low levels of haplotype diversity presumably because random mtDNA lineage extinction processes eliminate diversity at a greater frequency than in situ mutations are occurring (Awise et al., 1984; Awise, 1989). In most fish populations where high mtDNA diversity has been evident, the diversity has been traced to gene flow with divergent stocks through either natural or man-aided events.

In salmonids, current literature suggests that populations with high mtDNA diversity have been shown to have experienced gene flow with surrounding, but divergent, native stocks (Nielsen et al., 1994; Cronin et al., 1993; Williams et al., in press a) or with non-native stocks (Williams et al., in press b). The very high level of mtDNA diversity detected in Redfish Lake kokanee from Fishhook Creek indicates that the population has something unusual occurring relative to other salmonid populations. While mutations cannot be ruled out, the most likely explanation for such high diversity lies with gene flow. Moreover, where more than a single haplotype is observed, haplotypes typically occur as a predominant haplotype with one or more closely related haplotypes. Clustering of the multiple haplotypes (along with other populations and haplotypes examined in this study) allows one to determine whether the multiple haplotypes form a single closely-related group (suggesting in situ derivation) or are broken into two or more divergent groups (suggesting gene flow with non-native populations). The Redfish Lake kokanee show the latter (Figs. 3 & 4, Table 7) in that all nine observed haplotypes do not form a single closely related group. This suggests that at least some of the high mtDNA diversity in Redfish Lake kokanee from Fishhook Creek appears to be the result of gene flow via man-aided events.

Nevertheless, it is noteworthy that haplotypes S3 - S8 form a single clade in both the distance based analysis (Fitch-Margoliash, 1967) with a constant molecular rate of change and the parsimony analysis. These two analyses use very different algorithms for computation of dendograms. Thus, the concordance of both of these analyses, placing these 6 haplotypes together to form a closely related group, is striking. However, the data do not provide an unequivocal view that their source is either indigenous or introduced to Redfish Lake.

Our overall conclusions must also take into consideration the selection of regions for amplification. Recently, Cytochrome B in particular has been criticized for its use in

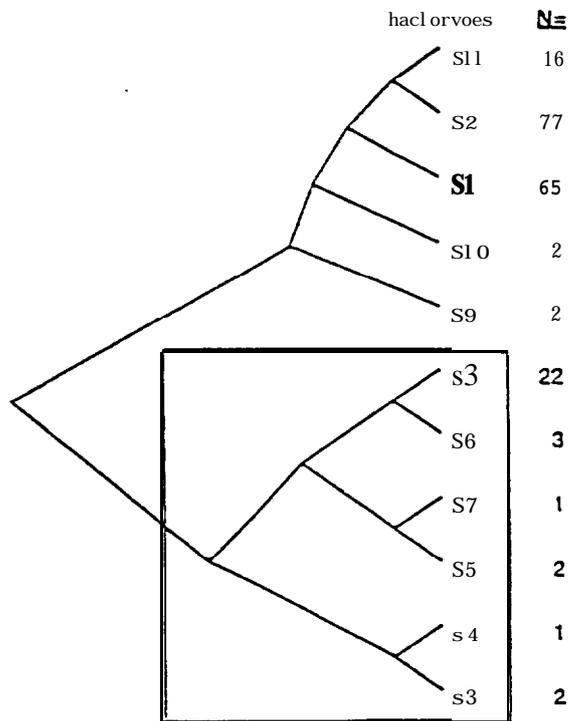


Figure 3. Distance-based dendrogram of *O. nerka* mtDNA haplotypes Fitch-Margoliash analysis with constant molecular clock.

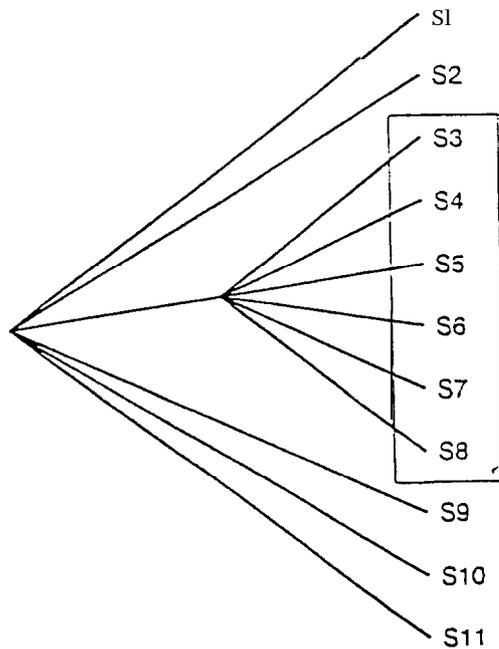


Figure 4. Parsimony cladogram of *O. nerka* mtDNA haplotypes.

phylogenetic work due to its strong sequence conservation throughout vertebrate phyla (Meyer, 1994) and particularly slower evolution in salmonids. Three of the four regions which we examined appear similar with the fourth faster in terms of detectable molecular sequence change. There are several other regions which have been investigated by other authors for salmonids (Thomas and Beckenbach, 1989; Nielsen et al., 1994) and they would be a logical place to enlarge this data set and the overall number of haplotypes. There are other four-base combinations which are unavailable for investigation with this technique due to the lack of a restriction enzyme with that recognition site on the DNA strand. Despite these concerns, we were able to obtain discrimination which has been useful for providing insight into stock origins.

We will be working to increase both sample size within each population and the number of populations. Full screening of all enzymes for each region for each population is also a goal. This will assure that data summarized in this document is leading us to appropriate conclusions. In addition, we hope to investigate the usefulness of microsatellites for addressing this intraspecific differentiation question even more definitively.

Task III - 0. *nerku* Population Life History

The third task, to differentiate 0. *nerku* populations or subunits based on life history characteristics was divided in three parts: a) The rate of embryo development of early and late spawning Fishhook Creek kokanee was compared for differences. b) Gill raker counts of kokanee sampled in 1992 from Snake River populations were compared, and c) behavioral and physiological comparisons were undertaken under laboratory conditions to characterize sockeye and kokanee migratory behavior.

Task III (a) Rate of Development

Egg development data from the 1991 kokanee brood year indicated there was a possible difference in rate of development between samples from the early (22 Aug.) and late (5 Sept.) portions of the Fishhook Creek population when adjusted for temperature differences. Such a difference would indicate that more than one deme exists within Fishhook Creek kokanee. The study was repeated with the 1992 brood and the sample time difference between the early (14 Aug.) and late (8 Sept-) segments was increased to assist in detection of differences.

Ten females were taken from the early portion of the run and each egg lot divided in two and fertilized with a separate male. Eggs were transferred in ice chests to the Eagle

Laboratory and placed in separate containers for incubation in well water at 12°C. Incubation to yolk absorption was assessed in temperature units from biweekly samples of five eggs per container. Dry weight of yolk stores remaining was the criterion evaluated. Rate of yolk absorption between the early and late segments, however, was nearly as great as the difference in the previous brood, but did not demonstrate the expected pattern of compensation, or later spawn requiring fewer temperature units (Fig. 5). The discrepancy between the two years made conclusions **uncertain** about the presence of more than one deme among Fishhook Creek kokanee based on development rates. Observations from the two years were different, which suggests that if more than one deme is present in Fishhook Creek, any given brood year could be homogeneous.

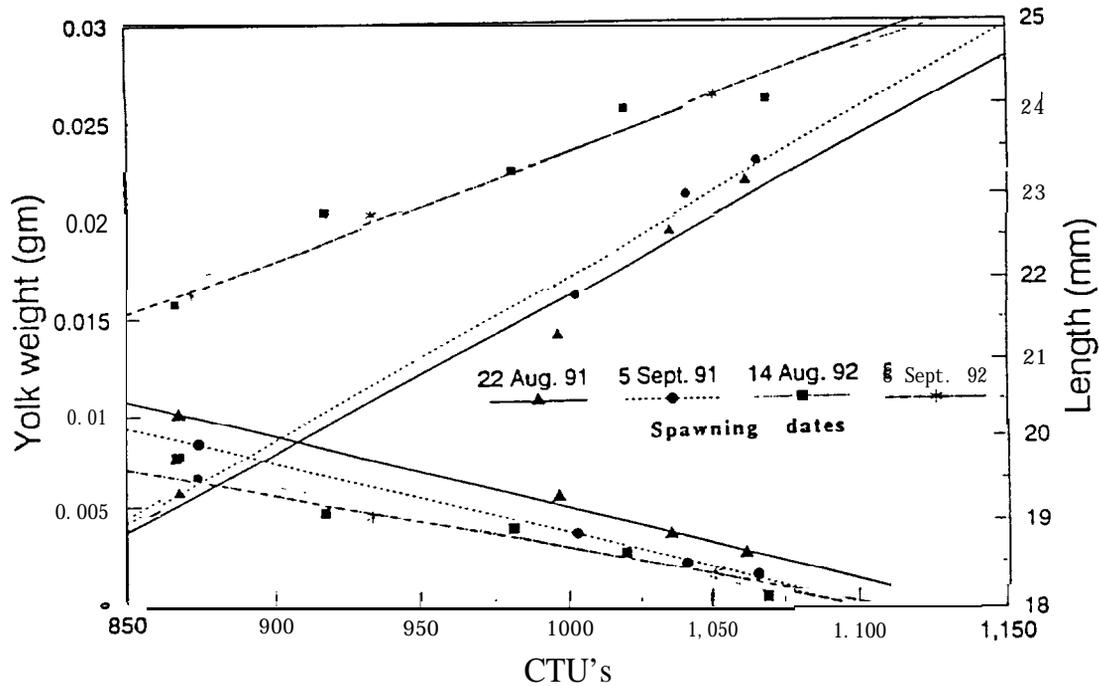


Figure 5. Yolk utilization in Fishhook Creek early and late 1992 kokanee **brood** alevins incubated at Eagle Laboratory under near constant temperatures.

Finally, the development rate of eggs spawned from the one 1992 resident beach spawner and fertilized with the beach spawner males (sampled in Task I) was compared to the sockeye egg development rates of the 1991 spawners to assess differences that could be used to characterize the resident and anadromous forms of *O. nerka* using the beach spawning site. Peak resident beach spawner activity appeared to occur in the first or second week of November, whereas the sockeye beach spawners have been reported to

spawn mid-October, the same as the maturation timing of recent sockeye. Resident beach spawner eggs incubated at a mean temperature of 11.9 °C required 927 centigrade temperature units (CPU) to reach yolk absorption (100 CTU < Fishhook Cr. kokanee). In contrast the sockeye incubated at a mean temperature of about 10 °C required nearly 1100 CTU when adjusted downward to the resident sockeye incubation temperature. The faster incubation rate of larvae from resident beach spawners would compensate for their later spawning time and synchronize spring emergence timing of the fry with that of sockeye.

Task III (b) Kokanee Gill Raker Counts

Gill raker counts were made on kokanee taken at the various locations for DNA samples. Analysis of gill raker counts from the 1992 kokanee samples showed differences between lake systems (Table 9), but their range was overlapping (Fig. 6). The outmigrant gill raker counts were close to Fishhook Creek kokanee. The resident beach spawners were too few to characterize, but appeared similar to Alturas Lake kokanee. Differences between the basin lakes may be valid differences related to the limnological variation among the systems. Generally the number of gill rakers in other kokanee populations is greater than sockeye, and often as high as 39. Snake River kokanee data appear to be lower than expected, especially in oligotrophic conditions associated with the Stanley Basin lakes.

Table 9. Gill raker (1st rt. arch) counts from samples taken from the 1992 spawners.

<u>Population</u>	<u>Count Mean</u>	<u>SD</u>
Stanley Lk	30.2	1.4
Redfish Beach spawners	31.0	1.8
Alturas Lk	31.3	1.8
Redfish LA-Fishhook	33.2	2.0
Redfish Lk Outmigrants	34.1	1.7
Deadwood Reservoir	34.2	1.7
Payette Lk	34.7	2.2
Wallowa Lk	36.2	1.8

Task III (c) Behavioral, Physiological and Genetic Differences Among Anadromous and Resident *Oncorhynchus nerka*

Recovery of the Snake River anadromous sockeye is centered on captive brood stock developed from progeny of sockeye returning to Redfish Lake and from outmigrants leaving the lake. Another potential source from which anadromous stock might be

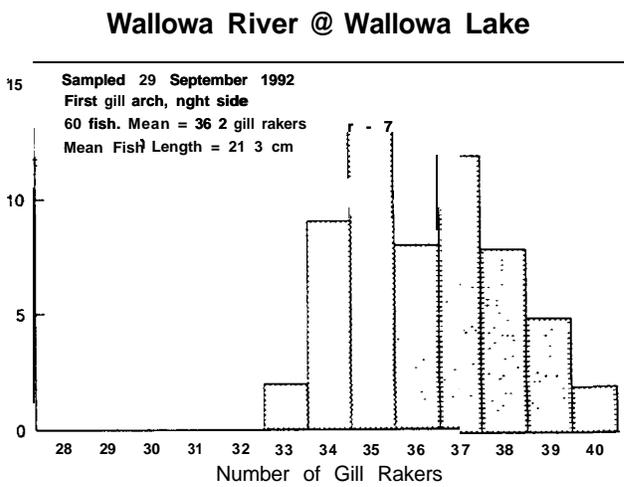
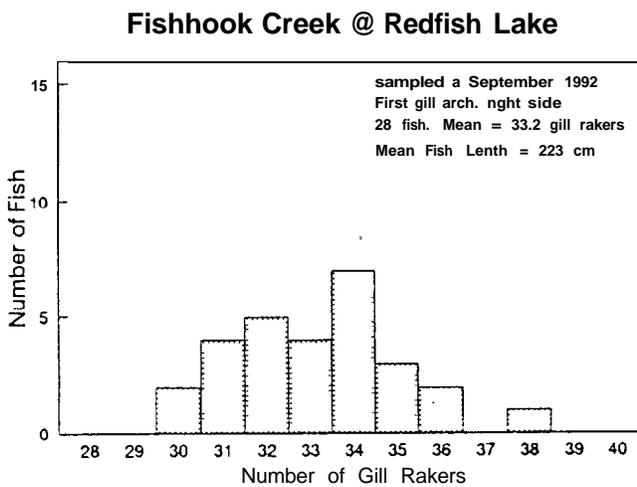
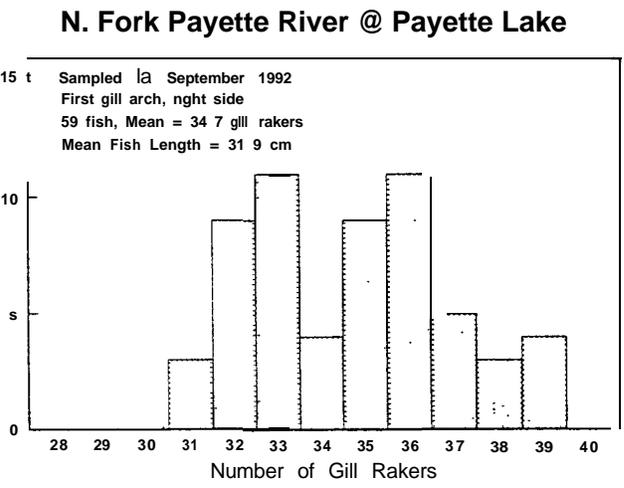
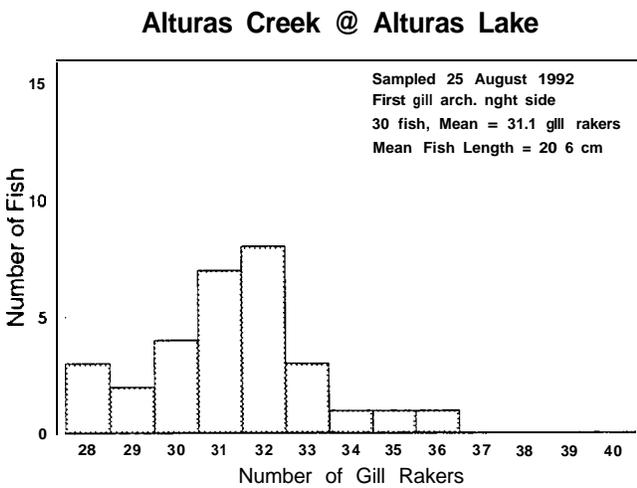
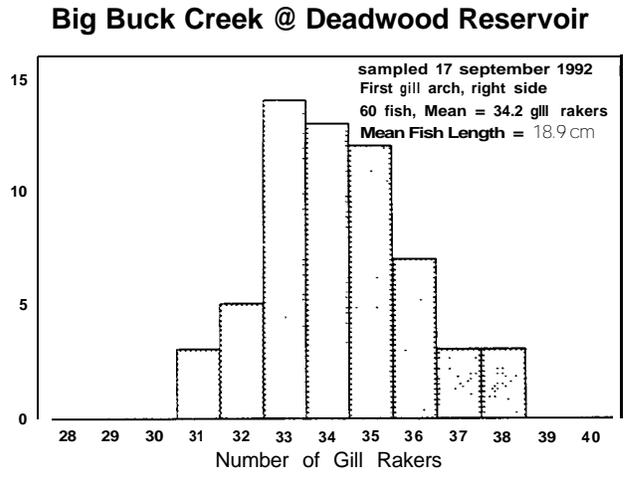
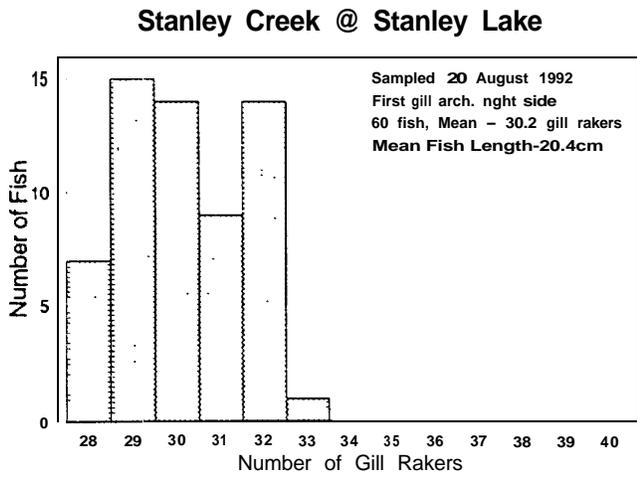


Figure 6. Kokanee gill raker counts from Snake River lake systems.

developed, however, is the kokanee, the resident form of sockeye. Deschutes River kokanee are thought to maintain an anadromous run (Nehlsen et al., 1991) that returns as adult size sockeye.

Although resident sockeye are present in nearly all of the Stanley Basin lakes, none of the populations demonstrate potential anadromy to the extent of those in Redfish Lake, with the exception of Alturas Lake stock. The ultimate fate of these migrants, however, is uncertain, and some may migrate downstream no further than the reservoirs.

The present study to characterize differences between the anadromous and resident forms of sockeye was undertaken to develop a better understanding of what stimulates migratory behavior, and provide information that might assist in initiating migratory behavior among resident sockeye. If kokanee can be induced to contribute migrants, they would be valuable in restoration management of anadromous sockeye populations approaching extinction. Research in Task II (c) was focused on physiological, behavioral, and genetic characteristics of sockeye, kokanee and sockeye/kokanee hybrids.

Task III (c) Methods

In 1991, three groups of kokanee were raised at the University of Idaho Aquaculture Research Institute (UI-ARI). Gametes from kokanee spawned at Fishhook Creek entering Redfish Lake, and Deadwood Reservoir, on a tributary of the Payette River, were incubated at the University or at the facilities of IDF&G at Eagle, Idaho, and later transferred to the lab. Twenty females were spawned at Fishhook Creek, ten from the early segment and ten from the late segment two weeks later, and ten at Deadwood Reservoir.

In 1992, three groups of kokanee, one of sockeye, and one sockeye/kokanee were similarly incubated and raised at the UI-ARI. Wenatchee Lake sockeye from Eastern Washington and Wenatchee sockeye/Deadwood kokanee hybrids were incubated at NMFS in Seattle and transferred to the UI-ARI for rearing. Two of the kokanee groups were from Fishhook Creek, representing the early and late spawners, separated by three weeks, and the third kokanee group was from Deadwood Reservoir. The Wenatchee Lake sockeye were taken from four spawners. The sockeye hybrids were crosses between two Wenatchee Lake sockeye females and twenty males, pooled in lots of ten. All other groups were from ten females, each spawned with a different male.

The three 1991 kokanee groups were kept separated from each other, and reared under identical conditions. Initially, the alevins were reared in 55.8 liter circular tanks each with 1.9 liters/minutes water flow. Water temperature was recorded daily. When the fish reached approximately 2 g, they were transferred into 1,100 liter circular tanks and irrigated with 5.8 liters/minute water flow. Fry were fed approximately 2% body weight/day ration of Oregon Moist Pellet (O.M.P.). Beginning in November of 1992 the late spawned kokanee were fed on a reduced ration at 1% body weight per day to reduce growth rate. Fish growth, mortality rate, total biomass, average individual weight, feed amount and feed conversions were recorded monthly. Fish were kept indoors under electrical lights controlled to simulate natural photoperiod (SNP).

In 1992, all five groups of *O. nerka* were reared under identical conditions as the 1991 study, in separate tanks, with the exception that in November of 1993 flows were increased from 5.8 to 9.0 liters/min. Fish growth and ration size was calculated monthly. Feeding rate, however, was maintained at 1% body weight/day ration of OMP to control growth, approximating Redfish Lake migrant fingerling size.

Physiological Measurements

Smoltification in anadromous salmonids can be quantified using techniques which measure serum Na⁺/K⁺ ATPase levels, serum thyroxine (T3) levels, 24hour saltwater survival of smolts, histological techniques which measure chloride cell densities, external coloration, saltwater preference, and tissue guanine levels (Lam, 1985; Saunders et al., 1985; Blackburn and Clarke 1987; Foote et al., 1992). Physiological studies were conducted only on the 1992 fish, and survival in a 24-hour saltwater challenge test and assessing interlamellar chloride cell density were used to quantify smolt readiness.

Saltwater challenges were conducted by placing four fish from each of the five groups in **30** liters of 3% saltwater for 24hours in a tank containing 30.3 liters of 3% saltwater (Instant Ocean salt solution), and placing a second lot of four fish in freshwater at identical temperature, as controls. After 24hours the number of live fish in each tank was recorded. The 24hour saltwater challenge was conducted every three weeks on the following days: 1 1/29/93, 12/20/93, 01/10/94, 01/31/94, 02/21/94, 03/14/94, 04/04/94, and 04/25/94.

Chloride cell density measurements were undertaken by staining with osmium tetroxide and zinc iodine (Chevalier et al., 1985; Madsen 1985; Madsen and Korsgaard, 1989; Lubin and

Rourke, 1991). Two comparisons of chloride cell densities were made among the five groups. The first comparison was before any group survived the 24-hour saltwater challenge. The second left gill arch was dissected from 20 fish, four from each of the five 1992 stocks, stained, mounted, and the interlamellar chloride cell density determined. The second comparison was made after all groups survived a 24-hour saltwater challenge.

Behavioral Measurements

On March 15, 1993, the 1991 fish were transferred outdoors and put into separate 1,870 liter circular tanks under natural light for migratory behavioral observations after Schadt (1985) and Whitman (1987). The standpipes were modified by adding a 45° elbow to the top concentrating the spill of the exit flow. The fish were observed for migratory behavior (e.g., drifting or moving with the current, swimming near the surface) and the number of fish exiting through the modified standpipe was recorded. Outmigration started within hours after the fish were introduced in the tank.

On February 15, 1994, the 1992 fish were transferred outdoors to the 1,870 liter circular tanks for observations of migratory behavior. The 1994 study started earlier to preempt migratory behavior. The five groups were each fin clipped for later identification, mixed into three identical groups, and replicate groups placed in the three separate tanks. In addition to the 45° elbow, cylinder cowling was placed around the standpipe to create an additional barrier before exiting the tank. The cowling proved too good of a barrier and after 2 weeks (2/15/94 - 3/1/94) the cowling was removed. The number of smolts migrating from each of the tanks was recorded daily. A chi-square test of homogeneity was used to determine if the proportion of any stock migrating out of the tank was different from the proportion of that stock originally put into the tank-

Genetic Measurements

DNA fingerprinting was undertaken and the degree of band sharing was calculated to determine if quantifiable characteristics could differentiate between anadromy and resident behavior in both sockeye and kokanee. **DNA** mixes were used for the comparisons (Spruell et al., 1994) as described above. The samples were (1) cut with restriction endonuclease, **Hae** III, and probed with Jeffrey's 33.6 oligonucleotide probe, (2) cut with restriction endonuclease, **Dpn** II, and probed with Hpa I oligonucleotide probe, and (3) cut with restriction endonuclease, **Dpn** II, and probed with Per oligonucleotide probe described previously under nuclear DNA.

Task III (c) Results

Growth, physiological, behavioral and genetic characteristics of the 1991 and 1992 test groups of *O. nerka* from Redfish Lake and Deadwood Reservoir in Idaho, and from Wenatchee Lake, Washington, showed definite differences. Growth performance can be highly variable and an extensive assessment of growth was not the subject of this study. Comparisons were made only within brood years, and examined just for relative performance when reared under the same conditions. Physiological assessment was limited to smoltification. The behavioral tests were also limited to migratory readiness, and the genetic analysis examined stocks for relatedness and for differences between migrators and non-migrators.

Growth

A one way ANOVA comparing the mean individual weights of 1991 fish at smoltification, showed significant differences ($p < 0.002$). Deadwood Reservoir kokanee grew from 5.07 g to 44.7 g **in** 11 months, and appeared to demonstrate an inherently slower growth rate than the early Fishhook Creek stock which grew from **3.6** g to 51.6 g in 11 months. Late Fishhook Creek kokanee grew from 3.6 g to 49.8 g over the same time period and were not markedly different than the early stock.

The 1992 fish also showed significant weight differences ($p < 0.001$) at the lower ration size (1% body weight/day). Wenatchee Lake sockeye grew largest, from 0.48 g to 12.4 g in 15 months, followed by sockeye hybrids from 0.35 g to 10.4 g. The early Fishhook Creek kokanee grew from 0.73 g to 10.2 g, followed by the late Fishhook Creek kokanee from 0.33 g to 8.7 g in 15 months. Deadwood Reservoir kokanee were again the smallest, growing from 0.45 g to 7.7 g over the 15 months.

Physiological Tests: Saltwater Challenge

Every three weeks from 1/29/93 through 4/25/94, a 24-hour saltwater challenge was performed on a sample of fish from each of the 1992 groups. No fish survived 24 hours in 3% saltwater in tests from 11/29/93 through 1/31/94 (Table 10). On 2/21/94, 100% of the Wenatchee Lake sockeye survived 24 hours; 67% of the sockeye hybrids survived; 50% of the Deadwood Reservoir kokanee; 75% of the late Fishhook Creek kokanee; and 0% of the early Fishhook Creek kokanee survived. The test was repeated on 2/25/94 in order to verify saltwater tolerance. Again 100% of the Wenatchee Lake sockeye survived, but different percentages of the other stocks survived. The results were complicated by the small sample sizes. A chi-square test of homogeneity did not reveal significantly increased

survival in any stock between the two dates. However, over the next six weeks all groups were able to survive 24-hours in the saltwater.

After each 24-hour saltwater challenge, the weights and lengths of each fish were recorded. A student's t-test, two-sample test assuming equal variances, comparing mean weights and lengths between survivors and non-survivors on 2/21/94, 2/25/94 and 3/14/94 showed a significant difference ($p < 0.02$) between the mean weights and lengths of survivors and succumbing fish (Table 11). Surviving fish were significantly heavier and longer than non-surviving fish. The size difference between surviving and non-surviving fish, created a confounding factor between 24-hour saltwater adaptability and stock origin.

Physiological Tests: Chloride Cell Density

After the 4/25/94 saltwater challenge, fish gills were dissected, stained, and interlamellar chloride cell densities were calculated on an average of 150 interlamellar spaces per fish. A single factor ANOVA comparing interlamellar chloride cell densities between the stocks revealed a significant difference ($p < 0.001$) between stocks. Mean comparisons were then calculated using Fisher's Least Significant Differences (Ott, 1993). Wenatchee Lake sockeye, hybrids, Deadwood Reservoir kokanee, and late Redfish Lake kokanee from Fishhook Creek had a significantly higher interlamellar chloride cell density than early Fishhook Creek kokanee. Late Fishhook Creek kokanee and Wenatchee Lake sockeye had a significantly higher interlamellar chloride cell density than either Deadwood Reservoir kokanee or the hybrids (Fig. 7).

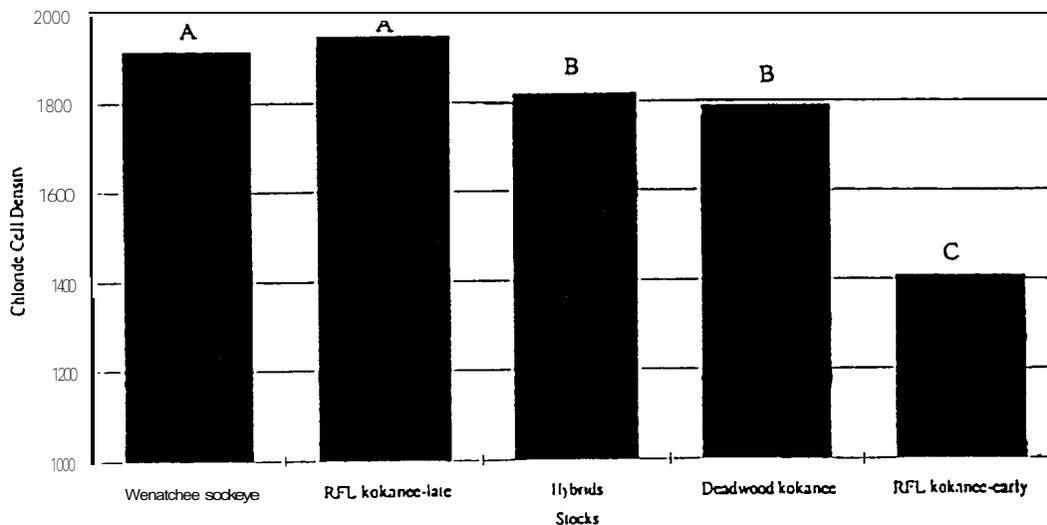


Figure 7. Mean interlamellar chloride cell density for each 1992 O. nerka stock after each stock survived 24-hour salt water challenge.

Table 10. Survival of 1992 O. nerka stocks to 24-hour saltwater challenge.

Date	Wenatchee Sockeye		Hybrids		Deadwood Kokanee		RFL-Early		RFL-Late	
	Number	Survive	Number	Survive	Number	Survive	Number	Survive	Number	Survive
11/29/93	4	0%	4	0%	4	0%	4	0%	4	0%
12/20/93	4	0%	4	0%	4	0%	4	0%	4	0%
1/10/94	4	0%	4	0%	4	0%	4	0%	4	0%
1/31/94	4	0%	4	0%	4	0%	4	0%	4	0%
2/21/94	3	100%	6	67%	4	50%	4	0%	4	75%
2/25/94	3	100%	4	25%	5	0%	4	50%	4	0%
3/14/94	2	100%	6	100%	7	86%	11	54%	6	100%
4/04/94	2	100%	2	100%	3	100%	10	100%	14	100%
4/25/94	6	100%	6	100%	6	100%	6	100%	6	100%

Table 11. Mean weights and lengths during 24-hour saltwater challenge.

Stock	Date	Mean Weight (gr.)		Mean Length (mm.)	
		Survive	Succumb	Survive	Succumb
W Sockeye	1/31/94	---	7.0	---	101.5
	2/21/94	7.0	---	105.0	---
	2/25/94	6.3	---	95.3	---
	3/14/94	6.5	---	97.5	---
	4/04/94	11.0	---	103.0	---
Hybrids	1/31/94	---	5.5	---	91.3
	2/21/94	4.5	1.5	88.0	70.0
	2/25/94	6.0	4.0	97.0	79.0
	3/14/94	5.8	---	94.3	---
	4/04/94	9.5	---	105.0	---
Deadwood	1/31/94	---	4.8	---	87.8
	2/21/94	3.5	2.5	87.5	75.5
	2/25/94	---	4.5	---	83.4
	3/14/94	5.0	4.0	90.0	84.0
	4/04/94	8.7	---	70.3	---
RFL-Early	1/31/94	---	5.3	---	86.8
	2/21/94	---	5.3	90.3	88.7
	2/25/94	7.5	5.5	93.0	---
	3/14/94	9.0	6.4	91.8	89.0
	4/04/94	9.5	---	103.6	---
RFL-Late	1/31/94	---	---	---	86.8
	2/21/94	4.7	---	88.7	85.0
	2/25/94	---	4.0	---	87.7
	3/14/94	4.8	4.0	89.0	---
	4/04/94	9.0	---	101.0	---

A combination of 0.2% acid fuchsin/0.12% methylene blue stain provided an excellent contrast between the Wenatchee Lake sockeye gill filament specimens and the rest of the fish. The Wenatchee Lake sockeye gill filaments were in healthy condition following the 24-hour saltwater challenge prior to 100% tolerance. Other stocks had gill filaments and especially lamellae that appeared fluid filled and swollen from exposure to saltwater. The squamous epithelial cells of the lamellae separated from the pillar cells in many areas.

Behavior

The three 1991 groups transferred to migration tanks outside the UI-ARI on March 15, 1993, were kept outside until June 3, 1993. Numbers, weights, lengths, and condition factors of fish exiting the tanks were recorded. Two factor ANOVA's using unweighted means were performed on the data (Howell, 1982) from migrators and non-migrators. There was a significant weight and length difference ($p < 0.001$) between the migrators and non-migrators. Migrators were larger than non-migrators in 1993. However, there was no significant difference in condition factors between the stocks nor a significant condition factor difference between migrators and non-migrators. It was interesting that the number of 1993 migrators from each of the three tanks was low (7%). A chi-square test of homogeneity was conducted on the data to determine if one stock migrated significantly more than the others, and there was no evidence that the proportion of any stock migrating was different from any other stock.

The five 1992 groups were fin clipped and transferred to migration tanks outside the UI-ARI on February 15, 1994. The fish were kept outside until May 10, 1994. During that time the numbers, weights, lengths, and condition factors of fish exiting the tanks through the modified standpipe were recorded (Table 12). Three separate two factor ANOVA's using unweighted means solution were performed on the weights, lengths, and condition factors comparing stocks and migration behavior (Howell, 1982). There were significant length ($p < 0.01$) and weight differences ($p < 0.01$) between migrators and non-migrators. The migrating fish were significantly smaller than non-migrating fish in 1994 (Fig. 8), and had a significantly lower condition factor (< 0.001) than non-migrators.

Genetics

The final comparisons between groups of 0. *nerka* and between migrating fish and non-migrating fish compared **mixed** stock DNA fingerprints. Fingerprint comparisons were made on both the 1991 and 1992 0. *nerka* stocks. While analysis showed identity of the different stocks sampled, DNA mixes showed no unique bands that characterized or

appeared in all migrating or non-migrating fish. Migratory/non-migratory behavior was not correlated with a specific banding pattern.

Table 12 Mean weight, mean length and mean condition factor of 1994 *O. nerka* migration behavior test

Stock (1992)			Mean		
	Number	%	Weight (gr)	Length (mm.)	Cond. Factor (gr/mm ³)
Wenatchee Sockeye-Migrate	323	63%	10.9	112.2	0.76
Non-migrate	187	37%	14.6	120.4	0.82
Hybrids-Migrate	525	66%	7.7	100.1	0.75
Non-migrate	267	34%	10.3	111.7	0.77
Deadwood Kokanee-Migrate	496	54%	8.9	104.7	0.72
Non-migrate	425	46%	13.6	117.1	0.83
RFL Kokanee-Early-Migrate	590	66%	6.9	97.2	0.72
Non-migrate	307	33%	9.2	103.3	0.81
RFL Kokanee-Late-Migrate	656	63%	8.9	105.3	0.75
Non-migrate	379	37%	12.2	114.0	0.81

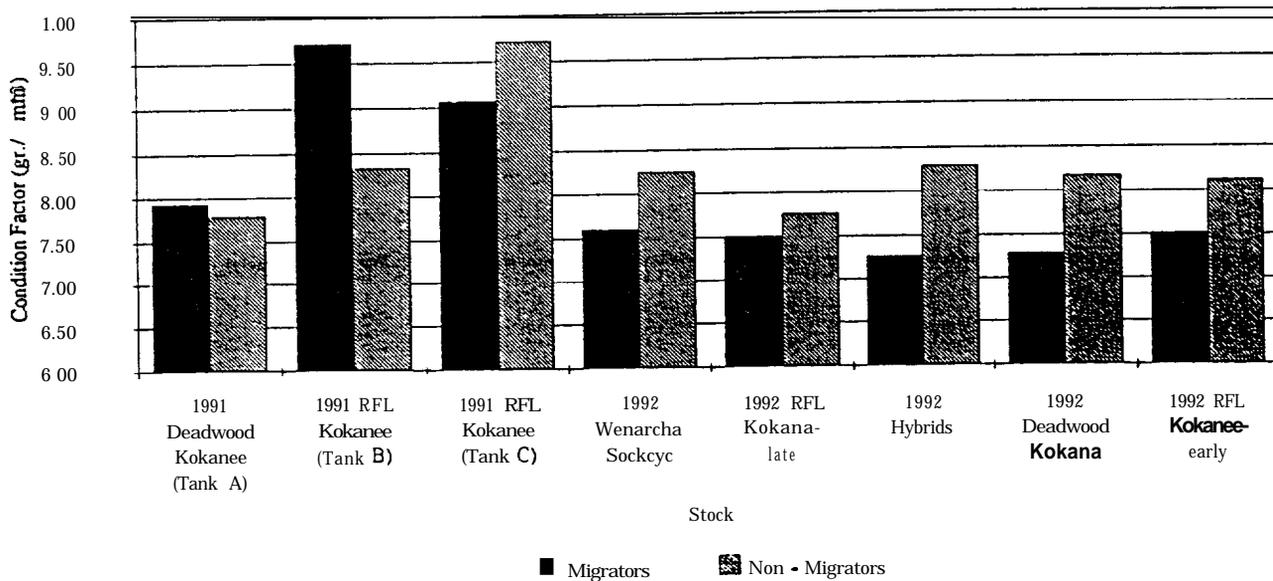


Figure 8. Mean condition factor of *O. nerka* stocks separating migrants and non-migrants. In six of eight stocks, migrants had lower condition factor than non-migrants.

Task III Discussion

Development rates of the early and late run segments of Fishhook Creek kokanee showed little evidence of compensation for time of spawning. This would suggest that if separate demes are present within the population of Fishhook Creek kokanee, it is not readily apparent by their incubation strategy. Of course, if the window of opportunity for optimum spring emergence timing is variable with patterns of ice coverage, there may be no reason to compensate for time of spawning when the range in spawning time is effectively no longer than three or four weeks.

Gill raker differences among the stocks sampled were quite marked. Such a difference is curious from the standpoint of Redfish Lake. The resident beach spawners had low counts compared to the outmigrants, in which apparently beach spawner progeny are well represented. Sample size of beach spawners was too low to provide confidence in the counts, but the number of gill rakers in beach spawners was low enough that one would not attribute their contribution to the outmigrant population as plentiful. The range of counts among the sampled populations would suggest that populations were well established and demonstrated long-term isolation.

With regard to physiological differences among the stocks tested, some responses were unexpected. Sympatric *O. nerka* stocks in saltwater challenges during different seasons have shown a circannual cycle of increased survival during the spring and decreased survival during late summer and fall (Foote et al., 1992). In this study, sockeye, kokanee, and sockeye/kokanee hybrids each exhibited a circannual cycle. Sockeye exhibited saltwater adaptability before the hybrids and kokanee as shown by Foote et al. (1992).

It was interesting that all of the stocks tested demonstrated some tendency toward migratory behavior as defined by the criteria used in this study. The kokanee were by all the tests conducted, not markedly different from sockeye, with the exception of their lower growth rates. Over the course of the study, there were differences attributed to stock origin, but not to anadromous or resident life history.

The fish fed 2% body weight per day were three times larger than even the largest sockeye smolts known over their Pacific range. These large fish showed low migratory tendencies in the migratory tests, which may have been because of their large size, but perhaps they were tested too late and had already begun to revert to fresh water preference by the time of

the first tests. Since size is a factor that affects timing of migratory readiness within a population, their abnormally large size may have readied them for migration earlier than March.

Growth rate was reduced in the 1994 studies, and migratory tendencies were tested earlier in the season than the previous year. By March, 1994, at the beginning of the migration season, these fish were equivalent in size to large natural smolts in Redish Lake. The pure sockeye had grown significantly larger than any of the other stocks. The sockeye hybrids were intermediately sized between pure sockeye and pure Deadwood kokanee, as expected.

It is noteworthy that in 1994, the early Fishhook Creek kokanee were significantly larger than the late Fishhook Creek kokanee. The late group, however, showed earlier saltwater tolerance and a higher chloride cell density than the earlier group, which appeared to demonstrate that these groups are separate sub-populations with different behavior patterns that may have a genetic basis. These results are consistent with the 1991 embryo development rate differences that suggested the presence of sub-populations differentiated by spawning time.

The ability of each of the 1994 stocks to survive a 24-hour saltwater challenge was also varied. Foote et al. (1992) reported that there was a difference in the timing of saltwater adaptability in pure sockeye, sockeye hybrids, and pure kokanee. Sockeye developed saltwater adaptability earliest in the spring, before sockeye hybrids and kokanee showed any saltwater tolerance. In this study, 1994 groups were saltwater challenged every three weeks from November 1993 through April 1994. No fish survived the 24-hour saltwater challenges until 2/21/94. In fact, during the first challenge (1/29/94), all fish were dead within 2 hours of the beginning of the test. Clearly not even the pure sockeye were ready for marine existence. By 1/31/94 some of the fish lived overnight, but died by the 24th hour. On 2/21/94, 100% of the pure sockeye test subjects survived 24 hours in the saltwater, while only a portion of the sockeye hybrids and pure kokanee survived. However, eventually all groups survived saltwater tests, irrespective of any anadromous parentage.

These data showed a trend consistent with the Foote et al. (1992) results in that sockeye smolted earliest. However, timing may be simply a stock variable, and not related to sockeye or kokanee parentage. Sockeye were also largest, and the difference in stock

timing of saltwater tolerance may have been a size dependent response. certainly, within the present study, the largest fish within a group were first able to withstand saltwater. Early Fishhook Creek kokanee were the exception. While nearly as large as sockeye, they showed the latest onset of saltwater tolerance.

Interlamellar chloride cell density also showed variation consistent with life history differences. Wenatchee Lake sockeye had a significantly higher interlamellar chloride cell density than the sockeye hybrids, Deadwood Reservoir kokanee, or early Fishhook Creek kokanee. The hybrids had the same interlamellar chloride cell density as their Deadwood Reservoir kokanee paternal stock.

Again, it is noteworthy that the late Fishhook Creek kokanee had interlamellar chloride cell densities equal to the sockeye. The fact that they showed such similarity to sockeye may indicate they have greater potential to contribute to the anadromous form than the early Fishhook Creek kokanee. Moreover, closer similarity of the late Fishhook Creek kokanee with sockeye, in contrast to the early Fishhook Creek kokanee, may reflect greater gene sharing with the anadromous beach spawners, because it is this segment of the kokanee population that would first experience potential overlap with the later beach spawners.

The differences in readiness to migrate between the 1991 and 1992 broods appears size related, with the larger fish less ready to leave the lake than fish of normal smolt size. In 1993, about 7% of the fish left the tanks, where as in 1994 over 60% migrated, and all showed as much migratory tendency as the pure sockeye.

O. nerka decrease their condition factor when getting ready to smolt (Hoar, 1985). The weight, length, and condition factor of the fish that had migrated out of the tanks compared to their non-migrating counterparts was significantly less. Migrating fish were significantly shorter and lighter in six of the eight groups, and with a significantly lower condition factor in five of the eight groups. These results indicate that one of the factors responsible for kokanee periodically assuming migratory behavior in natural systems may be associated with growth, reduced ration, or crowding.

The genetic comparisons between stocks of *O. nerka* and between migrating and non-migrating fish demonstrated the usefulness of the mixed DNA fingerprinting technique for analysis of population differences. Mixed DNA fingerprints provided a population profile of the individuals, but showed no distinct differences between migrating fish and non-

migrating fish. There were no banding pattern differences that could be associated with migratory behavior.

Physiological comparisons between pure sockeye, sockeye hybrids, and pure kokanee, lead to the conclusion that characteristics necessary to become anadromous are retained in the kokanee population. Different stocks of kokanee demonstrated markedly different patterns among the parameters tested, but this should not be interpreted to mean that such differences indicate progressive separation between anadromy and resident tendencies. Even after isolation in Kootenay Lake from anadromous forms for several thousand years, Kootenay Lake kokanee retained the season cycle in seawater adaptability (Foote et al, 1994). Although migratory tendencies may be reduced in some resident populations, there is no evidence that would indicate resident forms denied successful migration eventually lose all anadromous tendencies. To the contrary, there is good reason to believe that environmental circumstances favor polymorphism in *O. nerka*, *with the* balance shifting often enough to perpetuate both forms. It is more likely that the differences observed in the present data demonstrate that stock responses are site specific to environmental stimuli, and in their home range the right combination of variables will periodically motivate the appropriate responses. Sockeye definitely were at one end of the range of responses observed, but in each case there were kokanee that were as responsive as the sockeye.

Summary:

The capture of kokanee on the sockeye spawning beach verified that at least a third *O. nerka* population resides in Redfish Lake. The population, referred to as resident beach spawners, spawns later but very near the timing observed for sockeye at that location. Their population size doesn't appear to be more than five hundred. They spawned in 1992 in a limited section of the beach and were observed in an area estimated to cover 2000 m². Cleaned gravel was noted in only three or four locations in that area, ranging from 0.2 to 1.5 m².

Fairly large numbers of *O. nerka* transplanted in Redfish Lake raises the issue of stock purity within the **system**. However, the various forms of *O. nerka* in Redfish Lake are definitely closely related and different from geographically more distant populations. On the local scale, sufficient differences exist in both nuclear and mtDNA among Redfish Lake forms of *O. nerka* to indicate that different stocks are represented by Fishhook Creek kokanee and sockeye beach spawners. DNA patterns show differences that are functional in separating sockeye from kokanee among outmigrants from Redfish Lake.

On the broader picture, Redfish Lake and Alturas Lake populations of *O. nerka* are very similar, and significantly different from *O. nerka* populations sampled elsewhere in the Columbia or outside the Columbia River Basin. Stanley and Pettit Lake kokanee, however, were atypical of the other Stanley Basin populations, and since Stanley Lake was eradicated and subsequently restocked with fry from sources outside the Basin, it appears that the original population may have been altered by transplants. In general, the differences observed between Stanley Basin populations and geographically distant sockeye or kokanee populations are extensive and testify of a close relationship among the Stanley Basin *O. nerka* populations.

The conclusion that the beach spawning sockeye and resident beach spawners are identical stocks, however, would not be warranted. While the resident beach spawners appear remarkably close to the sockeye, and may show strong similarity with the outmigrant DNA patterns, there is good reason to believe they are more distantly related to their progenitor anadromous form than what would be described as residuals separated by one generation. The beach spawner resident *O. nerka* forms appear temporally displaced from the beach spawning sockeye by a week or two. Moreover, while they have created a niche by spawning later (early November) than sockeye (mid-October), they have had to increase development rate per unit of temperature (150 CPU less than the anadromous sockeye) to still have the same window of opportunity for emergence timing in the spring as the sockeye.

One would expect them to have greater success by adopting such a strategy because they can avoid having their redds dug up by the deeper digging sockeye, and they can then spawn on the sockeye redds which provide much easier digging than harder substrate, undisturbed by the sockeye. Thus, they have the security of being able to avoid competition with sockeye for spawning area, and can bury their eggs deeper because of the prepared substrate. Also, the beach spawning resident stock appears to spawn primarily at night which would be a behavioral adaptation to avoid predation by diving birds, and a pattern not expected of fish recently separated from the anadromous strain.

The biochemical alteration demonstrated by an accelerated development rate is the strongest evidence that these resident forms are not first or second generation descendents of the anadromous stock, but have been separated long enough to have evolved a behavior pattern that is characteristic of a different population. Egg size has been shown by Linley (1993) to be a very conservative trait. While egg size of kokanee is generally smaller than in the anadromous form, their egg size remains nearly as large as the anadromous strain, whereas fecundity in the smaller fish is reduced proportionally to their size from that of sockeye. Moreover, embryo size is reduced nearly proportional to the reduction in egg size, resulting in about the same time required for yolk absorption by both large and small embryos within a population. Therefore, differences in development rates between beach spawning sockeye and the resident forms cannot be explained just by differences in egg size alone. Adaptive separation between the beach spawning sockeye and resident form suggests that the latter could be considered another Redfish Lake kokanee population, but still closely related to, and most representative of, their progenitor anadromous sockeye.

While kokanee show a much reduced migratory tendency than sockeye, and demonstrate different degrees of genetic segregation from their progenitors depending on the extent of isolation, they should not be considered an evolutionary unit separate from sockeye. The fact that they exist as a component part of nearly all sockeye systems and can produce migrants, speaks strongly of their capacity to develop anadromous forms under certain circumstances, and a potential that continues to be demonstrated (Kaeriyama et al., 1992; Foote et al., 1994).

Moreover, sockeye colonizing new lake systems may very well produce resident forms as first generation progeny. Migratory orientation is largely genetic (Groot, 1965) and when the outlet of the adopted lake is located in a direction markedly different from that of the parent lake, smolts predisposed to migrate to the outlet of their parental lake will trap themselves behaviorally, and residualize in the new system. Residuals, therefore, may remain resident

for several generations before producing enough variation in their spring orientation behavior to successfully exit the lake and establish a new anadromous run, especially if it is a complex lake system. Residual populations created in such a manner will still retain anadromous tendencies and, given the appropriate stimuli, redevelop through selection an anadromous strain, preadapted for their adopted system.

Kokanee in Fishhook Creek demonstrate sufficient temporal separation to isolate them from the present beach spawning forms in Redfish Lake. Strategy to recover Redfish Lake sockeye, therefore, should not exclude the opportunity to reestablish the Fishhook Creek sockeye strain as the other primary sockeye population in the lake. Waiting for natural events to reestablish the Fishhook Creek strain, however, would most likely be impractical under the severe mortality experienced by migrants in the Snake and Columbia rivers. Inducing migratory behavior by kokanee/sockeye crosses would assist the process by encouraging a larger number of migrants from the kokanee population. Success inducing migratory behavior in Lake Ozette kokanee by using sockeye sperm (LaRiviere, 1993) provides supportive evidence to justify such an attempt to stimulate anadromy among Fishhook Creek kokanee, and thus take advantage of the preadapted genes for Fishhook Creek timing rather than using other stock sources. Results from the present studies demonstrated that stock hybrids were intermediate in some of the characteristics of sockeye and kokanee, as expected, and the late Fishhook Creek kokanee were already very similar to the sockeye in salt tolerance and migratory readiness.

The amount and quality of spawning/incubation habitat available in Fishhook Creek is far superior to the sockeye beach as a spawning area for sockeye. It is suggested that if pre-1900 populations of anadromous sockeye numbered in excess of 5000, it would have been possible only if Fishhook Creek was used as their major spawning area. The poor quality of spawning habitat on the beach with the lack of upwelling incubation flows would appear to eliminate that area as an effective spawning habitat to sustain high spawner densities.

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