Independent Populations of Sockeye Salmon in Lake Ozette

Posted - March 31, 2008

Last Revisions - June 7, 2004

Draft for Public Review

DRAFT

The Puget Sound Technical Recovery Team:

K. Currens, Northwest Indian Fisheries Commission; R. Fuerstenberg, King Co. DNR; W. Graeber, Washington DNR; K. Rawson, Tulalip Tribes; M. Ruckelshaus and N. Sands, NMFS–Northwest Fisheries Science Center; and J. Scott, Washington Dept. of Fish and Wildlife

DRAFT

EXECUTIVE SUMMARY

The first task of the Puget Sound Technical Recovery Team (TRT) in developing a recovery plan for Lake Ozette sockeye salmon is to delineate independent populations within the evolutionarily significant unit (ESU). In this context, NOAA Fisheries defines an independent population following Ricker's (1972) definition of a "stock," which is a group of fish of the same species that spawns in a particular lake or stream (or portion thereof) at a particular season and which, to a substantial degree, does not interbreed with fish from any other group spawning in a different place or time. For the TRT's purpose, to "not interbreed" to a "substantial degree" means that spawning aggregations are isolated to such an extent that exchanges of individuals among the populations do not substantially affect the population dynamics or extinction risk of the different groups (independent populations) over a 100-year time frame.

The Puget Sound TRT reviewed geographical, migration, genetic, life history, demographic, and habitat characteristics of anadromous sockeye salmon in Lake Ozette. We concluded that extant spawning aggregations in Lake Ozette are different subpopulations within a single population. It seems likely that differences also existed historically among other subpopulations, which have since been extirpated, either at different spawning beaches or in tributaries. In addition, significant genetic differences may have evolved between fish from different brood cycles as a result of the temporal isolation between spawning adults that are almost exclusively four years old.

Genetic data provided the best evidence of isolation among aggregations, but we considered it weak evidence of independence because of the magnitude of the differences. Estimates of F_{ST} (a measure of genetic divergence) and migrants per generation between beach spawning aggregations ranged from 0.007 - 0.015 and 31 - 73, respectively. By comparison, differences between fish from the same spawning area but different brood cycles were as large or larger. Times since divergence between different natural spawning aggregations from the same brood cycle on Olsen's Beach and Allens Bay (3 – 12 generations) were generally similar to estimates between Umbrella Creek hatchery fish and Olsen's Beach, where most of the brood stock originated 1-3 generations ago. Estimates of time since divergence from the genetic data corresponded closely with known times of divergence between Olsen's Beach 1996 and Umbrella Creek and Olsen's Beach 1996 and Olsen's Beach 2000, which gave us confidence in other estimates. Independent populations under our definition would have diverged approximately 25 or more generations ago.

Both recently collected DNA data and older allozyme data, however, showed measurable divergence between spawning aggregations. In addition, although the inferences were necessarily weaker, potential differences in peak spawning time between beach spawning aggregations and differences in incubation temperatures between beach and tributaries suggested that subpopulations exist in Lake Ozette now and probably were more extensive historically.

TABLE OF CONTENTS

EXECUTIVE SUMMARY iii

LIST OF FIGURES v

LIST OF TABLES vi

INTRODUCTION 1
Definition of a Population Used in This Approach 1
Indicators of Population Structure 2
Data Quality 4

METHODS AND RESULTS 4
Geographic Distribution 4
Genetic Attributes 6
Past Analyses 6
New Analyses—Methods 6
New Analyses—Results 7
Life History Characters and Spatial Synchrony 11
Habitat Characteristics 12
Population Structure Decisions 16

CONCLUSIONS 17
Data needs and remaining uncertainties 17

LITERATURE CITED 18

DRAFT

LIST OF FIGURES

Figure 1. Sockeye salmon spawning areas in the Lake Ozette ESU. 5

Figure 2. Genetic similarity of sockeye and kokanee salmon aggregations in Lake Ozette based on nine microsatellite loci..... 9

Figure 3. Multidimensional scaling of coancestry genetic distances between Lake Ozette sockeye salmon. 10

Figure 4. Timing and abundance of lakeshore spawning sockeye salmon in 1978-1979 spawning season (from Dlugokenski et al. 1981)..... 12

Figure 5. Seasonal changes in temperature for Lake Ozette (adopted from Beauchamp et al. 1995). 14

Figure 6. Water temperatures in the Ozette River and three inlet tributaries of Lake Ozette (adapted from Bortleson and Dion 1979)..... 15

DRAFT

LIST OF TABLES

Table 1. P-values from pairwise chi-square tests for allelic homogeneity for nine microsatellite loci (below the diagonal) and F_{ST} values with 95% confidence intervals (above the diagonal) for *O. nerka* in Lake Ozette..... 8

Table 2. Migrants per generation (above the diagonal) and time since divergence, t, (below the diagonal) for sockeye salmon in Lake Ozette..... 11

Table 3. Evidence and strength of inference for population structure in Lake Ozette sockeye salmon. 16

DRAFT

INTRODUCTION

The first task of the Puget Sound Technical Recovery Team (TRT) in developing recovery plans is to delineate independent populations within each of the three evolutionarily significant units (ESUs) assigned to us. Understanding the size and spatial extent of populations is critical for the viability analyses that are a necessary step in recovery planning and conservation assessments for any species. This report describes the delineation of populations and subpopulations for the Lake Ozette sockeye salmon ESU. The TRT has completed this delineation for Puget Sound chinook salmon and an additional report will address Hood Canal summer chum.

The populations we identify represent what we believe to be the historical populations of sockeye salmon within the Lake Ozette ESU. Anecdotal evidence suggests spawning aggregations of sockeye salmon in different areas of the lake and its tributaries may have gone extinct. Little data is available to determine whether these were independent populations. For each of the independent populations that we identify in this document, the TRT will in future documents characterize its present and historical status and viability and describe targets for abundance, productivity, life history and phenotypic diversity, and spatial distribution for spawning and rearing. These are necessary to answer the question, "What are necessary population characteristics that will add up to persistence of the ESU?"

Definition of a Population Used in This Approach

The definition of a population that we use in this report is described in detail in the viable salmonid population (VSP) document (McElhany et al. 2000). The VSP document defines a viable salmonid population as

> …an independent population of any Pacific salmonid (genus *Oncorhynchus*) that has a negligible risk of extinction over a 100-year time frame due to threats from demographic variation (random or directional), local environmental variation, or threats to genetic diversity (random or directional).

In this context, NOAA Fisheries defines an independent population following Ricker's (1972) definition of a "stock," which is a group of fish of the same species that spawns in a particular lake or stream (or portion thereof) at a particular season and which, to a substantial degree, does not interbreed with fish from any other group spawning in a different place or in the same place at a different season. For our purposes, to "not interbreed" to a "substantial degree" means that they are isolated to such an extent that exchanges of individuals among the populations do not substantially affect the population dynamics or extinction risk of the different groups (independent populations) over a 100-year time frame.

In many animals, reproductive isolation (or the level of "not interbreeding") can vary from very little, which occurs between pairs of fish in the same spawning aggregation, to nearly complete and permanent isolation, which occurs between different biological species. The "independent population" defined here, therefore, is a group of fish that can be identified by looking at measures of reproductive isolation. Within

independent populations, for example, groups of fish that are separated geographically or temporally may show some level of reproductive isolation but they are not isolated enough to meet the criteria for independence used here. The distribution and differences among these groups of fish, which we consider "subpopulations," has important consequences for characterizing the whole VSP, because they affect the spatial distribution and diversity of the whole population, which are two of key parameters for evaluating viability.

Indicators of Population Structure

Based on our definition of independence, the definitive information needed to identify populations is long-term migration rates between different geographical spawning aggregations and the demographic consequences of those migration rates. In practice, information on such migration (or "straying" as it is common known in salmon biology) is rare. Consequently, we used other kinds of information as potential indicators of reproductive isolation. Each type of information contributes to our understanding of where independent populations might occur, but none alone provides us with a definitive answer. We describe these below. Depending on the quality of the data quality and the genetic and demographic history of salmon in different regions, the usefulness of these indicators in any one area varies.

1. **Geography.** The boundaries of a salmon population will be defined, in part, by the spatial distribution of its spawning habitat. Physical features such as a river basin's topographical and hydrological characteristics dictate to a large degree where and when salmon can spawn and delimit the spatial area over which a single group of fish can be expected to interact. Geographic constraints on population boundaries (such as distance between streams) can provide a useful starting point from which to look more closely at the attributes of groups of fish within circumscribed geographic areas, but will not generally support strong inferences at finer scales (e.g., distinguishing separate populations within a small river basin.) In addition, biogeographic characteristics and historical connections between river basins on geological time scales can also be informative in defining population boundaries.
2. **Migration rates.** The extent to which adults move between sites will affect the degree of reproductive isolation and, therefore, demographic independence between sites. Straying estimates are the primary indicators available of the amount of connectivity between spawning aggregations. Stray rate estimates are particular to a group of fish and the season and streams in which they are made, thus they provide useful information about straying under current conditions. In contrast, data are not available to obtain estimates of the magnitude of their variation over long time periods (e.g., 100 years). Compared to mark-recapture and other direct estimates of straying, indirect estimates, such as genetically based estimates of intergroup isolation, can be

DRAFT

used to estimate straying that has occurred between groups of fish, integrated over longer time periods than direct estimates.

3. **Genetic attributes.** Neutral genetic markers are useful in identifying salmon populations because they indicate the extent of reproductive isolation among groups over longer periods of time. The observed patterns of variation in neutral markers can be difficult to interpret, because they may reflect anthropogenic sources of migration from hatchery practices or offer a characterization at a single point in time of populations that are changing. Consequently, they should be interpreted with caution. Adaptive genetic differences among groups of fish (as indicated by quantitative traits or molecular markers) are more difficult to document than discrete marker differences, but they may offer good supporting evidence for distinct populations.
4. **Patterns of life history and phenotypic characteristics.** Phenotypic traits based on underlying genetic variation (rather than environmentally induced variation) are informative in identifying populations. Although most life history and phenotypic traits are influenced by environmental variation, they are also controlled by genetic differences. Consequently, phenotypic variation may be used as a proxy for genetic variation, and it also may indicate similarities in the selective environments experienced by salmon in different streams.
5. **Population dynamics.** Abundance data can be used to explore the degree to which demographic trajectories of two groups of fish are independent of one another. All else being equal, the less correlated time series of abundance are between two groups of fish, the less likely they are to be part of the same population. Complicating the interpretation of correlations in abundance between groups of fish is the potentially confounding influence of correlated environmental characteristics. When groups of fish that are in close proximity are not correlated in abundance over time, it is likely that they are not linked demographically. The reverse is not always the case—when correlations in abundance between groups of fish are detected, more work is needed to rule out confounding sources of correlation.
6. **Environmental and habitat characteristics.** The biotic and abiotic characteristics of occupied salmonid habitat may also help define a population, if these ecological characteristics are associated with different selective environment that could lead to isolation between groups of salmon. If different groups of salmon experience different selective environments and there is very little migration between those groups, we expect the fitness and phenotypic characteristics of the groups in each of those environments to diverge. The relative strength of inference for this kind of information though is weak, because we generally do not know which environmental variables affect selection or whether those effects will be observed at the population level.

DRAFT

Data Quality

An important first step in analyzing and interpreting any of the population structure indicators above is to carefully screen the data and information for potential sources of error or bias. To minimize such error, we consulted with biologists familiar with the local geography, adult and juvenile sampling and enumeration methods and calculations, and history of hatchery releases.

METHODS AND RESULTS

Geographic Distribution

Lake Ozette sockeye salmon spawn in the Lake Ozette watershed, which defines the geographic boundary of the ESU (Gustafson 1997). Sockeye salmon historically spawned on beaches throughout the lake and may also have spawned in the tributaries. Currently, nearly all the spawning in the lake occurs on two beaches: 1) Olsen's Beach (referred to as Elk Creek in some reports) located on the eastern shore north of Siwash Creek, and 2) Allens Bay north of Allens Slough on the western shore (Figure 1). Limited or intermittent spawning may also occur on the south shore of Baby Island (Meyer and Brenkman 1995), Umbrella Point, and Ericsons Bay (Dlugokenski et al. 1981, Makah Fisheries Management unpublished data). Presence of ready-to-spawn sockeye salmon in Boot Bay near Quinn Creek may suggest intermittent or historical spawning near there also (Dlugokenski et al. 1981, Jacobs et al. 1996). No known geographical or ecological barriers exist to migration of sockeye salmon among these locations. Distances between locations range from 3-12 km.

No historical abundance data exist for sockeye salmon in the tributaries of Lake Ozette, but the Makah Tribe and others have documented anecdotal information of sockeye salmon in some tributaries (reviewed in Jacobs et al. 1996). A spawning run of sockeye salmon has successfully colonized one tributary, Umbrella Creek, as a result of accidental (and later intentional) releases of artificially propagated sockeye salmon from the beach-spawning aggregations (Makah Fisheries Management 2000). This supports the hypothesis that sockeye salmon could have spawned in the tributaries as well as beaches. Small numbers of adult sockeye salmon have also recently been observed in Big River, prior to any hatchery introductions to Big River, which occurred in 2000 .

Kokanee salmon, a nonanadromous life history variant of *Oncorhynchus nerka*, also occur in Lake Ozette and spawn in the smaller tributaries, such as South, Siwash, Quinn, and Crooked creeks. Genetic evidence indicates that the kokanee salmon are reproductively isolated from the anadromous sockeye salmon (Gustafson et al. 1997), but both life history forms occupy the same habitat as post-emergent fry and while rearing in the pelagic zone of Lake Ozette until the sockeye salmon migrate seaward. Kokanee salmon are not part of the Lake Ozette ESU.

DRAFT

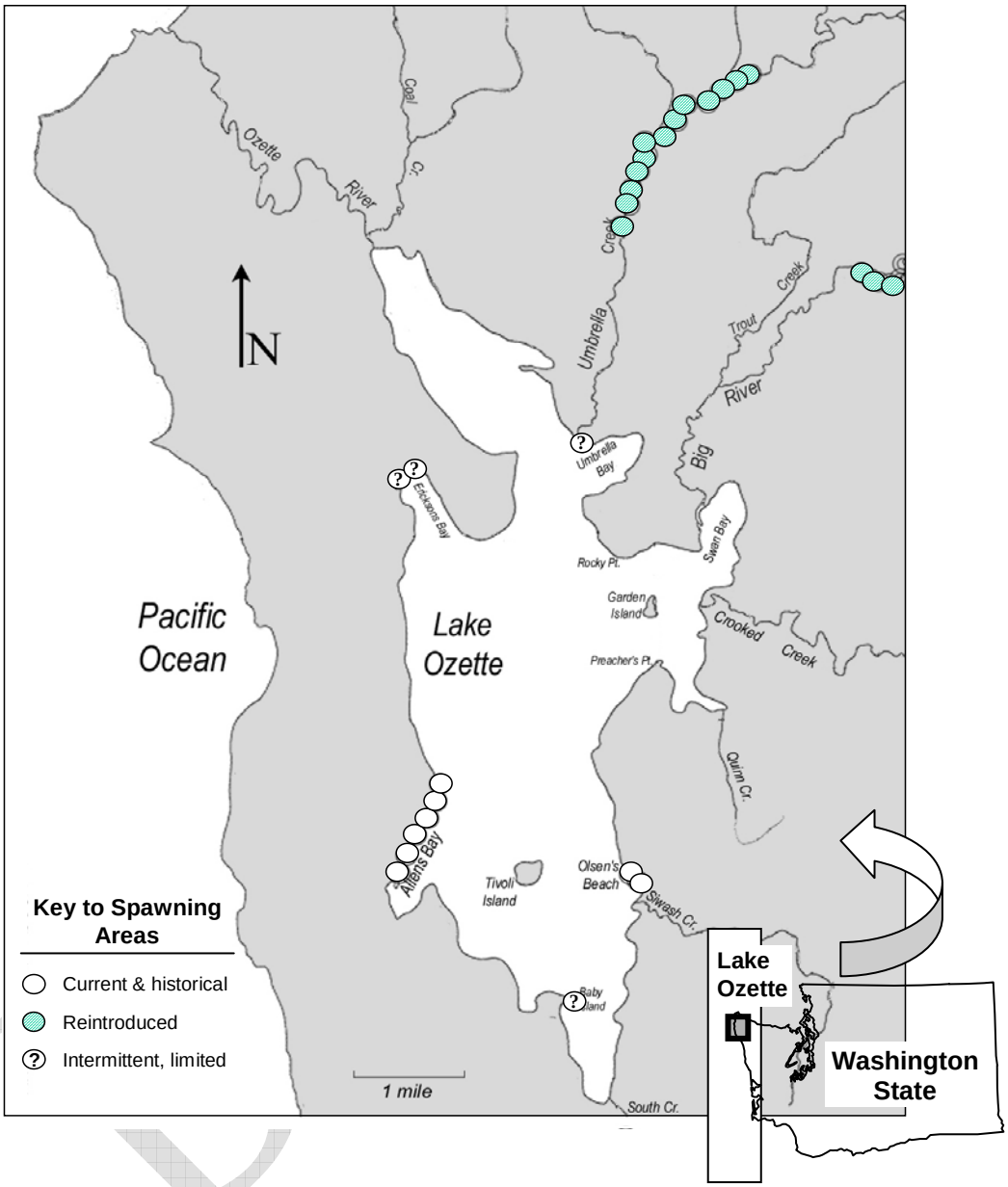

Figure 1. Sockeye salmon spawning areas in the Lake Ozette ESU.

Genetic Attributes

Past Analyses

Genetic data provide the strongest inferences about population structure in Lake Ozette. Analyses based on allozyme variation indicated that Lake Ozette sockeye salmon were genetically distinct from other sockeye salmon populations in Puget Sound (Winans et al. 1996) and Washington coast (Gustafson et al. 1997). Within the Lake Ozette ESU, Hershberger et al. (1982) documented genetic variation at two of 37 allozyme loci and based on variation at *PGM-1**, suggested that two genetically different groups of sockeye salmon were present in Lake Ozette. They hypothesized the groups might be isolated by differences in run-timing. Gustafson et al. (1997) documented significant differences in allozyme frequencies among the Allens and Olsen's beach-spawning aggregations in samples collected in 1995. Significant difference also existed between these 1995 samples and two samples collected in other years. Because the spawning destination of the fish in the samples taken at the Lake Ozette weir were unknown, however, they could not determine whether these differences were due to interannual variation or geographical differences among spawning aggregations.

Introductions of sockeye salmon and kokanee salmon into Lake Ozette do not appear to confound interpretations of current genetic patterns, but they remain an important consideration. Fingerling sockeye salmon were introduced from Baker Lake in 1937 and Quinault Lake in 1983; kokanee salmon were introduced from Lake Crescent in 1940 and from an undocumented source in 1958. Despite these introductions, sockeye and kokanee salmon appear to have retained distinctive genetic characteristics (Gustafson et al. 1997, Figure 2).

New Analyses—Methods

To add to these existing analyses, the Makah Tribe and Washington Department of Fish and Wildlife analyzed samples from sockeye salmon collected from Olsen's Beach in 1996, 1999, and 2000; Allens Bay in 2000, and Umbrella Creek in 2000 at nine microsatellite DNA loci (*One-100*, *One-101*, *One-102*, *One-103*, *One-105*, *One-108*, *One-110*, *One-114*, and *One-115*). Data and results of their analyses are available in Crewson et al. (2001).

For the TRT's analysis of these data, we calculated seven indicators of independence of the spawning aggregations. These were (1) pairwise *P*-values from chi-square tests for significant differences in allele frequencies; (2) Reynolds coancestry d genetic distances (Reynolds et al. 1983); (3) Nei's unbiased genetic distances (Nei 1978); (4) F_{ST}, a measure of genetic population structure, as estimated by θ (Weir and Cockerham 1984); (5) *M*, the number of migrants per generation, which is the pairwise analog of $N_e m$ (Slatkin 1993), and (6) *t*, the time since divergence of two populations from a common ancestral population (Wier 1996). We examined patterns of genetic differences by constructing dendrograms based on the genetic distances and the UPGMA

clustering algorithm (Sneath and Sokal 1973) and by ordination of Reynolds coancestry distance (Reynolds et al. 1983) using the multidimensional scaling (MDS) algorithm (Lessa 1990). We examined the robustness of the dendrograms by generating bootstrap values for each node based on 1000 resamplings of the allele frequencies.

We estimated the number of migrants per generation, M, using $M = (1-\theta)/2\theta$ (Slatkin 1993) and the divergence time of each pair of populations, t, by $t = -2N_e \ln(1-\theta)$ following Wier (1996, p. 194) . Calculation of time since divergence assumes that the populations have been completely isolated since diverging from a common ancestral population t generations ago, that mating is random within populations, that sockeye have discrete generations, and that θ is estimated from a neutral genetic trait. Violations of these assumptions mean that exact estimates of t are difficult to obtain. Limited or episodic gene flow is likely to have occurred between spawning aggregations, which biases estimates of t. Ozette sockeye salmon return, spawn, and die almost exclusively as four-year olds with little reproductive exchange between brood years (Makah Fisheries Management 2000). Most Pacific salmonids, however, do not have discrete generations, but Waples (1990) showed that violating this assumption is not likely to greatly affect estimates of t over long time periods. The microsatellite loci used to estimate θ were presumed to be neutral and unlinked to loci under selection, but this assumption has not been tested. Comparisons of t for divergence of Umbrella Creek hatchery fish and Olsen's Beach based on genetic data provided an internal control, however, for our estimates because the historical time of divergence is known.

We estimated the genetic effective sizes, N_e, of the populations in two ways and used these to calculate a range for t. From demographic data in the Lake Ozette Hatchery and Genetic Management Plan (HGMP), we estimated the joint inbreeding effective size of all populations, using $N_e = gN_b$, where g is the mean generation time and N_b is harmonic mean of the annual effective breeding number of spawners. We calculated the annual effective breeding number from N, the annual census number of spawners to Lake Ozette, while assuming $N_b : N = 0.3$, which is in the middle of the range of $N_b : N$ values in the scientific literature (Waples 1990, Waples et al. 1993, Ford et al. 2001). We also calculated a variance effective population size for Olsen's Beach sockeye salmon based genetic drift over one generation (1996-2000) following Waples (1991) and doubled it to estimate the joint effective population size between two different geographical aggregations.

New Analyses—Results

All genetic analyses showed major evolutionary divergence between sockeye salmon and kokanee salmon in Lake Ozette (Table 1, Figure 2). Kokanee and anadromous sockeye salmon aggregations consistently formed distinct groups in cluster analyses. Genetic divergence of the two forms of *O. nerka* was nearly ten times greater than divergence of anadromous sockeye salmon aggregations from each other. This supported the conclusion that they are different ESUs. The following discussion therefore focuses only on genetic differences among spawning anadromous sockeye salmon aggregations in Lake Ozette.

Table 1. P-values from pairwise chi-square tests for allelic homogeneity for nine microsatellite loci (below the diagonal) and F_{ST} values with 95% confidence intervals (above the diagonal) for *O. nerka* in Lake Ozette. Unshaded entries are anadromous sockeye salmon; shaded entries are kokanee.

	Olsen's Beach			Allens Bay		Kokanee	
Sample Location	1996	1999	2000	2000	Umbrella	Crooked	Siwash
Olsen's Beach							
1996		0.0222 (0.0069, 0.0431)	0.0069 (-0.0060, 0.0239)	0.0136 (0.0003, 0.0313)	0.0002 (-0.0081, 0.0113)	0.1146 (0.0709, 0.01699)	0.1087 (0.0669, 0.159)
1999	0.000		0.0072 (-0.0004, 0.0138)	0.0155 (0.0079, 0.0238)	0.0193 (0.0107, 0.0288)	0.1101 (0.0760, 0.1443)	0.1041 (0.0723, 0.1361)
2000	0.183	0.000		0.0068 (0.0006,0.0150)	0.0067 (0.0004, 0.0158)	0.0877 (0.0537, 0.1248)	0.0810 (0.0488, 0.1150)
Allen's Bay 2000	0.001	0.000	0.000		0.0094 (0.0003, 0.0249)	0.1061 (0.0676, 0.1489)	0.0968 (0.0603, 0.1347)
Umbrella Creek 2000	0.138	0.000	0.000	0.001		0.093 (0.0576, 0.1340)	0.0869 (0.0539, 0.1221)
Crooked Creek	0.000	0.000	0.000	0.000	0.000		-0.0008 (-0.0037, 0.0014)
Siwash Creek	0.000	0.000	0.000	0.000	0.000	0.648	

DRAFT

Microsatellite allele frequencies were significantly different between sockeye salmon from Olsen's Beach, Allen's Bay, and Umbrella Creek hatchery fish (Table 1). Significant temporal differences also occurred between sockeye salmon from different brood cycles on Olsen's Beach but not between generations of the same four-year brood cycle. Allele frequencies were also significantly different between sockeye salmon from Umbrella Creek and Allens Bay and between Umbrella Creek and Olsen Beach in two out of three comparisons. The magnitude of the statistical differences between Olsen's Beach and Allens Bay, as judged by *p*-values, was similar to the differences between Umbrella Creek hatchery fish and sockeye salmon from Olsen's Beach and Allens Bay, which provided the source brood stock for the hatchery program less than 20 years ago.

Pairwise F_{ST} values ranged from 0.0002 to 0.022. The largest F_{ST} values were between sockeye salmon from different brood cycles on Olsen's Beach and between different brood cycles from Olsen's Beach and Umbrella Creek. In contrast, the smallest F_{ST} values were between sockeye salmon from the same four-year brood cycle on Olsen's Beach (1996-2000) and likewise between the same brood cycles from Olsen's Beach and Umbrella Creek.

The dendrogram of genetic similarity based on Nei's genetic distance illustrates a similar pattern of diversity (Figure 1). Umbrella Creek hatchery fish and Olsen's Beach sockeye salmon from the same 1996-2000 brood cycle are genetically most similar. These fish are more similar to sockeye salmon from Allens Bay in 2000 than to sockeye salmon from Olsen's Beach in 1999. This same pattern is represented in two dimensions by multi-dimensional scaling of genetic distances (Figure 2).

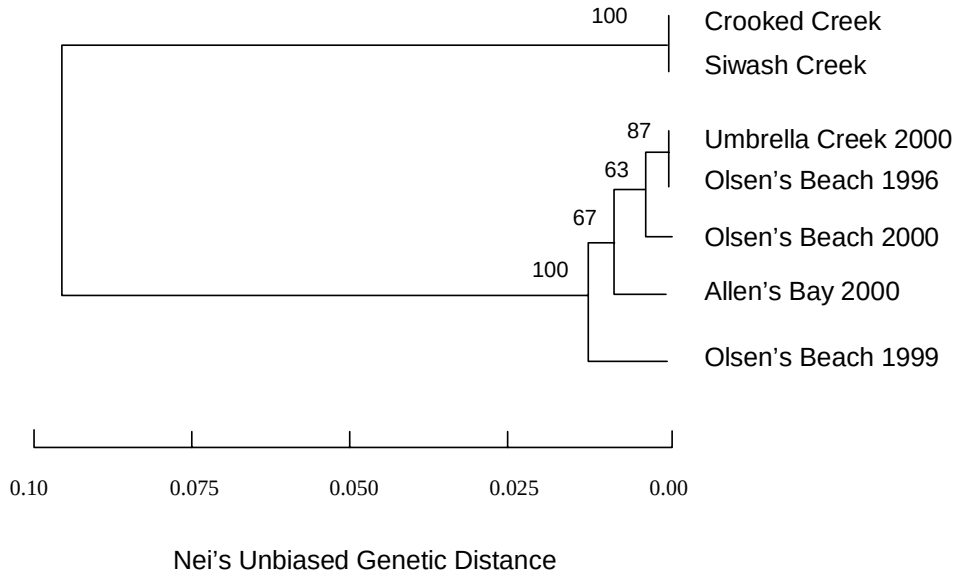

Figure 2. Genetic similarity of sockeye and kokanee salmon aggregations in Lake Ozette based on nine microsatellite loci. Numbers on branches are the percent bootstrap support. Similar results were obtained using Reynolds coancestry distance.

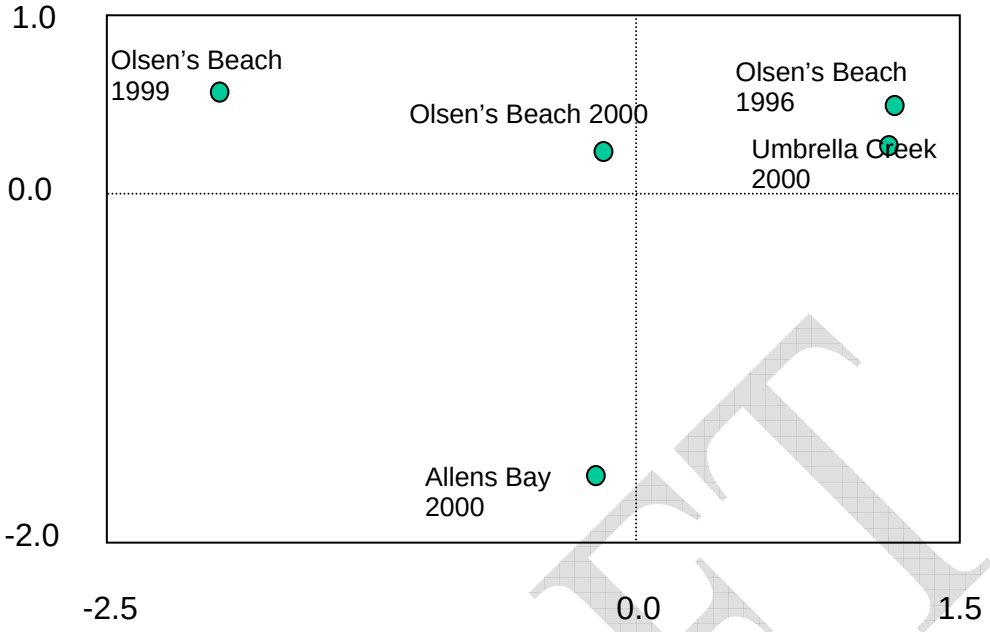

Figure 3. Multidimensional scaling of coancestry genetic distances between Lake Ozette sockeye salmon.

The number of pairwise migrants per generation, *M*, ranged from 22 to 2500 (Table 2. The greatest independence was detected between Olsen's Beach sockeye salmon in 1996 and 1999 and least independence was detected between Umbrella Creek hatchery fish and Olsen's Beach, which was the main source of brood stock for the hatchery program. The number of migrants per generation between Olsen's Beach and Allens Bay ranged from 36 to 73.

Based on demographic data from 1988-1999, the mean inbreeding effective population size for all sockeye salmon in Lake Ozette was 850. In contrast, the variance effective population size for Olsen's Beach based on genetic drift over one generation was 128. Using these effective population sizes to calculate time since divergence, the shortest divergence times were between Umbrella Creek hatchery fish and Olsen's Beach from the same brood cycle (0-1 generations). This estimate corresponds closely with the known founding of the Umbrella Creek hatchery strain. Olsen's Beach sockeye salmon were the principal source of brood stock for the Umbrella Creek hatchery strain 1-3 generations ago. The longest divergence times were between sockeye salmon from different brood cycles from Olsen's Beach (8-27 generations) and different brood cycles of Umbrella Creek hatchery fish and Olsen's Beach sockeye salmon (10-33 generations). Over all brood cycles, divergence time between sockeye salmon from Olsen's Beach and Allens Bay ranged from 3 to 27 generations. Divergence time estimates between Allens Bay and Umbrella Creek varied from 5 to 16.

DRAFT

Table 2. Migrants per generation (above the diagonal) and time since divergence, t, (below the diagonal) for sockeye salmon in Lake Ozette. The upper range of *t* was estimated using N_e from the demographic data and the lower range was estimated using N_e from the genetic data from Olsen's Beach.

	Olsen's Beach			Allens Bay	
Sample Location	1996	1999	2000	2000	Umbrella
Olsen's Beach					
1996		22	71	36	2500
1999	(6-38)		68	31	25
2000	(2-12)	(2-12)		73	74
Allen's Bay 2000	(7-23)	(8-27)	(3-12)		52
Umbrella Creek 2000	(0-1)	(10-33)	(3-11)	(5-16)	

Life History Characters and Spatial Synchrony

Historically, most demographic data on adult sockeye salmon has been collected as fish pass a weir on the Ozette River while entering the lake. Without means to distinguish fish from Olsen's Beach, Allens Bay, or other spawning areas, it has been impossible to look for temporal synchrony between locations in spawner abundance and differences in adult migratory behavior. Sockeye salmon from Ozette return to freshwater between late-April and mid August, from three to ten months before they spawn (Makah Fisheries Management unpubl. data, Jacobs et al. 1996). The sockeye spawn from mid-November to as late as early February. Timing of entry into the lake may be affected by water temperatures in some years, high temperatures can delay entry (LaRiviere 1991, Jacobs et al. 1996). The percentage of sockeye entering during the daytime has been seen to be highly correlated with lake level. In addition, discrepancies between total abundances observed at the weir and numbers of fish on the spawning grounds suggested unexplained mortalities or undiscovered spawning areas. These challenges have prevented investigators from examining correspondence between entry into freshwater and spawning characteristics at different beaches.

Dlugokenski et al. (1981) suggested that sockeye salmon spawning at Allen's Bay and Olsen's Beach might be different subpopulations based on spawn timing (Figure 4). Peak spawning on the eastern shore (Olsen's Beach) was over a month earlier than spawning on the western shore (Allen's Bay), but 60% of the total spawning occurred at the same time between December and January. More recent observations of spawning on Olsen's Beach and at Allen's Bay suggests that spawning coincides at both beaches (summarized in Jacobs et al. 1996; Makah Fisheries Management unpubl. data).

DRAFT

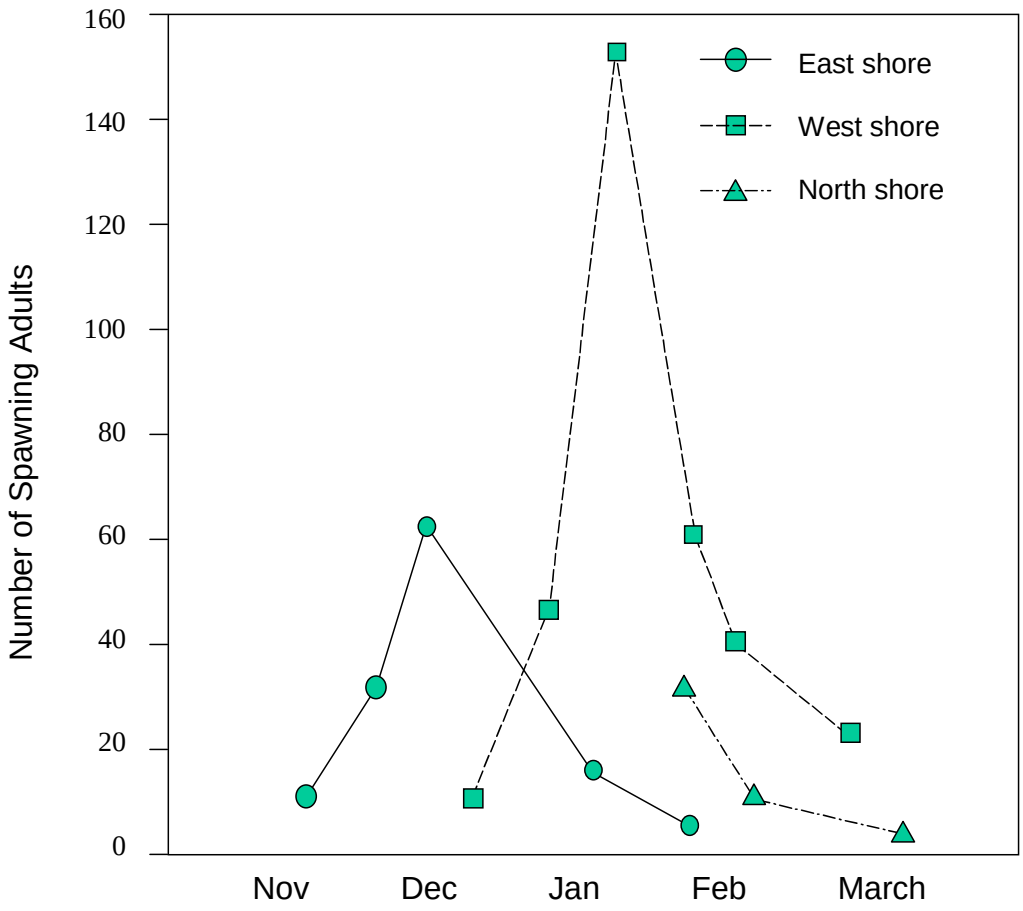

Figure 4. Timing and abundance of lakeshore spawning sockeye salmon in 1978-1979 spawning season (from Dlugokenski et al. 1981).

Habitat Characteristics

All spawning locations for Lake Ozette sockeye salmon are within the same ecoregion, which suggests that no major differences exist in regional habitat characteristics related to elevation, land cover, and geology. The major differences in spawning areas among streams and beaches are size, gradient, and substrate. We could find no evidence in the literature that the magnitude of these differences is associated with boundaries of independent populations. Big River and Umbrella Creek are the largest systems where sockeye currently spawn in Ozette, ranging from 15 to 25 km in main stem length and having multiple tributary creeks. The main stems are low and moderate gradients, declining an average of 3 m/km of length. Tributaries and headwaters are steeper, dropping 15 to 40 m/km. The smaller streams, which are predominantly used by kokanee salmon, such as Siwash, South, Elk, and Crooked Creeks are shorter, averaging 2-10 km in length and have steeper gradients (Figure 13 in Jacobs et al. 1996). Streambeds in low to moderate gradients tend to be composed of gravel and

sand, whereas streams with steeper gradients have more boulders and cobbles (Bortleson and Dion 1979).

One potentially significant habitat difference for lake-type sockeye salmon is between tributary and lake spawning habitat. Burgner (1991) noted that in sockeye salmon inlet spawning typically occurred earlier than beach spawning or spawning in lake outlet streams, which usually have higher water temperatures. Strong selection could exist in some lakes for fry emergence timing that would allow fry from different incubation temperature regimes to migrate to the lake and exploit common feeding and rearing opportunities (Godin 1982, Brannon 1987, Burgner 1991). Emergence time is heritable in salmonids and may be affected by genotype-environment interactions (McIntyre and Blanc 1973, Currens unpubl. data) that could lead to adaptive differences among populations. In Lake Ozette, warming and stratification of the lake during the summer (Beauchamp et al. 1995) may lead to periods in the October and November where because of the differences in mass shallower beach spawning waters have not cooled as rapidly as stream temperatures. By the time most spawning and incubation begins in late November or December, lake waters have cooled to 11 C^o (Figure 5, Beauchamp et al. 1995) but will not reach their coolest temperatures of 7-8 C^o until February and March (Makah Fisheries Management 2000). In contrast, inlet and outlet streams have already cooled to 7-8 C^o (Figure 6, Bortleson and Dion 1979). It is unclear whether this difference of could have led to adaptive differences between historical tributary and beach spawning populations. The successful introduction of lake spawning fish into Umbrella Creek, however, suggests that adaptive differences for this trait may not have been large enough to prevent successful reproduction of lake spawning fish in tributaries. This is another factor that support both the hypothesis that there has been in the past a tributary spawner component to the population and that the ESU contains one population, not several isolated populations.

DRAFT

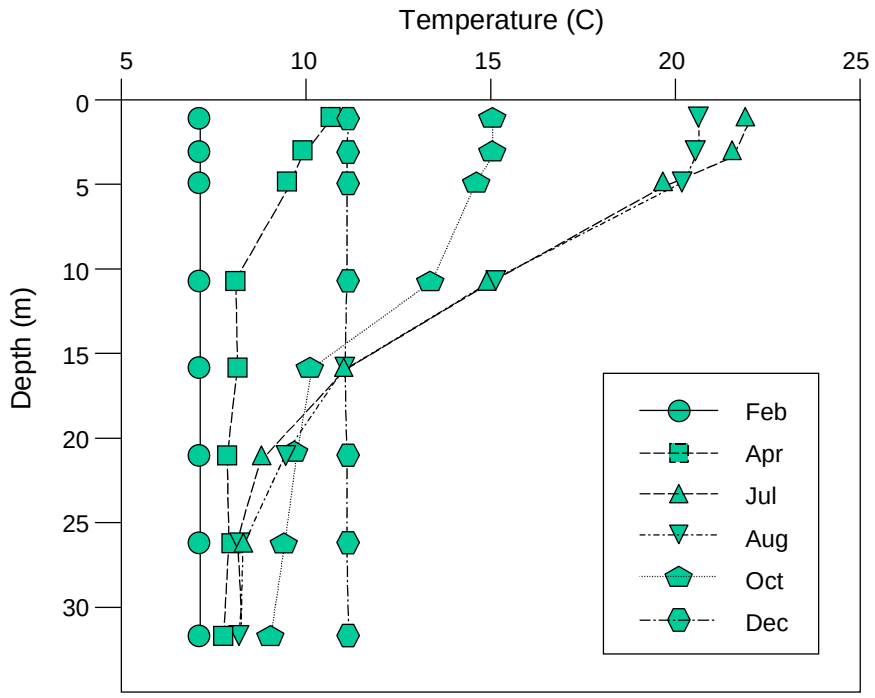

Figure 5. Seasonal changes in temperature for Lake Ozette (adopted from Beauchamp et al. 1995).

DRAFT

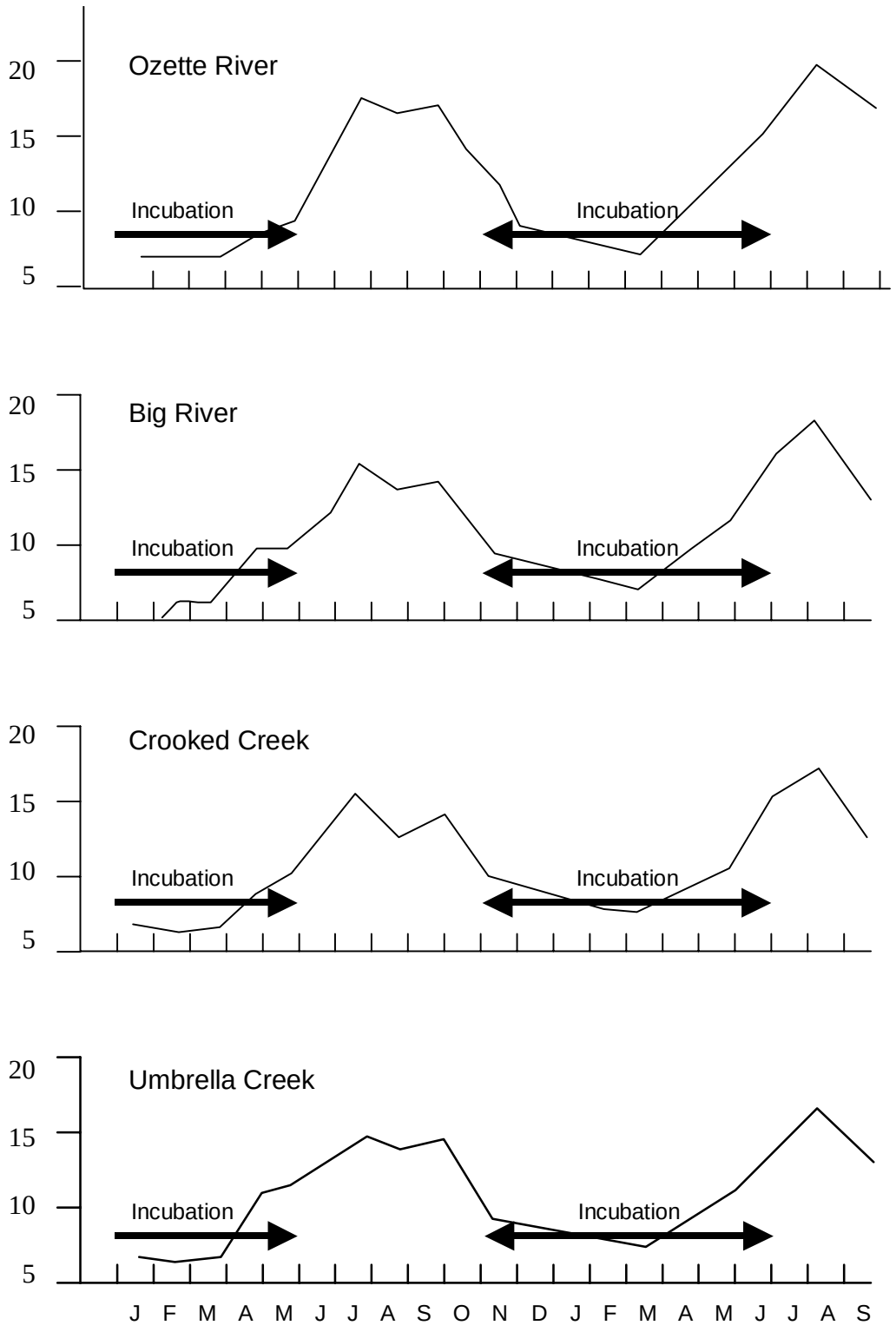

Figure 6. Water temperatures in the Ozette River and three inlet tributaries of Lake Ozette (adapted from Bortleson and Dion 1979)

Population Structure Decisions

Strong evidence of population structure for any of indicators 1-4 would provide reliable inference of independent populations in Lake Ozette. Corroborating evidence from multiple indicators would also strengthen our inference. Similar logic applies to evidence of subpopulation structure. Table 3 summarizes the available evidence for and against independent populations in Lake Ozette.

Table 3. Evidence and strength of inference for population structure in Lake Ozette sockeye salmon.

Indicator	Evidence	Strength of Inference	
		Population	Subpopulation
1. Geography	No geographical barriers; distances between aggregations small	Weak	Weak
2. Migration rates	No data available	--	--
3. Genetic attributes	Statistically significant divergence of aggregations; divergence recent (< 100 yrs); genetic migration rates high.	Weak	Strong
4. Life history characteristics	Possible difference in peak spawning among aggregations	Weak	Weak, but possible
5. Population dynamics	No data available	--	--
6. Habitat characteristics	Possible 4-5 C° difference in incubation temperatures between tributary and lake	Weak	Weak, but possible

Strength of inference for independent populations in Lake Ozette is weak in four of the six indicators and data do not exist to test for it in two indicators. Genetic data provided the best evidence of differences among aggregations, but we considered it weak evidence of independent populations because of the magnitude of the differences. Estimates of F_{ST} and the related estimates of migrants per generation, and time since divergence between different natural spawning aggregations on Olsen's Beach and Allens Bay were generally similar to estimates between Umbrella Creek hatchery fish and Olsen's Beach, where most of the brood stock originated 1-3 generations ago. Estimates of time since divergence from the genetic data corresponded closely with known times of divergence between Olsen's Beach 1996 and Umbrella Creek and Olsen's Beach 1996 and Olsen's Beach 2000, which gave us confidence in other estimates (Table 2). In contrast, independent populations under our definition would have diverged approximately 25 generations ago.

DRAFT

Strength of inference for subpopulation structure among spawning aggregations is much stronger. Both recently collected DNA data (Table 2) and older allozyme data (Gustafson et al. 1997) showed measurable divergence between spawning aggregations. In addition, although the inferences were necessarily weaker, potential differences in peak spawning time between beach spawning aggregations and differences in incubation temperatures between beach and tributaries suggested that subpopulations exist in Lake Ozette now and probably were more extensive historically.

The strongest evidence of genetic differences among anadromous sockeye salmon in Lake Ozette occurred between different brood cycles (Table 2). Sockeye salmon in Lake Ozette return to spawn and die almost exclusively as four-year olds, which limits potential genetic exchange between fish in the four different four-year brood cycles that return to the lake. Temporal isolation maintained by the age structure of these fish may have allowed genetic differences to evolve between fish in different brood cycles. It would be useful to continue to monitor the age structure for any divergence in the predominately 4-year old return pattern.

CONCLUSIONS

Based on the genetic data, we conclude that extant spawning aggregations in Lake Ozette are different subpopulations within a single population. It seems likely that differences also existed historically among other subpopulations that have since been extirpated, subpopulations occurring at different spawning beaches or tributaries.

Data needs and remaining uncertainties

If geographical subpopulation structure were real, we would expect year-to-year differences among generations to be smaller than differences between geographical areas. Existing data support this, but only one between-generation comparison was possible (Olsen's Beach1996 and Olsen's Beach 2000). More between-generation comparisons are needed. Likewise, more comparisons between brood cycles will allow us to determine the temporal genetic structure within the population. Both of these may be possible by using DNA from archived scale or tissues.

DRAFT

LITERATURE CITED

Beauchamp, D. A., M. G. LaRiviere, and G. L. Thomas. 1995. Evaluation of competition and predation as limits to juvenile kokanee and sockeye salmon production in Lake Ozette, Washington. N. Am. J. Fish. Manage. 15:193-207.

Bortleson, G. C., and N. P. Dion. 1979. Preferred and observed conditions for salmon in Ozett Lake and its tributaries, Clallam County, Washington. U.S. Geological Survey Water Resources Investigations 78-64.

Brannon, E. L. 1987. Mechanisms stabilizing salmonid fry emergence timing. Can. Spec. Publ. Aquat. Sci. 96:120-124.

Burgner, R. L. 1991. Life history of sockeye salmon *Oncorhynchus nerka.* Pages 3-117 *in* C. L. Groot and L. Margolis (eds.). Pacific salmon life histories. University of British Columbia Press, Vancouver, B.C., Canada.

Cavalli-Sforza, L. L. and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 21:550-570.

Crewson, M. J., M. J. Haggerty, A. C. Ritchie, S. F. Young, J. B. Shaklee, K. P. Currens, and W. E. Eldridge. 2001. Genetic Characterization of Lake Ozette Sockeye Salmon for Enhancement Recovery Strategies. Final Report Research. Hatchery Scientific Review Group, IAC Grant 01-038, Interagency Committee for Outdoor Recreation, Olympia, Washington.

Dlugokenski, C. E., W. H. Bradshaw, and S. R. Hager. 1981. An investigation of the limiting factors to Lake Ozette sockeye salmon production and a plan for their restoration. U. S. Fish and Wildl. Serv., Fish. Assist. Office, Olympia, WA.

Godin, J. G. J. 1982. Migrations of salmonid fishes during early life history phases: daily and annual timing. Pages 22-50 *in* E. L. Brannon and E. O. Salo (eds.). Proceedings of the salmon and trout migratory behavior symposium. School of Fisheries, University of Washington, Seattle , WA.

Gustafson, R.G., T.C. Wainwright, G.A. Winans, F.W. Waknitz, L.T. Parker, and R.S. Waples. 1997. Status review of sockeye salmon from Washington and Oregon. NOAA tech. memorandum NMFS-NWDSC-33. 282pp.

Ford, M., P. Budy, C. Busack, D. Chapman, T. Cooney, T. Fisher, J. Geiselman, T. Hillman, J. Lukas, C. Peven, C. Toole, E. Weber, and P. Wilson. 2001. Upper Columbia River steelhead and spring chinook salmon population structure and biological requirements. Final Report. National Marine Fisheries Service, Northwest Fisheries Science Center, Seattle.

DRAFT

Hershberger, W. K., J. Seeb, and P. Aebersold. 1982. Genetic analysis of the sockeye salmon (*Oncorhynchus nerka*) returning to Lake Ozette. Unpubl. Rep. To Steve Joner, Makah Fisheries Management, from W. K. Hershberger, School of Fisheries, University of Washington.

Jacobs, R., G. Larson, J. Meyer, N. Currence, J. Hinton, M. Adkison, R. Burgner, H. Geiger, and L. Lestelle. 1996. The sockeye salmon *Oncorhynchus nerka* population in Lake Ozette, Washington, USA. U.S. Dept. Inter. NPS Tech. Rep. NPS/CCSOSU/NRTR-96/04.

Lessa, E. P. 1990. Multidimensional analysis of geographic genetic structure. Syst. Zool. 39:242-252.

McIntyre, J. D., and J. –M. Blanc. 1973. A genetic analysis of hatching time in steelhead trout. J. Fish. Res. Bd. Canada 30:137-139.

Makah Fisheries Management. 2000. Lake Ozette Sockeye Hatchery Genetic Management Plan. Biological Assessment prepared for Bureau of Indian Affairs Section 7 Consultation.

Meyer, J., and S. Brenkman. 1995. Water-quality conditions in the Lake Ozette Watershed and potential impacts on salmonids. Draft Report. Olympic National Park, Port Angeles, WA.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Reynolds, J., B. S. Weir, and C. C. Cockerham. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. Genetics 105:767-779.

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

Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy. W. H. Freeman & Co., San Francisco, CA.

Waples, R. 1990. Conservation of Pacific salmon. II. Effective population size and rate of loss of genetic variability. Journal of Heredity 81:267-276.

Waples, R., O. W. Johnson, P. B. Aebersold, C. K. Shiflett, D. M. VanDoornik, D. J. Teel, and A. Cook. 1993. A genetic monitoring and evaluation program for supplemented populations of salmon and steelhead in the Snake River Basin. Annual Report of Research to the Division of Fish and Wildlife, Bonneville Power Administration, Project 98-096.

DRAFT

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

DRAFT