

Date: August 20, 2002

To: Independent Scientific Review Panel

From: M. S. Powell, Principal Investigator (BPA Project 199009300)

Re: Responses to ISRP Comments on Mainstem/Systemwide Project Proposal

Following are the ISRP comments on the Mainstem/Systemwide Project Proposal (BPA 199009300, and project responses to these comments. ISRP comments are presented in *italics*, project responses are provided in normal text.

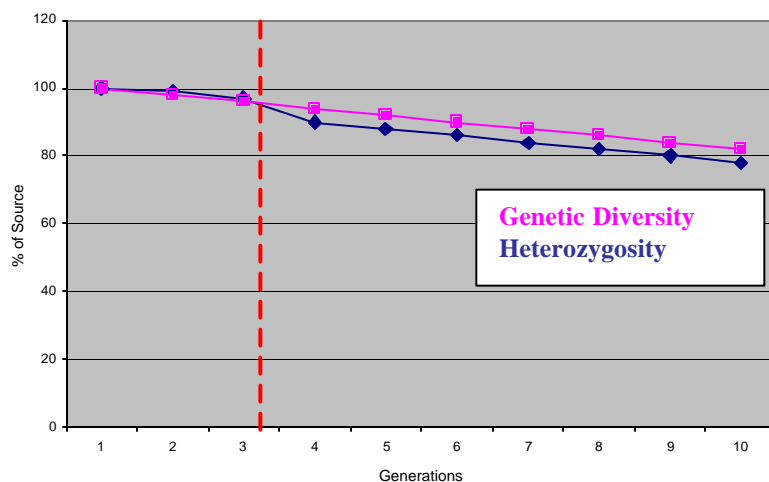
ISRP Comment 1:

The ISRP's primary concern was why results of past monitoring were not presented? There are some obvious issues of concern such as what has been variation of family size in families of sockeye? Or, has mortality in the captive brood programs (sockeye or Chinook) been random among families? How has data been used to structure mating schemes? In the absence of any presentation of these data, the reviewers cannot comment on the timeliness of these analyses or adequacy of analyses.

Response:

Because of time constraints with the oral presentations, the majority of past monitoring efforts were not presented in detail but rather summarized in several slides showing both empirical data and theoretical projections of both genetic diversity and heterozygosity. For example, Figure 1 below was taken directly from the presentation given in Portland on July 17th.

Figure 1. Genetic Diversity and Heterozygosity in Redfish Lake sockeye.



The figure illustrates both genetic diversity and heterozygosity among captive Redfish Lake sockeye over time as examined using nuclear RFLPs and microsatellite loci. Genetic diversity and heterozygosity has not significantly eroded from estimates of

diversity among the original 23 sockeye, outmigrants from different year classes and residual sockeye taken into the captive program. The red dashed line indicates the extent of present empirical data (approximately 3 overlapping generations). Data points beyond the dashed line are theoretical projections of the loss of genetic diversity and heterozygosity if the Redfish Lake population remains “closed” (no new genetic input) at the current effective population size (the harmonic mean of N_e over the past 3 overlapping generations). The figure points out in summary that past genetic monitoring efforts have apparently thus far been successful at reducing or minimizing the loss of genetic diversity. However, this should be accepted with caution since there has not been an “unmanipulated” family line of surviving Redfish Lake sockeye kept to compare to. We can only speculate what could happen based upon numerous studies and general precepts in population genetics which have shown drift to be an overwhelming influence on the loss of genetic diversity among small populations. Moreover, the figure also points out a theoretical loss of diversity over time simply due to drift. This will likely occur despite our most enlightened conservation efforts.

Some loss of genetic diversity in Redfish Lake sockeye is however already evident as observed in mitochondrial DNA, mtDNA, (see the 2002 completion report to BPA). Mitochondrial DNA is more sensitive to population bottlenecks than nuclear DNA since mtDNA reduces effective population size to $\frac{1}{4}$ of N_e estimates using nuclear DNA. In the beginning of the multidisciplinary conservation effort on Redfish Lake sockeye, 6 compound mitochondrial haplotypes were observed among anadromous or residual sockeye (the listed populations). At present only 3 of these maternal lineages remain with one, designated H25, unique to Redfish Lake. Genetic drift due to extremely small numbers of returns has eliminated 3 of the maternal lineages from the surviving population. The loss of these maternal lineages is not a result of any shortcomings within the breeding program but ostensibly illustrates the limits of our capabilities to “save” genetic diversity given the constraints of our smolt-to-adult return ratios.

Monitoring of outmigrants and returning sockeye has failed to show any significant difference among family groups with respect to differential success and/or mortality. However, this is not likely to be shown if the spawning of individuals is done such that population or familial substructure is minimized. We spawn individuals across family group, across maternal lineage, across year class and across origin to minimize the risk of inbreeding, to maximize breeders and to remove any artificial substructure within the remaining population (this serves to maximize effective population size too) (for reviews on this subject see Ballou, Gilpin and Foose 1995; Roff 1997). It is important to note that mating schemes are also used to also minimize fluctuations in family size (to equalize them) by crossing whenever possible using multiple spawn crosses and dividing reproductive effort (eggs or milt) among crosses. For example a female’s eggs are split evenly into 2 or more lots to be fertilized separately with milt from two or more males (or cryopreserved milt). The complete details of all captive mating schemes are discussed within monthly technical oversight committee meetings for Redfish Lake sockeye. These discussions and presentations of spawn crosses are recorded and are available for public viewing and comment. Genetic data is generated yearly on returning adults in a “real-time” fashion such that genetic identities of each prespawn adult can be assessed before

they are released to spawn volitionally or held for captive spawning. The genetic composition of sockeye released to spawn volitionally as compared to captive sockeye has remained non-significantly different. Thus, sockeye within the captive program are a genetic representation of their wild counterparts in Redfish Lake.

Current genetic studies underway and those proposed for FY 2003 include intensive genetic typing of outmigrant assemblages to address questions of possible differential family success and differential success among release strategies.

ISRP Comment 2:

The proposal provides additional information on reports and publications that have resulted from these studies to address some of the ISRP's FY00 comments. One hopes that with the long-term dataset that is being generated by this study that additional peer-reviewed publications will arise from the work. The proposal has a long-term monitoring component that is needed to provide consistency and insights into the Redfish Lake sockeye captive broodstock effort. This effort, while necessary, is largely routine by this time.

Response:

This monitoring program has an intended "date of obsolescence" of FY2008. This will allow for empirical data to be generated for Redfish Lake sockeye for 4 complete overlapping generations and 3 overlapping generations of captive Salmon River chinook salmon. Within that time frame, significant departures from theoretical losses of genetic diversity or heterozygosity over time will be evident within each program. This has a two-fold value to our conservation efforts. First, it allows for the assessment of our current mating strategies using pedigree information for sockeye and detailed genetic information for chinook (MAI discussed below). Second, it demonstrates whether or not theoretical projections regarding the rate of loss of genetic diversity and heterozygosity within captive programs have any predictive value.

In summary:

Are the mating strategies and genetic information used in our captive propagation programs minimizing the loss of genetic diversity? Yes, thus far we can't detect any significant loss other than in mitochondrial lineages. The long-term consequences of the loss of these maternal lineages is unknown.

Will this change once the populations are "closed"? Undetermined. We don't have enough information yet to address this question but future analyses through FY2008 will provide data necessary to address this question.

Do our current estimates for the loss of genetic diversity in these populations over time have predictive value? Undetermined. The theoretical rate of loss of diversity is eloquently demonstrated in numerous texts (example: Roff 1994) but the rate of loss given manipulation under captive management has not been examined in threatened and endangered salmon populations. We simply don't really know what will happen over a long period of time in a closed, captive population.

ISRP Comment 3:

The most interesting aspect of the proposal, which could have been more fully described, is the more recent use of microsatellite loci analyses to develop pedigrees, identify parentage, and to set up MAI (Maximal Avoidance of Inbreeding) matrices to guide captive breeding options for severely depressed chinook populations in the East Fork of the Salmon, West Fork of the Yankee Fork, and so on. This approach has very strong applied conservation biology implications and deserves to be better described in the proposal with respect to its methods, application, and management implications.

Response:

Breeding matrices both simple and complex have been used to slow down the rate of inbreeding in small populations and have a long history with domestic animal husbandry (see Falconer 1989; Ballou, Gilpin, and Foose 1995 and references therein). Breeding schemes are also used extensively in laboratory animal husbandry and more recently in zoos and other conservation applications (Flesness and Mace 1988; Princee 1986). The idea behind the maximum avoidance of inbreeding or MAI is to genetically type individuals, keep pedigrees on all crosses and control reproductive success (i.e. maximize the number of breeders, equalize family sizes, etc.). Figure 2 (from the Portland Presentation on July 17th) illustrates the use of MAI as an example on captive East Fork Salmon River chinook salmon. Empirical data are shown to the left of the red line. Theoretical data are shown to the right. Three different breeding schemes are represented: 1x1 matings, commonly employed by hatcheries with large returns; 4x4 matings used in conservation circumstances; and MAI where the genetic identities of individuals in every cross are known. The loss of heterozygosity is tracked over time for each breeding scheme. Thus far, we have employed MAI on captive Salmon River chinook without any significant loss of heterozygosity. In the future when the population becomes “closed” (i.e. if captive efforts continue) the theoretical loss of heterozygosity will be minimized using MAI as compared to estimates using the other breeding schemes.

An example of the MAI breeding matrix used in 1998 for captive East Fork Salmon River chinook salmon is attached as Appendix A. During that year, no adults were expected to return. It was decided by the Technical Oversight Committee for Salmon River chinook salmon that prespawn adults currently held captive would not be released but instead be spawned in captivity. There were a total of 5 live males and 24 females. Additionally, cryopreserved milt was also available from 2-year old, precocial males from that year class and other year classes.

All individuals were genetically “typed” using a combination of nuclear and mitochondrial RFLPs and microsatellite loci. The genetic information was used to generate coefficients of kinship (described in Lynch and Walsh 1997) for each individual. An overall level of relatedness (kinship) was calculated for the population then divided into 5 separate bins or ranks. Ranks were then assigned to each possible cross between males and females. Cryopreserved sperm samples were also genotyped and ranked. A portion of the resulting matrix is shown in Appendix A. Females are listed at the top along with an abbreviated genotype, males and cryopreserved males are listed on the side. “1-5” denotes the rank assigned each possible cross with a “1” the most preferred

(indicating the most avoidance of inbreeding) and a “5” the least desirable (the most closely related individuals according to kinship). Green boxes indicate where crosses were actually performed.

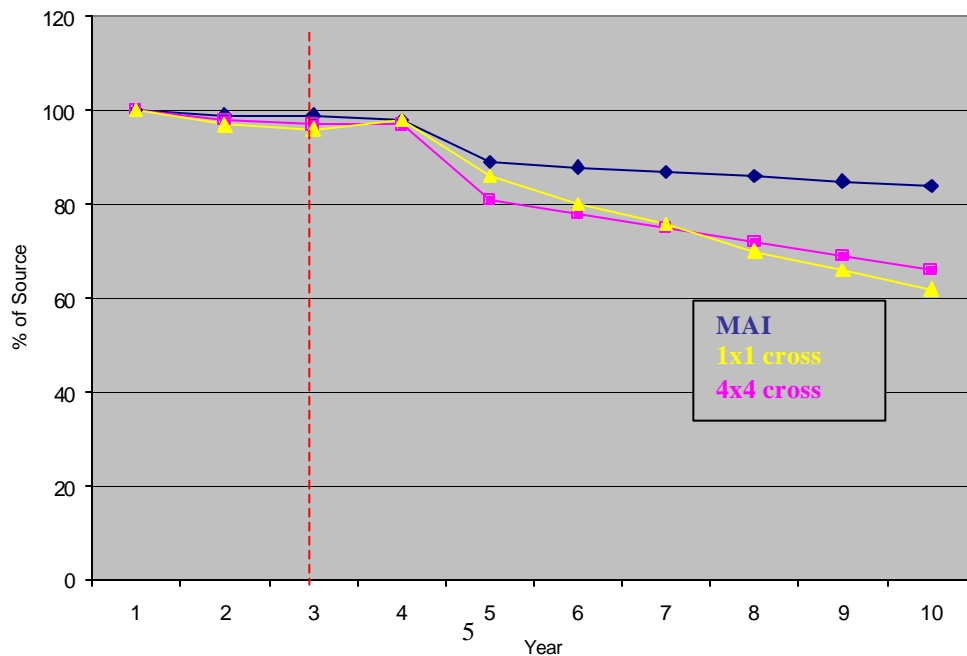
In practice, the most desirable crosses are not always in synchrony with respect to spawn timing. Moreover, some males and some females by virtue of their rare genotypes appear to have a higher proportion of desirable crosses. This is taken into account such that all available individuals are used an equal number of times and desirable crosses are made where logistically possible.

The utility of the MAI matrix can be illustrated with two examples. First, the matrix not only provides data for desirable crosses but also provides information where undesirable crosses were unavoidable (tracking pedigrees). Thus, the question about relative success of family lines or crosses can be assessed in the future. Second, in the case of the 1998 East Fork Salmon River chinook salmon, milt from 2 cryopreserved males were used to “reconstitute” rare alleles back into the surviving live population. The alleles were no longer present in any live individual (they had been lost due to drift). In this instance, MAI was used to infuse genetic diversity only present in a “gene bank” back into the live population.

We agree that MAI has significant value in conservation applications. This program will continue to employ these methods until the planned termination of the investigation in FY2008. At such time, MAI can be empirically evaluated for its utility in salmon conservation. Moreover, as stated above the predictability of our theoretical projections regarding the loss of genetic diversity can also be determined.

Our current and past efforts within the chinook salmon artificial propagation programs are presented at monthly Technical Oversight Committee meetings which are recorded by BPA and are available to the public for inspection and comment.

Figure 2. Heterozygosity loss over time using different spawning methods.



ISRP Comment: 4

This project also monitors the bi-catch of sockeye salmon in a sport fishery for kokanee in Redfish Lake and has demonstrated the bi-catch of anadromous or residual sockeye. While this concern is not the responsibility of this author, it is a concern that in a lake with a listed sockeye salmon stock, at an extremely depressed population size, that a kokanee fishery would be allowed at all. What impact is allowed on sockeye in this fishery and how is it justified?

Response:

The National Marine Fisheries Service is responsible for setting guidelines and justifying incidental take of listed sockeye in Redfish Lake. Genetic surveys of creel samples have in the past demonstrated that listed sockeye have indeed been reduced to possession during the kokanee harvest. However, the expanded estimates of the incidental listed sockeye harvest have remained lower than the maximum allowed by NMFS. That a kokanee fishery exists at all in Redfish Lake is an issue with the Idaho Department of Fish and Game who's mandate it is to provide fishing and hunting opportunities to its constituents (and concomitantly protect wildlife and fish as well).

It can be argued from an IDFG perspective that a sport harvest on kokanee reduces the competition of resources with anadromous and residual sockeye. Sockeye lakes in B.C. and Alaska typically show either a robust sockeye population which out compete sympatric kokanee or vice versa (Burgner 1991 and references therein). Thus, reduction of the kokanee population through harvest is a management alternative to reduce competition among sympatric populations. Kokanee harvest in Redfish Lake is closed in early fall before the return of anadromous sockeye. However, residual sockeye (which are similar in size to kokanee) remain in the lake. This is rather circular though: kokanee harvest exists because kokanee are there and competing with sockeye / kokanee were historically transplanted there and a weir is maintained to manage a sport harvest. Rainbow trout are also transplanted and managed in the lake for a sport harvest.

Furthermore, from an ecological perspective it could be argued complete elimination of the kokanee population in Redfish Lake would serve the listed residual and anadromous populations even better by removing all competition for food resources within that niche. After all, genetic data using mitochondrial DNA within this program has shown the kokanee population is heavily admixed with out-of-basin transplants.

From a genetic perspective, the kokanee are both temporally and spatially separated in spawn timing from the sockeye so genetic impacts are nil. Genetic analysis by of outmigrants by this program has also failed to show any contribution to outmigrant assemblages from the kokanee spawning in Fishhook Creek. However, there are cases of kokanee "reverting" to an anadromous life history (Burgner 1991) and historically it is

thought that Redfish Lake sockeye spawned up Fishhook Creek and not at their present location in the shallow waters around the lake.

The advantages of genetic monitoring of bycatch are more important during commercial harvest in the Columbia River. In 1999 during a 3-day limited gillnet fishery for sockeye in the Lower Columbia River, 11 Redfish Lake sockeye were identified for sale at roadside markets and 6 were subsequently genetically typed to Redfish Lake as an origin. This information along with a provenance and chain-of-custody were used by Oregon State Police to prosecute the offenders.

References

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Appendix A. Breeding Matrix for 1998 East Fork Salmon River chinook salmon.

		Females						
Fresh males		No tag	EE-02 BB a+	EE-04 AA a	EE-08 AA a	EE-09 AB a+	EE-10 AA a	EE-16 AB a
EE-11	AB a							
EE-14	AA a		3	5	5	1	5	4
EM-11	AB a		2	4	4	2	4	5
EM-13	AA a		3	5	5	1	5	4
EM-22	AA a		3	5	5	1	5	4
EM-26	AB a+		4	1	1	5	1	1
Cryopreserved males								
7F54	AA a+		5	2	2	4	2	2
272E	AA a+		5	2	2	4	2	2
4D03	AB a		3	5	5	1	5	4
7E58	AA a		3	5	5	1	5	4
5201	AA a		2	4	4	2	4	5