Proposal Number 8:
Evaluating The Reproductive Success of Natural- And Hatchery-Origin Columbia River Chum Salmon

Sponsor: **Washington Department of Fish and Wildlife**

INDIVIDUAL RESPONSES

1) Origin Of Question: HHS
Question: Who will be the principal investigators responsible for this project and what previous history do they have with leading BPA or similar projects?
Response: This project will have two co-principal investigators. One will be Steve Schroder, Ph.D., Fisheries Research Scientist, Washington Department of Fish and Wildlife. Currently Steve is a P.I. on BPA project No. 200105300 (Reintroduction of Lower Columbia River chum salmon into Duncan Creek) and also the lead researcher investigating the reproductive success of hatchery- and wild-origin spring chinook salmon for BPA project No. 199506424. The second co-principal investigator is Howard Fuss, Fisheries Research Scientist, Washington Department of Fish and Wildlife. Howard has led a number of NMFS' funded projects that took place in the Elochoman River (e.g. Migratory behavior and incidence of post-release residualism of hatchery-reared coho and chinook salmon released into the Elochoman River, Annual Report Number: FPA99-08; Stock characteristics of hatchery-reared salmonids at Washington Department of Fish and Wildlife Columbia River hatcheries, Annual Report No. H98-03; Migratory behavior and incidence of post-release residualism of hatchery-reared steelhead and cutthroat trout released into the Elochoman River, Completion Report 1996 – 1998). In addition, Howard is a co-principal investigator of a HSRG project that is evaluating the reproductive success of coho salmon spawning in Minter Creek, a south Puget Sound stream.

2) Origin of Question: ISRP
Question: Are the natural chum residing in the Grays River representative of wild chum or has there been extensive incorporation of hatchery chum into the basin so that the population is a homogenous mixture of hatchery and wild-origin genes?
Response: Introductions of "out-of-basin" chum salmon occurred in the Grays River in 1973 and 1974 when a total of 1 million fry originating from the Big Quilcene River, a Hood Canal-Puget Sound population, were transplanted into the basin. Moreover, in 1978 and 1979 a total of 102 thousand fry from an unknown donor stock were also released into the Grays River. Within-basin chum were artificially propagated from 1963 through 1974 at the hatchery. During this fourteen-year period an average of 88,000 chum fry were liberated each year (NOAA Technical Memorandum NMFS-NWFSC-32). When genetic samples collected in the mid- to late nineties from this population were electrophoretically analyzed they proved to be different from the constellation of chum populations that spawn below the Bonneville Dam. They were also distinct from, but related to, the coastal chum stocks that spawn in streams located in Willapa Bay and

Grays Harbor (Larry LeClaire personal communication). Because Grays River chum were not subjected to artificial culture for 18 years or 4 to 6 generations we believe that any effect of hatchery genes, either through domestication or by the introduction of out-of-basin stocks, has been significantly diluted or entirely alleviated. Consequently, non-hatchery origin chum returning to this basin should be representative of wild Lower Columbia River chum salmon. The present chum supplementation program in the Grays River started in 1998 and the first adult fish returned as 3-yr-olds in 2001. Thus, the earliest that progeny produced by hatchery adults spawning in the wild might return to the Grays would be in 2004. Data presented below indicate the magnitude of the current supplementation program.

Summary Of The Grays River Chum Salmon Recovery Program

Brood Year	Broodstock Used		Green Egg-To-Fry Survival	~ No. Of Fry Released Into The Grays
	No. Of Females	No. Of Males		
1998	46	47	91%	110,000
1999	68	71	92%	135,000
2000	119	120	95%	205,000
2001	184	183	93%	305,000
2002	164	162	93%	407,000

Because of its short tenure, and the number of fed fry that were released in the first several years, chum salmon returning to the Grays in 2004, 05, and 06 should contain relatively few NORs originating from hatchery parents. This may change in the future as additional hatchery adults are allowed to spawn in the river, however, in 2003, 04, and 05 homogenization of the chum Grays River gene pool will be as low as it can get with a program designed to allow adults produced from artificially cultured fish to spawn under natural conditions.

3) Origin of Question: ISRP
Question: If progeny originating from H x H, H x W, and W x W crosses have equal fitness at the F2 and F3 generations will this project be able to differentiate between the following two alternative hypotheses: a) That supplementation worked perfectly, or b) That this result occurred because there was no genetic or phenotypic differences between the wild- and hatchery-origin parents to begin with.
Response: Our ability to interpret such a result depends on the validity of the assumption that chum salmon in the Grays River represent a non-domesticated wild population. This seems like a reasonable assumption given the number of generations that have elapsed from the last time cultured chum salmon were released into the basin. Moreover, if we can start this project in 2003 the population will not contain any NORs that have originated from hatchery-origin parents. In 2004 and 05 some NORs containing genes from F1 hatchery fish will be in the population. Such fish will have gone through a single generation without experiencing hatchery selection pressures and therefore are probably closer genetically and phenotypically to wild fish than contemporary hatchery-origin

individuals. Consequently, we believe that if hatchery- and wild-origin chum parents produce similar numbers of "grand fish" it will mean that such fish passed on genetic programs that allowed their offspring to survive under natural conditions. Conversely, if differences exist in the survival of F2 fish, we will be able to assess whether a linkage between survival and other performance variables are related to the proportion of hatchery genes the fish carry.

4) Origin of Question: ISRP
Question: Will supplementation or straying interfere with the ability of the investigators to evaluate the fitness of H x H, H x W, and W x W fish as they move from one generation to the next?
Response: Our experimental design is based on introducing Grays chum salmon into an adjacent stream and providing both hatchery- and wild-origin fish with the opportunity to colonize this new habitat. Their offspring will be sampled and through msDNA-driven pedigree analyses we will know the proportion of fry that have H x H, H x W, W x H, and W x W origins. When these fish return as adults we will once again use msDNA and pedigree analyses to ascertain how many originated from each of these fry types (crosses) and by their individual parents. Clearly the introduction of adult strays or the straying of the adults produced by the project to other spawning locations can profoundly affect assessments about the fitness of the fish used in the original founding populations. One of the strengths of our approach is that straying of non-project fish into the Elochoman will be negligible. A very small remnant population of chum (5 to 20 fish per year) returns to this stream. Because every Grays River adult introduced into the Elochoman will be genotyped it will be possible to identify the few strays that may enter the stream. In such a situation, the effects of strays are minimized throughout the entire life of the project.

On the other hand, if this study were to take place in the Grays River Basin, project fish could stray into other portions of the basin and non-project fish could stray into any area that might be set aside to do this study. Unlike the Elochoman, this is not a trivial problem because more than 10,000 adult chum salmon returned to the Grays River in 2002. Future populations are likely to number in the thousands. Thus to estimate straying an extensive sampling program throughout the basin would have to occur. One could argue that fry produced from the project could be fin clipped or receive CWTs (so-called half tags). We anticipate that 300,000 fry are likely to be produced each year from our study population. Fin clipping or CWTing unfed fry is expensive, difficult, time consumptive, and physiologically stressful to the fish being marked or tagged. Moreover this task seems unnecessary since setting the experiment up in the Elochoman River basin eliminates the need for it.

Finally, the ability to detect adult strays from project fish relies on our capacity to genotype the offspring produced from our experimental populations. As we stated in our proposal, we plan to place modified fyke nets in a controlled-flow stream to capture fry soon after emergence. After capture, a consistent proportion of fry selected at random will be removed from each day's catch. The genotypes of these fry will be used to characterize the reproductive success of the adults that produced them. The genotypes present on the adults produced from these individuals will be compared to the genotypes that were present at the fry stage. In this manner we will be able to test whether H x H, H x W, W x H, or W x W fry survived to maturity at dissimilar rates. Clearly too, individual fitness estimates for the fish that produced these adults will be made. It is not possible to trap and enumerate juvenile chum in the same manner in any Grays River tributary. The flow regimes that occur in the Grays River basin are so extreme, that modified fyke nets, or other types of traps, could not be fished every day of the emergence season. In this circumstance, all the comparisons that we wish to make regarding survival and fitness would be put at jeopardy because of uncontrolled weather events. By building an observation and incubation stream at Elochoman, we do not have to face such sampling uncertainties, we would know exactly how many fry were produced and we would also have a reliable estimate of the genotypes of the produced fry.

5) Origin of Question: ISRP
Question: Is it possible to create a "control" reach where only W x W fish are allowed to mate so that the fitness of the resulting progeny can be compared to that achieved by H x H and H x W produced fish?
Response: As mentioned in our proposal, all the chum salmon produced from the hatchery supplementation program established at Grays River receive thermal marks. These are cryptic so it is impossible to visually determine whether a live fish is of hatchery or wild origin. Therefore it is not possible to set up a control reach where the researcher could be 100% sure that his fish were all wild or all hatchery. Even though such an approach is attractive and has been used by previous researchers, our experimental design doesn't require it. We plan to let both hatchery and wild parents spawn together in a common environment so that both types of parents and their offspring will experience identical conditions. The intent of this work is to compare the relative fitness of the founding parents through two generations. In this case, it seems more straightforward to simply compare the fish and their progeny while they reside and experience the same environmental circumstances.

6) Origin of Question: ISRP
Question: Can the project determine the growth rate (lambda) and genetic diversity of the population in subsequent generations?
Response: Yes both lambda and genetic diversity can be assessed if the project takes place in the Elochoman Basin. As suggested above, using a controlled-flow stream for a spawning area in the Elochoman and genotyping all the fish placed into it will allow us to track the reproductive success of each fish introduced into the stream over time. The abundance of chum returning to the Elochoman can be monitored and the genetic diversity of the adults returning to the stream will be

examined. As suggested above this would not be possible if the project were embedded in the Grays River basin which contains a larger co-existing population of chum. Strays from that larger population would likely spawn in the area sequestered for the project. In addition, project fish would also stray into other parts of the Grays basin and thus be unavailable for sampling. The ability to census fry genotypes would also be compromised because of the uncertain flow regimes that occur in the Grays basin.

7) Origin of Question: ISRP
Question: How will out-of-basin factors that affect survival and other demographic parameters by accounted for in the proposed experimental design?
Response: At present, both Federal and State biologists are monitoring the abundance of the chum populations in the Lower Columbia River. Those data can be used to see if our project fish increase or decrease in abundance in concert with other Lower Columbia River chum populations. Because we will know the ratios of the H x H, H x W, W x H, and W x W fry that are produced by our experimental adult populations we will be able to see if any of these survive at lower or higher rates than one another and how their productivity compares to other adjacent chum populations.

8) Origin of Question: ISRP
Question: What type of quality assurance and control plans will be instituted to ensure that the genetic information obtained from the study will be processed, managed, and interpreted properly?
Response: There are two major areas where quality control has to occur. First, sample collection, preservation, and identification, and second linking msDNA characterizations to specific samples. These concerns will be addressed by using the following steps that are being used in two other reproductive success evaluations that rely on msDNA and associated pedigree analyses.
a) Pre-printed uniquely numbered labels are inserted into vials filled with 100% ethanol
b) When samples are collected one person is used to collect tissues another is used to record the sample number associated with the tissues being preserved. The sampler provides the alphanumeric code to the recorder, the recorder repeats the code back to the sampler to ensure that the code was heard and recorded correctly. Such samples will be taken at the time adult fish are placed into the observation stream. Biological data on each fish will also be recorded at this time (sex, general condition, weight, Petersen tag number, section of the observation stream the fish will be placed into, date and time of processing, egg losses due to tagging if any occur, and scales for later age determination). Chum salmon males may through normal fighting behavior loose Petersen disks. Therefore we plan to insert PIT tags into each adult to provide another means of identifying each fish, such tags will be applied and their numbers recorded when DNA samples are being taken.

c) At least two tissue samples will be collected from each adult fish being sampled. To prevent spoilage, 80% or more of the volume of a vial will consist of 100% ethanol. Dual samples are taken so that one can be run and the other can be archived for error checking if needed.
d) When otoliths are collected from the fish another tissue sample (opercle punch or similar sample) will be included with the otolith. These samples will also be preserved in 100% ethanol. If a fish has lost its tags, additional DNA samples will be taken and placed into labeled vials. Post-mortem data will be collected on each adult, such information will include date and time of death of collection, weight, length, egg retention and testes weight.
e) MsDNA samples will be collected from whole fry. A consistent proportion (most likely between 1 to 3%) of the fry emigrating from each section on a given day will be preserved in 100% ethanol. Again, 80% or more of the sample will consist of ethanol. In addition the fry will first be killed by a heavy dosage of MS222. The fry will then be added to jars partially filled with ethanol. The specimens will be mixed with the ethanol by gently rotating the jar to ensure that each fry is well preserved, the jar will then be filled with ethanol. A label indicating the location, species, and date of collection will be added to the jar.
f) When the samples arrive at the laboratory the vial labels from each sample are recorded on an electronic spreadsheet that mimics the extraction template. At this time any discontinuities in sample numbers are noted and sample quality is also recorded. Fry samples are treated in an analogous fashion.

Finally we also enhance our ability to perform pedigree assessments by purposefully choosing loci or markers that are easy to score. We anticipate that 18 to 20 such loci will be used.

9) Origin of Question: ISRP
Question: A) Why can't a direct study of reproductive success occur in the Grays River or a weir-controlled part of the river? **B**) Will the behavior of spawning chum salmon in an observation stream be similar to what they would express under natural conditions? **C**) Would a direct study be infeasible?
Response: A) There are both pragmatic and experimental design considerations that make studying the reproductive success of hatchery- and wild-chum salmon in the Grays River basin impossible. First, the Grays River and its tributaries have, as alluded to above, extremely variable flow regimes. A USGS report on monthly flow regimes from October – May, over a six-year period, showed that average cubic feet/second volumes varied from 131 to 1,465 over this time period. Peak flows were not presented in this database. Our personal experience with the Grays River might put this type of variation into perspective. As part of an attempt to protect Grays River chum salmon an artificial channel referred to as Gorley Springs was built adjacent to the main stem of the river just above the confluence of the West Fork of the Grays River. This structure was protected from the river by 15 to 20-foot high berms along its entire course. We established a weir and adult trap at its mouth in 1998 and again in 1999. In December of

1999, the Grays flooded, breached the berms protecting the Gorley channel and shifted its main flow down the Gorley channel. In the process it destroyed the channel, damaged and flooded farmland and destroyed a house. When the floodwaters finally subsided we attempted to find our weir, not a single trace of it was found. The Grays watershed is heavily logged, its banks are steep sided and the lower river is channelized. Moreover, the flat river valley contains unconsolidated marine sediments that are deposited over a basaltic intrusion -- a geological situation reminiscent of marbles being placed on a steel plate. The combination of these environmental realities is that no area in the flood plain is immune to being inundated and completely altered by the river during its high-flow period that coincides with the time that chum spawn and incubate in the river. For our project to work, we have to know the number and origin of the fish placed into a natural spawning area, we also have to count and sub-sample the fry produced from those adults. We know of no location in the Grays where a weir could be placed that could withstand the vagaries of weather events to produce such assurances. Moreover, the placement of a weir and creation of natural spawning areas require permits and property ownership and in some circumstances easements through adjacent property. Other than the hatchery located on the West Fork, WDFW does not own land in the basin, and the ability to get permits to place weirs in a flood plain as dynamic as the Grays is problematic. Finally, many of the local landowners have denied WDFW employees and other researchers access through their property to get to the Grays River, even to sample carcasses. By placing the project in the Elochoman we eliminate a vast number of problems. We can establish a stable area for chum to spawn in, we avoid the straying concerns that were expressed above, we own the property, and permitting becomes feasible. Moreover, the trapping of fry and adults is possible, weirs and traps will not be blown out, detailed observations on the fish spawning in the stream will be possible and not affected by high sedimentation rates caused by flooding. In essence we will have a controlled environment in which to perform this study, not one that may completely disappear because of uncontrolled flooding and unpredictable weather patterns.

B) Chum salmon have successfully spawned in controlled-flow streams or spawning channels throughout the Pacific Northwest. Such structures have not only been used to supplement natural populations but also have served as important research areas that have investigated the effects of different gravel compositions on egg-to-fry survival rates, the effect of differing instantaneous spawner densities on reproductive success, the possibility of using controlled redd superimposition as a management tool, and on examining the mechanisms responsible for mate choice in this species. These studies show that chum can successfully spawn in controlled-flow areas and that they are able to express their normal behavioral repertoires in such settings.

C) We believe that a study whose intent is to track individual fitness values over multiple generations must be conducted in a location where project fish can be sampled and not diluted by straying. In addition it must be carried out in a spot where adult behavior and the production of fry can be assessed. The Grays River basin does not provide such a location. Tributaries in this basin are just as susceptible to very large fluctuations in water flows as the main stem. This is a challenging enough study to carry out without adding another layer of significant uncertainty by placing the project in a location that would be subjected to regular flooding and perhaps completely destroyed part way through the study.

10) Origin of Question: ISRP
Question: What proportion of wild- and hatchery-origin chum salmon returned to the West Fork of the Grays River in 2001 and 2002; what proportion of wild- and hatchery-origin chum salmon returned to Crazy Johnson Creek or elsewhere in the Grays basin in 2001 and 2002?
Response: We examined otoliths from adult chum salmon that had returned to the West Fork of the Grays River in 2001 and again in 2002. Additionally chum collected outside of the West Fork were examined in 2001 and otoliths from chum returning to Crazy Johnson Creek in 2002 were examined. The results of these evaluations are presented below:

Location	Year	Number Of Otoliths Analyzed	% Hatchery Or Otolith marked Fish In The Sample
West Fork Grays River	2001	366	63.4%
Grays River Excluding The West Fork	2001	200	4.5%
West Fork Grays River	2002	220	16.4%
Crazy Johnson Creek	2002	133	6.8%

All the otolith data presented above were obtained from fish that had spawned naturally in the Grays River. Many of the West Fork samples examined in 2002 came from adults that had spawned at the mouth or lower portion of the West Fork because this is where the majority of the chum spawned. In the Table below we present otolith decode information obtained on the fish that were used as broodstock in 2001. Almost all of these fish were collected below the hatchery or had volunteered into the hatchery adult pond. In that year we spawned 184 females and 183 males, 61.2% of the females were three-year-olds (112 fish) and 73.9% of the males were three-year-olds (136 fish). Recall that these three-olds could have potentially originated from our 1998 releases of fed fry from the hatchery.

Sex	Number of Three-year-olds examined	% Of three olds used as broodstock that were hatchery-origin chum
Females	112	96.4%
Males	136	91.9%

These decodes clearly show that hatchery-origin chum returned to the West Fork adjacent to the hatchery. Moreover, these data also indicate that many of the hatchery-origin adults returning in 2004 (as three-year-olds) may be second-generation hatchery fish. In summary, the above information suggests that we should have a good chance of obtaining hatchery-origin fish if we concentrate our collection efforts around the hatchery. Likewise if fish are collected from the lower part of the West Fork or in Crazy Johnson Creek we should be collecting mainly wild-origin chum salmon.

11) Origin of Question: ISRP
Question: Could establishing areas where only fish of the same origin reproduce with one another provide a more powerful way of assessing the reproductive success of hatchery- and wild-origin chum salmon? Could such areas be established in the Grays River basin?
Response: We do not believe so. For reasons already stated, it seems to us that it is more straightforward to allow adults of different origins to compete for mates or space and to follow the survival and reproductive success of their subsequent offspring through time. Confounding factors related to possible effects linked to location differences disappear when hatchery and wild fish are allowed to spawn in the same locality. As suggested above, logistical problems dealing with water flow, property acquisition, permitting, and access make the establishment of two spawning locations in the Grays impossible.

12) Origin of Question: ISRP
Question: A) What evidence is there that the selection of fish from the West Fork of the Grays River will provide mostly hatchery-origin fish? **B**) What evidence is there that the chum salmon collected in Crazy Johnson Creek will be largely wild-origin chum salmon?
Response: The tables presented in question 10 show that more hatchery fish are found in the West Fork of the Grays than elsewhere in the basin. Plainly, one of the things that must be accomplished to perform this study is to introduce both hatchery and wild fish into an area where they can simultaneously spawn. We have developed the following protocol to maximize the likelihood that we can achieve populations that are equally represented by both types of fish. First we will collect fish adjacent to the hatchery, each collected individual will be placed into its own collection tube (10” by 40” PVC pipe equipped with removable end pieces) and its sex and date of collection will be penciled on the outside of the tube. The tubes will be placed into the adult holding pond located at the Grays River Hatchery. At the same time chum will also be collected from the lower portion of the Grays or from Crazy Johnson Creek. They will also be placed in individual tubes, labeled and transported, and held at the hatchery. A portion of the fish from each collection will be spawned either on same day they were collected or the following day. Otoliths will be removed from the fish and decoded at the hatchery by using a portable lap wheel and dissecting microscope. These decodes will give us an estimate of the proportion of hatchery and wild fish obtained from each collection point. This information will be used to create experimental populations from the remaining captured fish that are made up of approximately half wild and half hatchery fish. We have used real-time otolith

decoding for over a decade and have developed procedures that can be used to rapidly (>12 specimens every 15 minutes) and accurately decode newly recovered specimens.

13) Origin of Question: ISRP
Question: If it is not possible to control the proportion of HOR and NOR spawners does that affect the ability of the proposed research to assess the reproductive success of HOR and NOR spawners?

Response: We will use the method described above in an effort to achieve balanced hatchery and wild populations. Our design however, does not depend upon having equal numbers of males and females of each type in the observation stream. Instead we seek to track changes in hatchery and wild genes across two generations and to track deviations from expected proportions of H x H, H x W, and W x W crosses. Up to 400 fish will be placed into the channel, 200 of each sex. At least 50 of these or 25% of each sex should represent one of the adult types. If any fewer than this number is added to the stream then it may become difficult to track generational changes. What will undoubtedly occur is that a different overall proportion of hatchery and wild fish will be placed into observation stream each year. However, with our ability to decode otoliths from chum originating from different locations in the Grays River, and the proclivity of hatchery fish to home to the hatchery, we should be able to produce populations that have fairly similar numbers of hatchery- and wild-origin fish.