

**RFS# 013**

**Analytical Approach for Determination of Effects of Hatchery Reform  
on Extinction Risk and Recovery of Salmon and Steelhead**

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**Proposal in response to RFP: Synthesis of Existing Analytical Approaches, or  
Development of a New Analytical Approach, for Determining the Effects of  
Hatchery Reforms on Extinction Risk and Recovery (FCRPS BIOP Action #184)**

by

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# **Analytical Approach for Determination of Hatchery Reform on Effects of Extinction Risk and Recovery of Salmon and Steelhead**

## **1. Project Summary**

We propose a three-part study, designed to provide information and perspectives that can be directly applied to the operation of hatcheries, and help understand the evolution of salmon in a hatchery. Our first objective is to develop a series of protocols that could be used for breeding salmon in hatcheries, based on explicitly stated policy objectives. This objective will be accomplished by combining the scientific literature on salmonid breeding systems and reproductive success with the policies and protocols of agencies wherever salmonids are bred. These investigations will result in a report reviewing the literature, summarizing the results of agency surveys, and a set of conclusions based on a variety of goals and perspectives.

We will utilize the information gathered in the Artificial Production Review and Evaluation (APRE) and Hatchery Genetic Management Plans (HGMP) processes. APRE reports will contain valuable information on the goals of hatchery programs, data on benefits and risks of each hatchery program, and hatchery-related data.

The product will be a quantitative and qualitative analytical framework that will incorporate existing studies and new studies, as they become available. For this purpose, we will develop a statistical model to test for differences in components of fitness-related traits in hatchery and wild environmental conditions.

We also propose to test methods of assessment of hatchery reform through various quantitative and population genetic methods.

## **2. Project Description**

### **Introduction**

Hatcheries in the Columbia River Basin were developed to sustain the commercial salmon fishery in the early 1900s, and later used to mitigate for habitat losses caused by river development. Protection afforded by the hatchery environment increased survival to the smolt stage in excess of 70%, compared to less than 10% under natural conditions. Consequently, commercial and sports fisheries have become increasingly more reliant on hatchery production, which has accounted for over 90% of the Columbia River chinook salmon harvested in 1990s. Hatchery production was successful enough that escapement levels were minimized to exploit excess returns for harvest. However, mixed populations of wild fish, which require greater escapement levels to be sustained were exploited at the same rate as hatchery fish, diminishing their numbers even further than what was apparent from loss of habitat.

Authorized under federal law (the Grand Coulee Maintenance Project, the Magnuson-Stevens Act, the Mitchell Act, and the Lower Snake River Compensation Plan), the National Marine Fisheries Service (NMFS) has used hatcheries to provide

increased commercial harvest of salmon from the Columbia River Basin. However, NMFS excluded hatchery fish in determinations of the status of populations in their review of salmon and steelhead populations under the Endangered Species Act (ESA), unless they were essential to the recovery of the population (Hard et al. 1992).

Consequently, this brought the use of hatchery fish in supplementation programs designed to strengthen native populations or help recover weak stocks into question. Adding to the controversy was the recent article in *Fisheries* by the Independent Scientific Advisory Board of the Northwest Power Planning Council (ISAB 2002), entitled Hatchery Surpluses in the Pacific Northwest. The article pointed out potential risks created by allowing hatchery fish to spawn in the wild, and suggested that such practices need careful review.

### **Reproductive Success**

Salmon species exist as more-or-less discrete and isolated breeding populations, differing in selectively neutral traits and a variety of adaptive, quantitative traits (Ricker 1972, Taylor 1991; Quinn 1999; Brannon et al. 2002). This “stock concept” is the cornerstone for conservation and management of Pacific salmon. It is widely recognized that the health of salmon species depends on the maintenance of the complex population structure (Scudder 1989; Riddell 1993; Waples 1995), characterized by great diversity and adaptation to local conditions among populations, but the geographic or ecological scale of the adaptation remain largely unknown.

The variation in reproductive success ( $V_k$ ) of individuals that also differ in heritable phenotypic traits is a fundamental part of natural selection and the evolution of populations.  $V_k$  is a function of the success of individuals at obtaining mates and producing viable offspring. Mature adults are subjected to sexual selection (e.g., intra-sexual competition and mate choice), resulting in variation in  $V_k$ . Mortality agents such as nest disturbance, predation, disease and abiotic factors may take different proportions of the fish from different families (i.e., individuals with the same parents), resulting in further variation in reproductive success among the adults of the previous generation. These processes are not unique to salmon but are common to animals (Emlen and Oring 1977; Wade and Arnold 1980; Clutton-Brock and Parker 1992; Andersson 1994), and are a natural component of evolution.

A number of traits have been documented or hypothesized to affect  $V_k$  in salmon. At the breeding stage, males actively compete for access to ripe females, and large males tend to dominate such competition (Gross 1985; Foote 1988; Quinn and Foote 1994). However, small males may adopt alternative reproductive tactics (e.g. sneaking rather than fighting) and successfully fertilize some eggs (e.g., Schroder 1981; Chebanov et al. 1983; Maekawa and Onozato 1986; Foote et al. 1997). The success of males using these tactics changes rapidly over the course of the breeding season as the operational sex ratio (OSR: ratio of sexually active males to sexually active females) increases (Quinn et al. 1996). Most research has focused on variation in  $V_k$  among males but reproductive success may vary considerably among females as well. Larger females have more numerous and larger eggs (producing larger fry, with higher survival rates) than smaller females (Beacham and Murray 1993), and they dig deeper (hence less vulnerable) redds (Steen and Quinn 1999), so there could be size-biased variation in female reproductive success. However, Holtby and Healey (1986) questioned this hypothesis that large

females are more productive than small females. Much of the high mortality rate for embryos is related to gravel scour, and this may vary greatly among families but be unrelated to female size. In addition to the factors affecting reproductive success in males and females, there is evidence that salmon tend to mate assortatively by size (Hanson and Smith 1967; Foote 1988; Foote and Larkin 1988); that is, large males tend to breed with large females, and smaller males with smaller females, as a result of both competition and choice. Finally, the date of spawning is heritable, hence the progeny of early spawners will be more likely to mature at the same time as their siblings than non-siblings (Siitonen and Gall 1989), leading to genetic isolation by time within populations (Leary et al. 1989; Gharrett and Smoker 1993; Bentzen et al. 2001). This may make them vulnerable to mortality agents affecting certain segments of the population (e.g., flooding during incubation: Thorne and Ames 1987).

Thus the breeding biology of salmonids is characterized by uneven contribution of adults to the next generation (i.e., selection), and this tends to reduce the effective (as opposed to absolute) population size. In addition, a series of connections between juvenile life history events may result in differential survival of sibling groups, magnifying the variance in reproductive success ( $V_k$ ) of the parental generation. Juveniles of most species establish feeding territories in streams, and the ability to acquire and hold territories is largely a function of size and prior residence (e.g., coho salmon, *O. kisutch*: Mason and Chapman 1965; Rhodes and Quinn 1998). Egg size controls the initial size of juveniles, and juveniles that emerge early (because they were spawned earlier) will have a size advantage when later-emerging juveniles try to compete with them for space and resources. Territoriality is also strongly related to prior residence, hence there may be great variation in territorial possession among families. Studies with Atlantic salmon and brown trout indicate the importance of these factors for survival (Brännäs 1995; Einum and Fleming 1999, 2000).

In addition to factors related to size and date of emergence, growth rate varies among families (e.g., Beacham 1989). Juveniles are also attracted to siblings (Quinn and Busack 1985) and sibship can affect patterns of foraging and aggression (Brown and Brown 1993), and distribution and growth (Quinn et al. 1994). The combination of family-specific variation in fry size, date of emergence, growth rate, social behavior, and distribution may lead to considerable variation among families in size at the end of their first summer in the stream. Size is positively correlated with survival through the winter in freshwater (e.g., in coho salmon, *O. kisutch*, Quinn and Peterson 1996) and at sea (coho salmon: Holtby et al. 1990; cutthroat trout, *O. clarki*: Tipping and Blankenship 1993; and steelhead trout, *O. mykiss*: Ward et al. 1989). Finally, there is evidence for family-specific variation in survival at sea independent of smolt size (pink salmon, *O. gorbuscha*: Geiger et al. 1997; chinook salmon, *O. tshawytscha*: Unwin, Quinn and Kinnison, in review).

### **Artificial Propagation**

Many factors have reduced wild salmon abundance, including impassable or injurious dams, overfishing, land use practices, and variation in marine and freshwater conditions driven by climate (Stouder et al. 1996; National Research Council 1996). To

offset the effects of these factors, salmonids have been produced in hatcheries in the Pacific Northwest for over a century. Considerable attention has been focused recently on the benefits and costs of such artificial propagation (e.g., Hilborn 1992; ISAB 2002). Some of these concerns pertain to the elevated fishing rates on mixed wild and hatchery populations (Wright 1993; Hilborn and Eggers 2000), and possible competitive interactions between populations (Nickelson et al. 1986; Nielsen 1994). These kinds of concerns can be difficult to resolve but are relatively simple to understand. However, genetic concerns regarding hatcheries are much more intricate and perplexing. Reviews of this subject (e.g., Hindar et al. 1991; Waples 1991; Ryman et al. 1995) identify three major issues: fitness of hatchery populations for post-release survival, interactions between wild and hatchery populations, and reduction in effective population size due to supportive breeding.

Most kinds of animals bred for human consumption (e.g., chickens and pigs) are given no opportunity to interact with wild members of their species (if they even exist). Salmonids are unusual in that we breed them in highly artificial environments but then expect them to range freely on the ocean to feed, grow and return (excepting completely controlled and closed aquaculture operations). A large and growing literature demonstrates that captive rearing induces a number of phenotypic changes in salmonids. Some changes such as color (Maynard et al. 1995) are largely if not exclusively environmentally induced but others may result from both genetic and environmental changes such as aggression (Berejikian et al. 1996; Rhodes and Quinn 1998), growth (Reisenbichler and McIntyre 1977), and anti-predation responses (Berejikian 1995). Changes in the timing of migration and breeding are largely under genetic control (Flagg et al. 1995; Quinn et al. in press).

There are many studies indicating that the survival rates of hatchery fish are lower than those of wild conspecifics; these differences are obviously attributable to many factors. Nevertheless, the potential for genetic changes in hatchery populations are cause for concern, not only for the fitness of the hatchery population but also for wild populations with which they might interbreed. The most convincing studies on this subject, conducted on Kalama River steelhead (Chilcote et al. 1986; Leider et al. 1990), showed that hatchery adults produced markedly fewer offspring, when spawning naturally, than did wild adults. In this case the hatchery stock was derived from another basin, thereby limiting the comparability of the offspring, so the generality of the finding is not clear. In addition, the hatchery had been operating for many years, so the extent of introgression prior to the study was difficult to ascertain. Finally, the study relied on protein electrophoresis and could assign the fish to population (hatchery or wild) on a probabilistic basis but could not assign them to specific parents. Thus, the traits associated with reproductive success (and the poor performance of the hatchery fish) could not be determined. Notwithstanding these drawbacks, the Kalama River work was extremely important and influential, and there is evidence elsewhere of poor performance of hatchery fish in natural environments (McGinnity et al. 1997).

Hatcheries greatly increase egg-to smolt survival but often have poor post-release survival. This is largely a result of rearing practices, and there are efforts to improve them (Maynard et al. 1995, 1996a, b; Brannon et al. 1999), but many phenotypic changes affecting survival have a genetic basis as well. In some cases, there has been

deliberate selection for a trait that will facilitate operations in the hatchery (e.g., spawning fish that mature early in the season and discarding those that ripen later: Ayerst 1977; Crawford 1979). More often the selection is inadvertent and less obvious. Since most fish survive in the hatchery, genetic changes probably result from one of two processes. First, hatcheries fail to cull fish of “poor quality” that would be selectively taken by predator or pathogens in nature, and natural processes cull them after release. After eliminating the obviously abnormal fish, most hatchery managers are unwilling to cull large numbers of their fish without a clear set of criteria. Even if they were willing to cull many fish, how would they know which ones were unfit? There is little opportunity in a hatchery to test the vigor of juvenile salmon at a production scale, so this process may be unavoidable.

The second obvious form of selection in hatcheries is the mating system, and this is the main “pressure point” by which we may practically affect salmon evolution in hatcheries. There seems to be little documentary evidence on the specific practices in hatcheries but it is widely acknowledged that staff often tended to select a small number of males for breeding based on size or more subjective criteria related to “quality”. The use of a few males was both a matter of selection and convenience; only a few males are needed to fertilize the eggs of many females. Females tended to be spawned until the capacity of the hatchery was reached. Thus, except for attributes related to timing, there may have been little variation in probability of breeding and reproductive success (other than fecundity) among females but great variation among males.

### **Goals of conservation**

Geneticists have expressed concerns about any loss of genetic diversity that might be associated with artificial breeding programs (e.g., Utter 1998), especially as supportive breeding in itself may cause a reduction in genetic diversity (Ryman *et al.* 1995). In the establishment of breeding protocols, the dominant philosophy has been to prevent loss of genetic diversity. For example, the Alaska Department of Fish and Game (ADF&G) Genetic Policy (Davis *et al.* 1985) stated (on p. 14) that “Fitness is enhanced by heterozygosity” and (on p. 15) that “Any loss of genetic variation will be accompanied by a concomitant reduction in fitness.” Highly competent geneticists wrote this report but the statements linking genetic diversity to fitness were made without reference to specific evidence. The report was also very vague with respect to specific protocols (i.e., how the goal of diversity was to be achieved) but it expressed the need to keep the effective population size high ( $N_e > 400$ ).

Maintaining diversity in order to maximize fitness is not the universal goal. In NMFS hatcheries in southern California (e.g., Scott Creek and San Lorenzo), policy is designed to more closely mimic natural patterns. The protocol is for each female’s eggs to be fertilized with milt from four males (randomly picked) and each male to be given a change to fertilize eggs from four females. However, some agencies do not have any uniform policies. Contacts at the Washington Department of Fish and Wildlife (WDFW) indicated that there is no formal policy statement and set of protocols regarding genetics in hatcheries, though individual facilities may have protocols that suit their particular needs (John Kerwin and Howard Fuss, WDFW, Olympia, pers. comm.).

In the Columbia River Basin, the Integrated Hatchery Operations Team (IHOT 1994) produced a report on policies and procedures for salmonid hatcheries. The evaluation of “Performance Standards” (p. 35) was all related to aspects of production such as number and size of smolts, date of release, water temperature, etc. However, the report contained a “Genetics Policy” (pp. 65-72); the objective was to “... maintain adequate genetic variation and fitness in populations” (p. 66). This policy was to be achieved by three goals:

- 1) “All fish produced and released meet identified management objectives for specific artificial production programs and follow genetic guidelines.”
- 2) “Monitor and evaluate implementation of genetic guidelines and genetic effects of artificially propagated fish on wild, natural, and cultured populations.”
- 3) “Foster open and frequent communications among managing entities to jointly resolve related issues.”

More specifically, the policy objective was to be achieved by “... broodstock collection guidelines designed to minimize selective pressures from hatchery practices.” A series of protocols were recommended, notably:

1. Use of 1:1 sex ratio and single-pair matings if possible (“sperm should not be pooled”)
2. Random matings with respect to fish body size
3. Use of jacks in proportion to their abundance
4. Absence of selection against fish whose appearance suggested “poor quality”

The philosophy was summarized in the suggestion that the hatchery manager should “... make sure that all mature parents contribute equally to the spawn taking.” If the total run exceeded the capacity of the facility then all fish should be spawned and equal proportions of the eggs discarded, rather than sacrificing the late fish and keeping all eggs from the early fish. This may be a sound policy if the objective is to maintain as much of the population’s genetic material as possible but it is in stark contrast to the natural patterns of breeding observed by behavioral ecologists (e.g., Hanson and Smith 1967; Schroder 1981; Gross 1985; Keenleyside and Dupuis 1988; Quinn and Foote 1994; Quinn et al. 1996). Is there room for improvement of breeding protocols in hatcheries?

Aldo Leopold wrote in “Round River” (1953), “If the biota, in the course of eons, has built something we like but do not understand, then who but a fool would discard a seemingly useless part? To keep every cog and wheel is the first precaution of intelligent tinkering.” To some extent, this is the present philosophy with respect to genetic resources: keep all the material and be sure nothing is lost. This may be wise when it comes to biodiversity at the species level but is it necessarily wise at the level of gene pools? Can you repair a machine by putting all the “cogs and wheels” into the case and shaking them up, as may result from such breeding protocols? Although the existence of

genetic variation in fitness-related traits among populations is unequivocal, genetic diversity as estimated from sampling variability in neutral genetic markers may have little relation to fitness variation.

### **Risks in the use of hatcheries for enhancement of natural populations**

It is important to make the distinction between artificial propagation and fisheries management, because in essence the hatchery controversy is really not about hatchery fish, but rather the manner in which hatcheries and hatchery fish have been used. That fact is often overlooked. Most allegations that hatchery fish pose serious risks to wild fish are based either on interpretations made from laboratory studies, translocations of poorly adapted fish into new environments, genetic changes in totally captive populations, or performance of first-generation hatchery fish in the wild. The effects of releasing artificially propagated salmon and steelhead on their own populations is rarely if ever addressed, and yet this is where the real question resides.

ISAB (2002) stated that substantial experimental evidence demonstrated that domestication selection can genetically alter hatchery populations in a few generations and that hatchery–origin adults returning from the ocean and spawning in the wild produced fewer progeny than adults of wild origin spawning in the wild. They also felt more limited evidence suggested that interbreeding between hatchery-origin adults and wild fish can reduce the fitness of the wild population. Such statements coming from the ISAB are of serious concern because hatchery fish make up nearly 90% of the chinook salmon and 60% of the steelhead returning to the Columbia River (Brannon et al. 2002). If hatchery fish present such risks to wild populations of salmon and steelhead, and if hatcheries are to continue their objective of sustaining salmon and steelhead runs in the Columbia River, significant measures in hatchery reform must take place. The key to addressing this need is the determination of what constitutes risk.

### **Risks Associated With Hatchery Operations.**

In the concerns shared by ISAB (2002), the hypothetical situation was put forth where interbreeding between hatchery-reared and wild salmon could result in incompatible genotypes, and thus the eventual loss of the fish targeted for recovery. In discussing the mechanisms responsible for risks to healthy salmon and steelhead populations, three areas of risk were identified as outbreeding depression, domestication selection, and inbreeding depression. It is important that these breeding scenarios be briefly reviewed.

(1) Outbreeding Depression occurs when non-local fish interbreed with the locally adapted population and introduce undesirable alleles, or breakup of co-adapted gene complexes. Outbreeding is recognized as a potential risk, and admittedly, management programs in the past ignored the importance of maintaining stock integrity. We are not aware that the extensive mixtures of genetically different stocks that have occurred over the past century have created incompatible genotypes, even where some genetic homogenization has taken place (Hard et al. 1995; Campton 1995). However, mixtures and translocation of hatchery fish throughout the Columbia Basin have been routine over

the years (Flagg et al. 1995, Peven 1992, Busack et al. 1979) and is still part of management practice. We agree that interbreeding caused from such translocations and mixtures of broodstock may threaten stock structure and population fitness (Brannon et al. 2002, Ayerst 1977, Fessler 1977, Leider et al. 1996), and changes resulting from outbreeding need to be identified and their impact described.

(2) Domestication is defined in this proposal as the consequence of unintentional selection in hatcheries, referred to by Campton (1995) as natural selection in the hatchery environment. More specifically, we classify these changes as unintentional changes to the genotype that have deleterious effects when the fish are returned to the natural environment. We emphasize unintentional changes because hatchery programs have undertaken many intentional changes, which we believe are reversible. For example, managers wanting to target certain fisheries, an earlier release date, or segregation between hatchery and wild stocks, purposefully moved hatchery spawn timing earlier. The result was to create hatchery stocks for those purposes, but in most cases, they are unsuitable for most natural stream systems, and excess production from such programs presented a potential risk to overlapping populations. Unintentional changes may also be reversible, but since they were unintentional, they are most often unknown and the effects unpredictable. These changes also need to be identified and their effects determined.

(3) Inbreeding was the third genetic concern identified by the ISAB, and this is related to the effective size of the population, ( $N_e$ ), which represents the real genetic diversity present, rather than simply the number of fish in the population. We recognize that this was a problem in many hatchery programs of the past, where single males were used on many females, which encouraged the reduction in  $N_e$ . With reduction in diversity comes the limitations on the ability of the population to adapt to changing environments or new challenges in species interactions. It will be important to be aware of what level of diversity exists in hatchery and wild populations, and to describe the nature of that diversity.

The genetic rationale used when characterizing the relationship of hatchery fish with their wild population is associated with these risks. The criteria are genetic distances (as in dendrograms) estimated from allele frequencies of variable enzymes or genetic loci traced by chemical means. It is presumed that the greater the differences in allele frequencies between the hatchery and wild fish in the population, the less fit are the hatchery fish. That conclusion is based on the assumption that differences in allele frequencies would also represent a similar degree of difference in functional life history traits that influence survival and reproduction. However, these supposedly neutral alleles are not subject to natural selection, so there is no reason or evidence that frequency differences would have any relationship to functional performance in the wild. If the argument is that the hatchery environment is less selective, permitting the presence of less advantageous traits, and thus greater diversity, there is no reason to doubt that these fish will return to the optimum phenotype once they are reproducing under selective forces of the natural environment, and that can be monitored. Conversely, if the argument is that the gene pool of hatchery fish is less diverse because of limited brood representation, it will also be detectable through monitoring programs. Loss of diversity is less apt to happen in present hatchery programs with larger brood populations. The desirable level of diversity in salmon and steelhead populations is uncertain, except that

genetic homozygosity is considered limiting. In natural populations, assortative mating, male hierarchies, and isolation on the spawning grounds also limits representation of gene pool among wild progeny. It is also important to realize that diversity is not the most important characteristics of the population. One only needs to look at the known innate characteristics of populations (spawn timing, embryo development rate, fry rheotaxis, smolt orientation, etc.) to understand that stock specificity is of critical survival value, and that it doesn't necessarily fit with maximum diversity. Therefore, the standard with regard to genetic diversity will be what exists in the natural populations where such information is attainable.

It is difficult to relate these risks to actual problems observed in the field because sufficient data has not been collected to demonstrate the changes that occur or the result of those changes on the characteristics of the population. However, the empirical evidence that was used in documenting the negative consequences of interbreeding between hatchery fish and wild fish by the ISAB (2002) is informative about the potential of such risks because it contained the most important references on the subject of deleterious effects that may be expected from interbreeding with hatchery fish. It is instructive, therefore, to re-examine those works to better understand the manner in which risk can be represented.

### **Cited Empirical Evidence of Biological Impairment**

Several respected authors have published on the subject of hatchery and wild fish interaction under laboratory conditions, which the ISAB has cited as evidence on the likelihood of biological impairment from allowing hatchery fish to spawn with wild fish. The first was the study by Johnsson and Abrahams (1991), where it was demonstrated that the progeny of a domesticated strain of rainbow trout crossed with wild steelhead showed in two of the four trials greater willingness to risk entering territory of a predator than did progeny of the pure steelhead strain. There was no evidence presented whether the behavior translated into greater fingerling mortality. However, the study alerts us to the influence that genetics can have on the behavior of the progeny from a cross of two different stocks. This was a captive strain of rainbow trout that had undergone 20 years of selective breeding for the qualities desirable in commercial aquaculture, so it demonstrated one potential effect that genotypic differences can have on potential vulnerable behavior when domestication in hatchery broodstock is assumed to have been extensive. It would not apply directly to interbreeding with hatchery salmon or steelhead broodstock.

Swain and Riddell (1990), and Riddell and Swain (1991) showed greater agonistic behavior among newly emerged hatchery coho salmon compared to wild coho grown in a common environment, and the difference was attributed to additive genetic variance. It was suggested that such behavior would be a disadvantage in the wild. In similar studies by Berejikian (1995), Berejikian et al. (1996), it was demonstrated that steelhead from a hatchery population showed less aggressive behavior in the first month compared to wild steelhead, but they became more aggressive over time. It is noteworthy that hatchery steelhead were more vulnerable to predation by sculpins than were their wild counterparts. The study design was well planned and the results deserve attention. Since

these were naive progeny of wild and hatchery fish, the difference between results appear to have been from domestication. The mechanism is uncertain. However, since the 1996 studies showed that wild fry were more aggressive up to the 30-day post-emergence period, it was apparent that the predator avoidance tests commenced at a time when hatchery fry were less aggressive. Conceivably, if submissive behavior was what made hatchery fry more susceptible to sculpins, the pattern should reverse as hatchery fish become more aggressive. It is also noteworthy that with some experience their ability to avoid sculpins improved markedly over their naïve performance, and would presumably continue to improve with further experience. How well these laboratory results translate to fish in the natural environment is uncertain, and what success hatchery fish may have in the wild cannot be predicted from studies on one or two behavioral components. For example, another study by Berejikian et al. (1999) with hatchery and wild coho half-sib progeny in growth and survival experiments suggested that the hatchery experience endowed the fish with a competitive advantage over the wild fish. Therefore, it is apparent that one must realize that laboratory results do not predict what the performance of wild or hatchery fish will necessarily be in the wild, but they do alert us to the fact that changes that can occur in the hatchery environment may translate into performance differences in the natural environment.

Swain et al. (1991) detected small divergences in the morphology of hatchery and wild coho in controlled studies, but attributed the difference largely to the rearing environment. Fleming and Gross (1993) also found morphological differences between wild and hatchery produced coho. Much of the distinction was geographical, suggesting region based differences, but some of the difference was also related to long-term hatchery propagation. Interestingly, the Chilliwack hatchery fish that have experienced only 4 generations of artificial propagation were often mistaken for the wild Chilliwack coho, which indicates the differences that one might associate with hatcheries occur over the long-term. The relative fitness implications of these differences are unknown, and in all cases they were associated with fish that had survived to maturation and returned successfully. It appears, therefore, that sufficient time exists for changes that might occur in hatchery fish to be intercepted and averted with proper breeding protocols.

From the study by Vincent (1960), ISAB concluded that high feeding rates in a hatchery, together with a feeding response rather than a fright response was additional ancillary evidence of acclimatization to the culture experience. This study indicates the potential for fish to genetically acclimatize to a hatchery environment, especially after 90 years of captive isolation as in the case of the hatchery brook trout in this study. We would agree that fish that show food oriented aggressive behavior in the hatchery may be more successful in that environment, and if under a decidedly selective regime, would tend to perpetuate a more aggressive behavior. The implications of such behavior on subsequent performance in the wild and the result of crossing with wild fish suggest that highly selected domestic strains may have a negative influence on wild fish, although that has not been defined. It does point out that the implications of using highly domesticated strains of trout in such programs should be viewed as potentially problematical because they are different than what occurs in the wild. In the case salmon and steelhead where domestication selection is to be avoided as much as possible, the examples from such studies with highly domesticated strains of trout at least provide evidence that traits associated with domestication are passed on to the progeny and thus alerts us to the need

to ascertain what effects even slightly altered traits may have on hatchery propagated salmon or steelhead and their progeny in the wild.

As demonstrated in the above citations, there are some contrasting results in laboratory studies on hatchery and wild fish performance, but in general there is evidence that unintentional genetic changes from the wild parental genotype can be expected to occur through long-term artificial propagation. These results are informative and suggest that revisions in fish hatchery technology should be considered that would help reduce the magnitude of such changes. The long-term implications of these changes on performance in the wild, or their effect from interbreeding with wild fish are unknown. Laboratory studies are most useful when employed to resolve problems found under field conditions. If hatchery fish performance in the wild is different from that of native fish, laboratory studies provide explanations under controlled conditions that may help resolve the causes and potential effects of such changes.

The following series of studies discussed here were performance comparisons made in field settings, and might be viewed as more substantive than those in the laboratory. Nickelson et al. (1986) reports on a program where hatchery pre-smolt coho originating from a different river system were planted in 15 streams and their performance compared to wild fish performance in 15 reference streams. It was concluded that hatchery fish were less productive because the resulting adults produced proportionally fewer juveniles, and thus it would be concluded that fewer F2 adults would materialize. Unfortunately, the study was dominated by variables other than the effects of artificial propagation; the hatchery stock was not from the streams studied, hatchery spawn timing was much earlier than the wild fish, the hatchery fish were released at a much larger size than the wild fish, and stocking densities of hatchery fish were much higher than wild fish density in the non-stocked reference streams. Although it was not possible to isolate the effects of just being exposed to the hatchery environment, it did point out the problem when a genotype is unsuitable for the target stream. Spawn timing and home-stream synchrony are indispensable stock characteristics that cannot be overlooked, and if they are ignored, it will affect the results apart from any influence of artificial propagation.

The studies of Fleming and Gross (1993), Chebannov and Riddell (1998), and Fleming et al. (2000) are often cited as evidence that some hatchery fish will spawn in the wild, but there was the intimation that hatchery fish may not generally spawn successfully. These studies showed that even in their first generation in the wild the spawning ability of hatchery origin females was nearly indistinguishable from wild females. Hatchery males tended to show greater variability, and appeared to be dominated by wild males in most cases. However, if hatchery males were on redds earlier than wild males they tended not to be displaced. Hatchery males tended to spawn less frequently when in highly dense spawning conditions, and reduction of hatchery male success in pairing was usually due to a size disadvantage with wild males. Other studies, such as Berejikian et al. (1997) have also shown that hatchery fish have the ability to spawn naturally and produce viable offspring. From these citations we can conclude that the spawning ability of first generation hatchery fish in the wild does not appear to be a major problem, and therefore subsequent generations would be expected to show similar performance as that of wild fish. It does leave some uncertainty about male

success, which deserves further study. Further, with various types of hatchery releasing fish for spawning in the wild, one must be able to assign them a value such as a “wild-equivalent recruitment value”, such that their reproductive potential is considered when determining escapement and productivity goals.

Reisenbichler and McIntyre (1977) compared performance of wild, hatchery, and wild x hatchery steelhead, using the Deschutes River steelhead stock. Although their final result they reported indicated that wild fish out performed hatchery fish, percent survivals were often very close. Growth differences were generally insignificant, and in half the cases the highest survival was shown among the hybrids (H x W). The fact that this occurred without blind tests and with traps that were not operated during certain periods of the year, brings into question that hatchery fish performance was actually less effective than that of wild fish. One can conclude, for example, that when 27,000 eggs are planted in each of two streams and only 245 H vs. 253 W and 344 H vs. 369 W fingerlings respectively are recovered in the traps, the numbers do not represent biologically meaningful differences. The main point is that given the natural variability in productivity from year to year in natural salmon and steelhead populations, the comparative success of the hatchery fish in this study does not leave the impression that such performance would be a decided disadvantage in the process of supplementation. There is some caution that must be given to such conclusions, however, since the wild group was a response of fish that had been cultured before release, and if these were fish that had never experienced hatchery propagation, their performance may have been diminished in that study. Also, it is uncertain what origins were represented in the hatchery gene pool, since translocation of hatchery fish was common in the past and Deschutes hatchery fish may have been influenced from such transplants in the past.

Chilcote et al. (1986) and Leider et al. (1990) are often cited as examples demonstrating poor performance of hatchery fish. These studies used Skamania hatchery steelhead from the Washougal River as their hatchery source and compared them with the wild Kalama stock in the Kalama River. Unfortunately, from the standpoint of researching the effects of artificial propagation, the two sources of fish used in the study were markedly different genetically, and had very different spawning times. Skamania hatchery steelhead represented an extreme departure from the characteristics of wild-type Kalama steelhead, so it is not surprising that their performance was also different. Essentially, the study showed the problem of asynchrony in timing when fish are planted in a new and unrelated environment. This was similar to the Nickelson et al. (1986) study. Emergence timing, and thus spawn timing, is one of the most significant factors responsible for survival success. Natural selection based on mean incubation temperature of the natal stream defines optimum mean spawn timing for the native population, and is the most significant element in establishing stock temporal differences among salmon and steelhead. Each native population has an optimum window in which to spawn that will assure the most beneficial emergence timing of the progeny for optimum survival thereafter. Similar performance differences would be expected with any introduced asynchronously timed population of steelhead, wild or hatchery, as what was demonstrated with the Skamania hatchery stock (Brannon et al. 2002). This study did little more than confirm the importance of synchrony between the fish and its environment, with little relevance on the isolated effects of artificial propagation, but

highlights the importance of hatchery reform necessary to eliminate such potentially harmful management method.

Hulett et al. (1996) concluded that hatchery fish did not perform as well as wild fish. Three year classes of winter steelhead in the Elochoman River were compared with a hatchery population made up of a stock mixture from Elochoman and Cowlitz rivers and Chambers Creek. The hatchery fish spawned earlier than the wild stock. As demonstrated above with the use of domesticated stock, the problem was exacerbated by different genetic stock and spawn timing, which completely overshadows the influence of artificial propagation. Reisenbichler and Rubin (1999) reviewed this and several of the above studies on hatchery fish performance and concluded that measurable decrease in performance as a result of domestication occurs immediately after a single generation in the hatchery, and is additive with succeeding generation. Some of the difference has to do with the perspective of the problem. For example Reisenbichler and Rubin (1996) view Nickelson et al. (1996) as negative evidence about artificial propagation, and while the hatchery fish used in the study performed poorly, we view that their performance was attributed to stock source and timing rather than the effects of artificial propagation.

Currens et al. (1997) argued that transfer of rainbow trout from the coast of Oregon into the Deschutes River Basin resulted in the high susceptibility of the introduced stock to a myxosporean parasite endemic to the Deschutes system, a parasite that the coastal rainbow had not evolved with. The parasite was absent in coastal watersheds. The transferred rainbow trout were a hatchery domesticated strain, and when they interbred with one of the Deschutes wild strains that were resistant to the parasite, the resistance of resulting progeny was reduced. However, the problem was introduction of a naive population, not artificial propagation. The fisheries managers were guilty of introducing a susceptible strain of rainbow trout to an area where an endemic parasite existed and for which that trout strain had no innate immunity. The same result would also have occurred if they had introduced a susceptible wild strain, but the point is that introductions of the wrong genotype can be deleterious.

It is clear from the literature that complex stochastic and deterministic processes related to breeding dynamics and survival of progeny result in differential reproductive success of adult salmonids with different phenotypic traits, and that these processes are essential to the long-term health of populations and indeed the species themselves, but that these processes are markedly different in matings in hatcheries. So many salmon are artificially produced in the Columbia Basin that hatchery practices are part of the “natural” selection and evolutionary biology of the species. Hatchery populations are on evolutionary trajectories that may reduce their fitness, and their interactions with wild populations are a serious conservation concern.

However, specific determinations on performance of hatchery and wild fish from the same parental population have not been made. There is no evidence comparing hatchery and wild fish of common ancestry from which it can be concluded that artificial propagation results in meaningful genetic alterations, or fewer progeny, or that interbreeding reduces the fitness of the wild populations. Such differences between hatchery and wild fish of common ancestry are possible, but the citations reviewed above do not provide relevant evidence of these effects. Most of the field studies cited demonstrate one common flaw. Management of artificial propagation was being

confused with the effects of artificial propagation. We agree that translocated stocks of hatchery propagated salmon or steelhead often did perform well when they were asynchronous with the receiving environment. However, this is attributable to faulty experimental design that complicated artificial propagation with translocation and genetic asynchrony. When the genetic predisposition does not fit the environmental template, any wild or hatchery fish will be confronted with insurmountable problems. We conclude that little conclusive decisive evidence exists to resolve (1) what the effects of artificial propagation are on the performance of hatchery fish in the wild, or (2) what the impacts of hatchery fish are on the wild population of the same ancestral origin. The limited evidence on the effects of artificial propagation on wild populations underscores the need to have well designed studies developed under Action 182 that will clarify what risks actually exist.

### **Proposed Assessment Methodology of Hatchery Reforms**

Studies of the natural history and behavioral ecology of salmon reveal complex stochastic and deterministic processes related to survival and breeding dynamics that result in differential reproductive success of individuals with different phenotypic traits. These processes are widely recognized as essential to the long-term health of populations and indeed the species themselves. For better or worse, artificial propagation is now an integral part of the overall evolutionary dynamics of salmon. A large fraction of the salmon in the Pacific Northwest and especially the Columbia River Basin are produced in hatcheries. Salmon populations in hatcheries are on their own evolutionary trajectories, and they interact with nearby wild populations, thereby affecting both groups. These interactions are consistently identified as priority concerns in salmon conservation (e.g., Waples 1991; Utter 1998).

There are many questions related to the genetics of hatchery and wild salmon but the core of the problem, and the least-studied aspect, is: what is the appropriate breeding protocol for salmon in hatcheries to minimize divergence from wild populations? The corollary of this question is: what are the patterns of evolution by salmon in hatcheries? Without addressing these uncertainties it will be difficult or impossible to reduce the deleterious genetic interactions between wild and hatchery salmon in a systematic manner.

Most recently, the Columbia Basin Fish and Wildlife Authority produced the “Mainstem and Systemwide Province Draft Artificial Production Program Summary” for the Northwest Power Planning Council in 2002 (at the CBFWA website, [www.cbfgwa.org](http://www.cbfgwa.org)). This document made reference, in numerous places, to the importance of genetic interactions between wild and hatchery salmonids. The report stated (our emphasis):

Studies need to be conducted to measure the relative fitness of hatchery fish spawning in the wild. Likewise, experiments need to be conducted to estimate the rate of domestication in a hatchery setting, the rate of readaptation to a natural setting, and to provide information into what traits are under selection in different environments.

With respect to breeding protocols in hatcheries, the report stated:

Conservation hatcheries should function in ways that reflect the latest scientific information and conservation practices to maintain genetic diversity and natural behavior and to reduce the short-term risk of extinction (Flagg et al. 1999, 2000b).

Initial, antiquated, production hatchery methodology probably had a more severe impact on fitness characteristics than present methodologies. Beginning in the late 1900s, reforms were championed to mitigate the effects of hatcheries on wild fish. These reforms to existing hatcheries included ... **adopting genetically appropriate mating protocols, ... and developing hatchery and genetic management plans to comprehensively guide operations and monitoring and evaluation programs.**

We believe that the genetic management plans and spawning protocols have been well intentioned but biased towards preservation of genetic diversity rather than natural attributes, and need to be re-examined with respect to the behavioral ecology of salmonids. Protocols also need to be evaluated against empirical studies of reproductive success and inadvertent selection in hatcheries. The issue of limiting production because of the risk of operation is though to be scaled according to the size of the production must be revisited with regards to alternative reform goals. Alternatively, alteration of production methods because the scale of the program cannot change will require a different implementation strategy. The goals of propagation such as producing early or late-run fish must be evaluated as a function of habitat requirement such as temperature profiles and physiological requirements of the fish. Finally, the policy for hatcheries on broodstock management, like promoting diversity in the spawnings, jack spawning in proportion to the run, etc., must be reviewed in the context of alternative hatchery and subbasin goals. Such goals might emphasize selection of desirable traits at the expense of diversity.

The present proposal under Action 184 is designed to develop a standardized analytical approach that will detect the effects of hatchery reform on extinction risk or recovery at the population level. It is of critical importance that the data generated for assessment be of a particular quality and be collected under conditions to make sure the data is comparable. Although not part of Action 184, we suggest several requirements for such data. There have to be studies on compatible groups of fish where the effect of artificial propagation is the only variable. This will involve studies on hatchery and wild fish of the same parental population, with similar spawn timing, age, and size. However, investigations on second generation hatchery fish in the wild must also be included. Most studies have focused on seed-stock, not fish produced in the wild. First generation hatchery fish carry the conditioned experience they acquired in the hatchery and they are naïve to natural stream conditions, so one should expect their performance to initially suffer compared with wild fish that have already gone through very high experiential costs. Second generation hatchery fish in the wild would no longer be subject to acquired conditioned behavior from the hatchery environment, and might be under natural selection to hone characteristics important in the natural environment. To do this effectively, given the growing literature on the subject, we will categorize the literature and reported effects in relation to the history of the stocks and management practices tested. We will highlight traits that are most affected by certain types of hatchery management. We also suggest that the data collected from such studies be compatible

among studies. In juvenile performance studies the physical measurements of the fish should be the same; weight, length, condition factors, width, and morphological criteria should be measured following standardize procedures. Survival and timing of behavioral indices should be comparable between studies. Similarly adult data should be gathered with the same protocol using consistent criteria and measurements. To make the analytical assessment as functional as possible, we suggest that considerable effort be placed on (1) studies of hatchery and wild fish of the same parental population, (2) assessment of hatchery fish raised under present hatchery production scenarios, (3) first and second generation hatchery fish in the wild, and (4) life history stage measurements to follow protocols established by BPA in this assessment process to provide the basic performance standard against which products of hatchery reform can be measured.

To develop a standardized analytical approach in the assessment of data from the several sources enumerated above, we propose a three-step process that includes:

- Identification of potential artificial propagation risks.
- Patterns of variability in reproductive success from studies of genetic parentage analysis or recruitment.
- Assessment criteria measuring risk and/or recovery potential.
- Rating of potential risks on the health of hatchery and wild populations.

We will then survey the Artificial Propagation Review and Evaluation (APRE) and Hatchery Genetic Management Plans (HGMP) reports on hatcheries that propagate salmonids to determine: 1) whether there are **Policy Objectives** with respect to genetics, 2) what **Protocols** are recommended to implement these objectives, and 3) whether the **Practices** in the hatcheries reflect these protocols. We will integrate the literature with information from agencies to develop risk profiles of specific management actions on quantitative traits and another set on potential domestication (unmeasurable) traits. Sets of protocols designed to achieve explicit policy objectives for genetics in hatcheries will be derived.

The first step, (1) identification of potential artificial propagation risks, fall under the three areas reviewed above as (a) inbreeding depression, (b) domestication, (c) outbreeding depression. Given awareness of the sources of such risks, and the importance of broodstock selection and breeding protocols to avert outbreeding and inbreeding depression, the most critical influence on hatchery fish will be domestication, but not exclusively. The sources of variation in the assessment process will help identify which of the three risk categories are involved. Risks associated with outbreeding and inbreeding can be recognized from allele frequency data of the progeny based on sources of broodstock and the breeding plan that was followed. Risks from domestication cover a range of behaviors, some of which will remain unknown until assessment techniques expose their influence. Domestication risks fall under two categories, (a) acquired, and (b) genetically based characteristics.

(a) In the process of hatchery propagation there are some risks associated with conditioned responses associated only with those characteristics acquired while in captivity. These include behavior conditioned to feeding regimes, conditioned to the presence of people, and similar phenomena that are apparent in the phenotype of the

hatchery generation that involve no genetic basis. These have an effect on success of the first generation of hatchery fish in the wild, but not on the progeny generations thereafter. These have significant survival influences and should be included in risk assessment, but only in the short-term. Our risk assessment will include this information as short-term acquired characteristics whenever it is available, and will identify the nature of such risk.

(b) The other concern of domestication of hatchery fish are the risks that have to do with functional genotypic traits, and understandably potential long-term implications on the progeny and the population that the hatchery fish are entering. These changes are considered to be of major concern and will not be detectable by standard techniques used in genetic electrophoresis. Functional genetic differences that may have no relationship with neutral allele frequencies will have to be assessed using quantitative techniques. Genotypic differences will be the main emphasis of risk assessment.

(2) The second step is assessment criteria measuring risk and/or recovery potential. In this step we have two analytical approaches; (a) quantitative trait analytical techniques and (b) a genotype-environment interaction analytical technique. Here we include assessment of acquired characteristics on the first and second generation of hatchery fish, the influence of acquired characteristics that also have a genotypic effect, and genotypic differences between hatchery and wild fish.

(a) The Quantitative Trait Analytical Technique (QTAT) will include what we refer to as juvenile competence and adult competence criteria. This will include standard measures of juvenile and adult performance in the wild after their introduction and in subsequent generations.

- Juvenile competence are those measured which include changes in incubation success and gamete viability including egg size, juvenile behavior, physical attributes, and survival.
- Adult competence, which includes survival, age at return, size, return and spawn timing, and spawning effectiveness.

In these competence assessments, the juvenile and adult these statistics will be assembled between first- and second-generation hatchery fish, and between second-generation hatchery and wild fish as performance criteria for comparison. The data sources referred to above and from the new analyses under Action 182, will be used in this assessment. The analysis will be a comparative assessment of the parameters identified, and a performance evaluation of (F1) hatchery fish versus (F2) hatchery fish or hatchery fish against wild fish compiled from the data.

Competence testing could also include the use of standardized tests on responsiveness to: 1) environmental components, such as temperature, oxygen, etc., and 2) performance traits such as stamina, predator recognition and aggression. The traits that would be used would be derived from the list of traits that are thought to be most affected by artificial selection and domestication, as determined from the literature review phase.

(b) The Genotype-environment Interaction Analytical Technique (GIAT) will involve what is referred to as Microarray Analysis or gene chip technology. Unlike protein electrophoresis, this is a new technology only recently employed in detecting differences in gene expression. Microarray analysis can detect the activity of a large

number of DNA sequences on the chromosomes. Microarray analyses are increasingly being used to investigate the influence of environmental stress and variability on gene expression in fish (Ju et al. 2002, Gracey et al. 2001). Microarray technology allows for the expression of thousands of individual genes to be monitored in parallel and allows researchers to ascertain what genes are affected by a given treatment or environmental stressor (Constans 2003). Given the concerns of hatchery and wild fish interactions, methodologies which can pinpoint which environmental factors drive differential gene expression will provide insight on what genes are responding to such challenges in wild and hatchery fish. Comparisons between fish of hatchery origin and wild may be able to show differences in gene expression that expose functional genetic differences. Gene expression may also determine how to modify hatchery techniques and thus minimize the risk of domestication. The primary objective of this work is to examine differential gene expression between hatchery and wild salmon or steelhead when exposed to hatchery and wild conditions. Since this is a new technology in fisheries, it will be necessary to develop the appropriate methodology for use in hatchery and wild fish comparisons, what factors are useful for analysis, and how they expose differences in the genetic expression.

(3) The third step in risk assessment will be rating of potential risks on the health of hatchery and wild populations. Risk levels have not been associated with the outbreeding, domestication, and inbreeding, and we recognize that even where risks can be identified, they still represent different levels of risk depending on the nature of the founding hatchery sources. Here the fish and the habitat are of equal interest because the performance of the fish is measured with respect to the environment in which it resides. We propose five risk levels associated with both the hatchery fish, and the receiving population; I, II, III, IV, and V, denoted as two classes such as II-V or I-III, etc. The lowest representing little or no long-term effect on the hatchery fish in the wild or the population, and up to the highest representing serious performance problems of hatchery fish and long-term impacts on the population. For example with the Skamania steelhead spawning 3 months sooner than the parental stock, the rating could be V-I where the early spawn timing of hatchery fish gives it no success in the wild (V) and there is no impact on the wild population (I) because there is no interbreeding. In this assessment we are assuming that the wild fish are the most fit for the environment under review. The rating system will depend on the sufficient data to make such assignments.

Quantitative methods of developing escapement and restoration goals that integrate hatchery and wild productivity, and harvest rates, will be developed for the Columbia River Basin. In this document, we will discuss productivity vs. abundance criteria, natural and hatchery productivity, limiting factors and setting escapement goals in the context of depressed populations, using productivity-based criteria. We will evaluate the benefits of including hatchery production in natural escapement estimation, and the quantitative issues resulting from the integration of hatchery and wild components into a single stock.

### **Quantitative evaluation**

Fitness in the hatchery and natural environment are not only expected to be different at the organismal level, but the traits that have the most influence of fitness are also expected to vary among environments. For example, fitness in the hatchery may be

most dependent on fecundity, whereas fecundity may play a relatively minor role in determining fitness in the wild.

We will compare fitness of hatchery fish and wild fish (type) and also investigate how differently genetic traits affect fitness. We will construct a statistical model where data on fitness-related traits thought to be susceptible to drift in hatcheries (e.g. run timing, fecundity, body size, etc.) are related to fitness indices (e.g. ratio of return size of progeny to release size as reconstructed from pedigree analysis). These data will be categorized by fish type. Within each fitness-related trait, the proposed model will allow the comparison of the trait effect on hatchery fish fitness to that on wild fish fitness. Further, we would like to quantify effects of these traits on fitness of fish type to see how differently traits affect individual fitness. To do so, we will build a function for fitness with respect to traits:  $W_T = f(\mathbf{X})$ , where  $W_T$  is fitness of type (i.e. hatchery vs. wild) and  $\mathbf{X}$  is genetic trait variables (vector dimension). We can build a linear model for the function  $f$ . Future models may incorporate non-linear effects as well.

In the linear model, the coefficients of the respective trait variables can be interpreted as the effect of the trait variable on fitness. By comparing the trait-specific coefficients in the hatchery fish fitness model with those in the wild fish fitness model, the potential magnitude of the rate of divergence of hatchery fish from wild fish can be computed. Significant differences in fitness-related trait variables and their coefficients between fish type may be useful background in the formulation of specific recommendation for the future hatchery management. For example, if a trait turns out a significant variable for fitness of hatchery fish but not for that of wild fish, it is implied the trait may be one of the agents that cause divergence of hatchery fish from wild fish. Thus, we can recommend that hatchery managers should change their practice to reduce the effect of the trait variable. Further, a coefficient for a particular fitness-related trait may significantly depart from the null hypothesis in both types yet also differ significantly from each other. In this case, management recommendations would depend on the direction of selection and its intensity. Variances of coefficients of fitness-related traits in the linear model will provide some indication of the expected response per generation.

### **Compatibility of data**

Finally, we will discuss the type of information needed and the format of that information to make the data comparable for the development of an analytical approach. We will develop a statistical model that will serve as a framework for testing for 1) differences in overall productivity between hatchery and wild fish, and 2) which variables are the greater determinants of fitness under hatchery and wild environment.

### **Relationships to other projects**

This project is related to ongoing efforts to reform hatcheries within the Columbia River Basin yet to our knowledge there is no specific effort to evaluate and develop protocols in the manner that we propose. There are studies using DNA parentage analysis to determine the reproductive success of salmonids (e.g., work of Berejikian and co-

investigators on competition between wild and captive-reared salmon at Manchester, Washington). However, to our knowledge there is no effort to link the specific breeding practices in a production hatchery with the quantitative trait variation as we propose.

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### **3. Qualifications of Participants**

#### **Dr. André Talbot (CRITFC)**

Ph.D. in population dynamics and quantitative genetics from the University of Dalhousie, Halifax, Canada. Dr. Talbot is the leader of the Production and Restoration Research Group at CRITFC and will act as the Principal Investigator of this study. He has broad expertise in conservation biology, including the important elements of biostatistics, population dynamics and quantitative/population genetics. Dr. Talbot has published key papers on artificial selection and domestication in cultured fishes. He has a particularly strong background understanding variability in reproductive success and habitat-based predictive models of fish production. His research, education, and experience provide the necessary skills for a critical appraisal of methodologies employed in sampling designs, monitoring systems, research programs, and resource management. In addition, he has extensive experience in analytical and statistical fisheries methods. He has assisted tropical and temperate research teams to develop research programs and management plans for integrated aquaculture as well as traditional and industrial fisheries operations. Dr. Talbot began working in International Development Biological Research in 1983 at Dalhousie University as a research associate and project co-ordinator for the Aquaculture Genetics Network in Asia (AGNA). While holding an Associate Scientist position at the University of Québec at Chicoutimi, he directed a firm specialising in international development from 1989-1994, with particular interest in fisheries resource monitoring, population dynamics, aquaculture and genetics. Within this framework, he managed projects, supervised graduate students, professional and technical staff in Canada and in tropical countries and collaborated on research in four continents. From 1994 to 1997, he accepted an overseas post as a Regional Unit manager and Scientific Advisor - Biostatistician for the Caribbean Fisheries Resource Assessment and Management Programme, a regional project financed by the Canadian International Development Agency. Most recently, Dr. Talbot has been employed as a conservation scientist since 1997 at the Columbia River Inter-Tribal Fish Commission, in Portland, Oregon (USA).

#### **Dr. Louis S. Adler**

Affiliate Professor, University of Idaho. Ph.D. in System Engineering, Purdue University, 1978. Extensive experience in management systems and control systems development. Dr. Adler is operations manager for Invensys-Pacific Simulation, Moscow, ID. Responsibility includes delivery of process optimization and energy related services in industry. His professional training and experience in mechanical and system engineering, software design, and process optimization is being applied in assessment of hatchery reform information. Application of Dr. Adler's expertise will be in information prioritization on the rating of potential risks on the health of hatchery and wild populations and how to integrate that information into prioritization models.

#### **Dr. Ernest L. Brannon**

Emeritus Professor, University of Idaho. Ph.D. in Fisheries (genetics), University of Washington, 1972. Dr. Brannon is presently researching new concepts in artificial propagation of salmon and steelhead through the use of engineered streams. Professor

and Director of Aquaculture Research Institute, and the Center for Salmonid and Freshwater Species at Risk, University of Idaho, 1989 to 2002, where research emphasis was on conservation fisheries, including hatchery propagation. Professor, University of Washington 1973 to 1989, where research was on salmonid life history and aquaculture, including performance of hatchery fish. Chief of Research from 1970 to 1973 and fisheries biologist from 1959 with the International Pacific Salmon Fisheries Commission. Research emphasis on salmon life history, rehabilitation, and hatchery fish behavior. Most recent BPA affiliated research was project number 98-004-03 (contract 98BI08319) on chinook salmon and steelhead population structure in the Columbia River system. BPA contact Greg Baesler.

**Dr. Saang-Yoon Hyun (CRITFC)**

Ph.D. and Post-Doctoral Fellow from University of Washington, with a strong background and research interest in quantitative science and fisheries management. With knowledge and skills in advanced bio-statistics and programming, he will conduct a sound quantitative analysis of fitness-related traits as they relate to actual measures of fitness in hatchery and wild populations. Dr. Hyun currently holds the position of Quantitative Fishery Scientist at the CRITFC.

**Dr. Madison Powell (University of Idaho)**

Dr. Madison Powell received his Ph.D. in the Systematics & Evolutionary Biology program at Texas Tech University in 1995 and is currently an Assistant Professor in the Department of Fish and Wildlife Resources and Department of Animal and Veterinary Sciences at the University of Idaho. Dr Powell is also the director of the Center for Salmonid & Freshwater Species at Risk at the University of Idaho. He supervises UofI molecular genetic laboratories at the Aquaculture Research Institute in Moscow, ID and at the Hagerman Fish Culture Experiment Station in Hagerman, Idaho. The laboratories' primary goals are to provide timely genetic information to applied conservation genetic questions, and provide genetic advice and consultation to state, federal, and tribal agencies regarding endangered fishes and fisheries management. Dr. Powell is currently the Principal investigator of several genetic projects examining reproductive success of hatchery and wild fish using microsatellite DNA analyses including (sockeye BPA project, Chinook captive broodstock project). Dr. Powell will assist in the development of the research study design, analyze data and report results.

**Dr. Dave L. Smith (University of Idaho)**

Research assistant professor, University of Idaho. Ph.D. in Fisheries (habitat simulation), University of Idaho, 2003. Dr. Smith specializes in salmonid habitat related issues. He is investigating the use of alternative hatchery practices to produce hatchery fish that are comparable in morphology and behavior to wild fish. Specifically, the concept of turbulence is applied to habitat use and the influence of habitat complexity on flow structure. In addition, the influence of habitat on gene expression is investigated using microarray technology. This information is being used to develop hatchery rearing

strategies that result in similar gene expression between hatchery and wild fish as encouraged by rearing habitat.

**Dr. Thomas Quinn (University of Washington)**

Ph.D. from University of Washington, currently Full Professor in the School of Aquatic and Fishery Science. Dr. Quinn has extensive experience in research of the behavior, ecology and evolution of salmonid fishes, with emphasis on migrations, life history patterns, reproduction, habitat requirements and conservation. He has published several articles in first-class refereed journals on the reproductive success of wild and naturally spawning salmon, rapid evolution of reproductive isolation in the wild, reproductive success of introduced salmon, and the evolution of life histories in introduced New Zealand chinook and sockeye. Dr. Quinn has 167 relevant refereed publications, making him a regional expert in this area. Dr. Quinn will review the evolutionary ecological literature relevant to this project, identify evolutionary and selective consequences of management actions pertaining to hatcheries and assist in the development of the analytical model.

## APPENDIX A:

### **Methodology in Microarray Techniques**

Approximately 300 hatchery fish (hatchery x hatchery cross) will be obtained from the Cle Elum hatchery during the spring timeframe. In addition, approximately 300 wild fish will be obtained from either the upper Yakima or Naches Rivers during the month of April or May as fish become available. Wild fish will be captured using beach seines, minnow traps, or with electrofishing gear. Wild fish will be staged at the Cle Elum hatchery and when 300 fish are collected both the hatchery and wild groups will be transported to the University of Idaho Aquaculture Research Institute using insulated tanks and supplemental oxygen. Driving time is approximately 3 hours. At the time of sampling for the hatchery and wild groups, 5-10 fish will be frozen in liquid nitrogen. These fish will serve as “controls.” If other hatchery:wild combinations are available they will be included in the analysis to increase the database.

Once fish are at the UI ARI both treatment groups will be reared for several weeks, and sampled weekly (5-10 fish) under hatchery and lotic conditions. Hatchery conditions will be simulated in 8-foot diameter tanks supplied with chilled filtered water. Lotic conditions will be simulated in a hydraulic flume capable of flows of 0.15 m<sup>3</sup>/s and supplied with chilled filtered water.

Sampled fish will be stored in liquid nitrogen and forwarded to the University of Idaho Hagerman Fish Culture Experiment Station for subsequent microarray analyses. Bone, heart, liver and brain tissue will be sampled, and processed to recover mRNA. Active genes from control fish will be used as templates for the synthesis of expressed sequence tags (ESTs) and “spotted” microarray plates. Messenger RNA will be recovered separately from sampled fish in each experimental group. Full-length cDNAs will be synthesized from recovered mRNA, labeled and used as probes to hybridize to microarray plates. Hybridization of labeled (fluorescent) probes will indicate where homologous RNA sequences exist between control and experimental fish as well as between controls. These steps allow for quantification of gene expression as a function of origin (hatchery or wild) and rearing environment (lotic or hatchery). Figure 1 depicts the experimental design of cross hybridization of each experimental group to microarray plates of ESTs from both control groups and the cross hybridization of control groups between themselves.

Fish that have been collected for microarray analysis will be incinerated after processing. There will be hatchery and wild fish remaining after all sampling has occurred. These fish will be exposed to a lethal dose of MS-222, frozen, and then incinerated as per accepted guidelines of the University of Idaho Animal Care and Use Committee.

(3) The third step in risk assessment will be rating of potential risks on the health of hatchery and wild populations. Risk levels have not been associated with the outbreeding, domestication, and inbreeding, and we recognize that even where risks can be identified, they still represent different levels of risk depending on the nature of the founding hatchery sources. Here the fish and the habitat are of equal interest because the performance of the fish is measured with respect to the environment in which it resides. We propose five risk levels associated with both the hatchery fish, and the receiving population; I, II, III, IV, and V, denoted as two classes such as II-V or I-III, etc. The lowest representing little or no long-term effect on the hatchery fish in the wild or the population, and up to the highest representing serious performance problems of hatchery fish and long-term impacts on the population. For example with the Skamania steelhead spawning 3 months sooner than the parental stock, the rating could be V-I where the early spawn timing of hatchery fish gives it no success in the wild (V) and there is no impact on the wild population (I) because there is no interbreeding. In this assessment we are assuming that the wild fish are the most fit for the environment under review. The rating system will depend on the sufficient data to make such assignments.

## APPENDIX B:

### **Differences between hatchery operations, artificial selection and domestication**

An example given of domestication (e.g. ISAB 2002) demonstrates the error that many mistakenly associate with artificial propagation. ISAB (2002) stated that the earlier return and spawn timing of hatchery reared adults, and their frequently younger ages at spawning, were commonly cited and accepted evidence of domestication in anadromous populations. The problem with that statement is that early timing is not the result of domestication, i.e. unintentional selection in the hatchery environment, from artificial propagation, but rather from hatchery management. Hatchery populations that return earlier do so because they are selected consistently from the early half of the returning run. Unless hatchery managers make sure they have representation of the population timing pattern, which is largely genetically controlled, timing can become earlier or later than the parental stock simply by when the hatchery selects their brood fish. Artificial propagation in itself does not result in earlier timing.

Moreover, younger age at return is similarly the result of hatchery management. High feeding rates, and sometimes warmer temperatures during rearing, induces more rapid growth and earlier emigration, which most often accelerates maturation a year sooner than what would be common in the parental native population. If size of fish released from the hatchery were the same as the wild fish in the adjacent stream, earlier age of return would not occur. Nothing in the act of artificially propagating fish will result in such changes if timing and migrant size of the wild fish are mimicked in the hatchery program. Management has actually purposely induced such changes in hatchery populations to achieve certain objectives, but mimicking natural population characteristics is also entirely within the ability of the hatchery program. Changes that can occur in salmon over relatively few generations of artificial selection underscore the ability of salmon to similarly respond to major environmental alterations through natural selection, and also demonstrate their inherent ability to accommodate change through both acquired and genetic mechanisms.

