

COLUMBIA RIVER INTER-TRIBAL FISH COMMISSION

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RE: ISRP comments on proposal 35014: Measurement of Quantitative Genetic Variation Among Columbia River Basin Chinook Propagation Programs

- 1. The selection of 6 hatchery stocks and 3 treatments receives little justification. In the initial years of these studies the number of stocks could be limited (see comments in b) and pre-study tests of the treatments could be conducted before assuming that these treatments will result in the "stress" expected, or that the stress does not simply kill all the fish. What preliminary studies have been conducted?*

As detailed in our response to ISRP question two, we have substantially decreased the sample sizes and number of stocks originally proposed. In order to accommodate the number of rearing containers necessary for a full factorial mating design (discussed below), we propose to decrease the number of stocks from six to four. Originally, our study design would have allowed for a comparison (within both spring and fall chinook salmon life history types) between stocks inhabiting the extreme geographic range within the Columbia River Basin, as well as a comparison between those stocks and an intermediate group. As modified our design will allow a comparison (within both spring and fall chinook salmon life history types) between the stocks inhabiting the extreme geographic ranges of the Columbia River Basin (i.e., an upriver versus lower river comparison). If significant genotype x environment interactions are observed in these comparisons, a subsequent proposal will be drafted to compare more geographically proximate stocks (e.g., Lyons Ferry versus Priest Rapids Hatchery fall chinook salmon).

Regarding the treatment conditions, we agree with the ISRP that treatments should be stressful enough to test reaction norms without resulting in high mortality. Temperatures ranging from 5.6°C to 12.8°C are within recommended ranges for spawning Columbia River chinook salmon (McCullough 1999), whereas 50% pre-hatch mortality occurs at temperatures above 14°C (Alderdice and Velsen 1978; Murray and McPhail 1988). Therefore, given that our overall sample sizes will be decreased by virtue of changes in study design, we will use 14°C as our temperature challenge. It is more difficult to predict a response to low oxygen concentration, however Silver *et al.* (1963) noted that developmental performance of embryos decreases at all dissolved oxygen levels below saturation. Steelhead embryos suffer complete mortality at

dissolved oxygen levels below 1.6 ppm. Davis (1975) noted that salmonid embryos exhibit distress, and are negatively affected at dissolved oxygen concentrations below 4.25 ppm. Therefore, we will use a 10°C, 4 ppm dissolved oxygen treatment to stimulate an oxygen stress response.

2. *We are uncertain about a number of aspects of the proposed half-sib breeding design.*
 - a. *Half-sib designs assume all males are independent; therefore, at least twice the number of males as females are needed.*
 - b. *The design as described cannot directly estimate GxE interactions. For Task 2a, how do you expect to partition the GxE effect?*
 - c. *There will likely be maternal effects that cannot be accounted for and there is no treatment replication within stock x family x treatment (i.e., no rearing container controls). Revising the design is likely to require more rearing containers and/or dropping some stocks to provide more containers.*

We have enhanced the design to respond fully to the potential problems identified. We have also substantially improved the description of analytical and statistical methods to make it clear to the reader what will be estimated in the course of this study.

3. *What is the value of maintaining the run-timing component within stocks?*

In order to avoid unintentional artificial selection, we intend to sample adults from throughout the period of adult returns at each sampled facility.

4. *The budget implies three years of study but the text does not make any such reference (other than a reference to using rainbow trout later). What is the expected duration of these studies?*

Initially, we proposed two phases for this proposal. Phase I would have compared genotype x environment interactions for six hatchery stocks, while Phase II would have compared genotype x environment interactions among natural origin and hatchery origin steelhead/rainbow trout stocks. Upon revision, we are seeking funding for a truncated version of Phase I only. Given that the experimental design has been modified, we are seeking funding at a reduced rate for a period of three years. We anticipate that the experimental portion of the proposal (rearing) will take up to a year, followed by a year of data compilation (e.g., arraying and X-raying individual samples for meristic data), and approximately six months of data analysis and report writing.

5. *Reliance on early development traits may not be appropriate. Phenotypic traits with strong relations to fitness (such as egg survival) may have very limited genetic variation. In which case, the outcome of this study may relate more to these specific traits than to a general feature of adaptive genetic variation. To minimize such a risk, it may be advisable to maintain the progeny during early growth stages and examine additional traits less associated with immediate survival.*

The point is well taken. Indeed, traits that are strongly associated with developmental physiology (such as degree-days from fertilization to hatching) generally have very high heritabilities and are essentially fixed for specific temperatures. However, egg survival has no heritability (one cannot select for survival), but survival is related to trait performance. We will attempt to evaluate several of these traits. In this study,

traits that affect survival rate and have a genetic basis will be monitored, and are expected to show variance due to treatment. Those traits are well described in the physiological and developmental literature, and heritabilities have been estimated for most of them for various species of salmonids. As the fish grow, genetic variance becomes a smaller fraction of total phenotypic variance for most polygenic fitness-related traits. We will be measuring juvenile morphological characters and growth rates well into the post-hatch period.

Secondly, we are testing an important mechanism of local adaptation. This study, the first of its kind in the Columbia Basin, will produce a first estimate of the scale of genotype by environment interaction across life history type and geography. It will be a very important element of the relationship between quantitative genetics (trait characteristics and performance in different environments) and molecular genetics (genetic distance among populations, life history types and ESUs). Genetic correlations between life-history and behavioral traits can cause reproductive isolation (Miyatake and Shimizu 1999). Thus, establishing a genetic component to the geographic and evolutionary history of chinook stock can help us understand the functional ecology of the species and the mechanisms for isolation within and among ESUs. Synchronization of emergence of the fry in the spring with favorable environmental conditions implies potential genetic differences in development rate and timing of spawning among populations when streams differ in water temperature profiles. Review of the literature indicated that GxE interactions for traits related to incubation and development is a significant component to total genetic variance.

- 6. There are issues in measuring GxE. First, the genotype being referred to is actually the family that will be composed of multiple genotypes. Here is where the real value of the molecular genetic studies could be used, but this aspect is not highlighted in the proposal. Second, if quantitative genetic methods are to be used to assess GxE interactions then there are specific breeding studies in multiple environments that can be used to estimate the interaction. Coupling these with the molecular genetic work could be a very original piece of research!*

Variance components are implicitly recognized in the proposal and variance within groups as well as main effects and their interaction terms will be estimated. We do assume that different populations from broadly different environments will be composed at least in part of different genotypes (as a result of local adaptation). We're not sure which specific breeding studies are being referred to. In this study, "environment" represents different temperature incubation and dissolved oxygen concentrations as well as incubation at the hatchery of origin. Differences in life history among chinook populations are primarily attributable to differences in stream incubation temperatures (this is particularly the case between spring- and fall-run chinook), and the need to spawn at different times of the year in order to meet the required degree-days for incubation (Brannon *et al.* 2002).

- 7. Task 4 seems to imply that the results of these detailed studies will be compared with the production history of the source hatcheries. The inherent assumption that past production history would relate to present genetic composition is weak and we question the utility of this part of the study.*

Given the decrease in stocks that will be analyzed in the revised proposal, we will not attempt to link juvenile performance of sample groups to performance of the original hatchery stock.

- 8. A final point for clarification is the authors' use of 'drift'. On page 9 Section 9, in the section on Genetic analysis of chinook salmon, the authors state "Differential success among family lines to environmental challenges will also be assessed by examining for*

changes in offspring genotype from that of the parents. Equalized familial representation across treatments will allow for the removal of variance associated with familial lines and variance due to drift.” It is not clear how these statements relate to the methods to be used and how genetic (presumably) drift relates to these analyses. Unless survival is very poor and/or highly variable between families, why does drift receive the profile it does in the proposal and why would equal family size control it?

The proposal should clarify who is actually conducting this research and the references cited in Section 2 should be complete.

We view this experiment as an ideal opportunity to study the rate of genetic drift and mechanisms by which genetic drift occurs at putatively neutral molecular loci. To do so, we will compare genotypes among subgroups of the same family reared at the hatchery of origin (local environment) as well as three additional environments: 10°C rearing water saturated with oxygen; 14°C rearing water; and 10°C rearing water at 4 ppm dissolved oxygen. The purpose of these comparisons is to determine whether environmental stress (temperature and oxygen challenges) and natal versus foreign (hatchery of origin versus non-stress 10°C rearing water with saturated oxygen at Hagerman) rearing environments result in greater rates of genetic drift at putatively neutral loci. Further, we intend to explore the mechanism by which increased genetic drift (if observed) occurs. For example, the signature of genetic drift resulting from increased variation in reproductive success under stressful conditions would be expected to differ from consistent but low reproductive success among all families. The results of this study would provide a useful means to scale neutral genetic differentiation observed within and among natural populations inhabiting stressful environments.

Regards,

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Bonneville Power Administration FY 2003 Provincial Project Review

PART 2. Narrative

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Steps to complete Part 2

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Project ID: (New Proposal)

Title: Measurement of Quantitative Genetic Variation Among Columbia River Basin Chinook Propagation Programs

Section 9 of 10. Project description

a. Abstract

To date, populations of salmonids within the Columbia River Basin have been targeted for conservation based on neutral genetic measures of reproductive isolation as well as observed phenotypic differentiation. It remains unclear if these guiding principles have adequately addressed underlying quantitative variation. Further, it is unclear whether hatchery programs have adequately maintained quantitative variation. We propose to undertake a “common garden” experiment using adults derived from two spring and two fall chinook propagation programs spanning the geographic range of propagation activities within the Columbia River Basin. By employing a full factorial mating protocol, in conjunction with an analysis of variance and covariance performed on fish of known relation, we intend to search for significant genotype x environment interaction and stock effects. The presence of such effects would presumably denote different distributions of quantitative variation among life history types and geographic regions spanning the range of chinook salmon within the Columbia River Basin. In addition, this study will: 1) quantify the presence and degree of adaptive genetic differentiation expressed by juveniles throughout the geographic and life history range of chinook salmon under propagation in the Columbia River Basin; 2) assess the degree to which heterozygosity at molecular (putatively neutral) loci can be used as a proxy for quantitative variation; and 3) examine the degree to which environmental stress affects genetic drift at putatively neutral molecular loci. The results of this study will provide guidance in identifying and prioritizing populations for conservation activities. Further, this proposal will test several proxies (e.g., heterozygosity and juvenile performance) that may be useful for rapidly measuring the adaptive capacity of stocks.

b. Technical and/or scientific background

Introduction

Within the Columbia River Basin, salmonids have been listed under the Endangered Species Act (ESA) as a number of Evolutionarily Significant Units (ESU). ESUs are created under the hypothesis that there are a number of populations that maintain gene flow at some level with one another, but are substantially reproductively isolated from other such population groups (Waples 1991). Further, it is assumed that these groups maintain the potential to diverge independently in evolutionarily meaningful ways, and that the loss of such groups would constitute an irreplaceable loss of an evolutionary legacy (Waples 1991).

To date, ESUs have been identified using neutral genetic measures of differentiation and by observed differences in life-history characteristics (e.g., spring versus fall chinook salmon). While significant genetic divergence at putatively neutral loci is typically a good measure of reproductive isolation (Coyne and Orr 1997), fluctuations in census and effective population size can obscure or inflate genetic distance, which in turn can be misinterpreted as evidence (or lack of evidence) of historical reproductive isolation (Hedrick 1999). In addition, the study of neutral genetic variation provides no information to assess the evolutionary significance of isolation (Hard 1995), or to determine whether or not observed divergence is biologically meaningful (Hedrick 1999). Further, while differences in life-history characteristics presumably reflect local adaptation, it is difficult to infer from simple observation whether these phenotypes arise in part from quantitative genetic divergence, or simply because of the effects of a different environment on similar genetic variation and architecture (Caswell 1983).

While population structure as inferred by neutral genetic variants provides a valuable contribution to the identification of potentially discrete spawning aggregates, we propose that a more rigorous assessment of quantitative variation is the next step in assessing the scale and biological units of conservation and assigning management priorities. Simply stated, the identification and conservation of quantitative variation is necessary to ensure that ESUs or management effectively identify evolutionary potential. The first step in assessing the presence of quantitative differentiation is determining the geographic and environmental scales typifying quantitative divergence. To do so, we propose to collect adults from a four hatchery programs from different Columbia River salmon ESUs. Adults within each sampled population will be mated in a full factorial design and reared in a series of common environments. Continuous physiological and meristic characters will be measured at emergence. An analysis of variance and covariance will allow estimation of both quantitative differentiation and heritability of meristic characters based on differential expression of these characters among individuals of known relation in a series of common environments (Falconer and Mackay 1996).

We submit that a crucial step in the conservation and recovery of anadromous salmonids is the identification and conservation of adaptive genetic variation. The scale and magnitude of adaptive differentiation observed in this study will provide managers a means to assess the geographic scale at which adaptive differentiation can be conserved, as well as a set of meristic measures with known inheritance rates with which to measure the potential for adaptive differentiation. These tools will enhance our ability to meaningfully identify and prioritize stocks for conservation activities.

Experimental Context

In order to assess the presence and degree of quantitative genetic differentiation within and among chinook salmon populations within the Columbia River Basin, we propose to perform a “common garden” experiment with four Columbia River hatchery stocks spanning the range of geographic and life history characteristics. Hatchery programs were selected based on the following criteria: 1) broodstock derived from within the ESU (e.g., representative of local quantitative variation); 2) samples are intended to span the gross range of life history types (e.g., sampling includes spring and fall chinook programs) across the geographic distribution of Columbia River chinook salmon; and 3)

hatcheries must have a “proven” production history, such that collection of adults for this project does not unduly compromise recovery or production goals. In order to maximize the probability of sampling the range of genetic variation encompassed by a stock, we intend to collect adults from throughout the period of adult returns. To avoid decreasing diversity within each program, only five females and five males will be collected at each hatchery in a full factorial mating design (every male will be mated to every female).

The eggs of each female will be divided into 5 groups and each group will be fertilized with a separate male from the same hatchery. Each individual family will be subdivided into 8 allotments. Assuming 5,000 eggs per female, this would result in roughly 125 eggs per allotment. Two allotments from every cross will remain at the original hatchery site, to be incubated in ambient hatchery conditions. The remaining 6 lots from each female will be transferred to the Collaborative Center for Applied Fish Science (CCAFS) in Hagerman, Idaho to be incubated. Three experimental treatments will be performed at the CCAFS; 10°C rearing water; 14°C rearing water; and 10°C rearing water at 4 ppm dissolved oxygen. For each family, a number of continuous traits will be measured through emergence (see 1c.). Experimental treatments were selected to test the developmental performance of different chinook salmon life history types spanning their geographic range within the Columbia River Basin. In order to elicit a response, we selected environments that we anticipate will result in stress, but will not result in greater than 50% mortality, hence maintaining a reasonable sample size for analysis. Temperatures ranging from 5.6°C to 12.8°C are within recommended ranges for spawning Columbia River chinook salmon (McCullough 1999), whereas 50% pre-hatch mortality occurs at temperatures above 14°C (Alderdice and Velsen 1978; Murray and McPhail 1988). It is more difficult to predict a response to low oxygen concentration, however Silver *et al.* (1963) noted that developmental performance of embryos decreases at all dissolved oxygen levels below saturation. Steelhead embryos suffer complete mortality at dissolved oxygen levels below 1.6 ppm. Davis (1975) noted that salmonid embryos exhibit distress, and are negatively affected at dissolved oxygen concentrations below 4.25 ppm.

Since temperature and oxygen exert a strong influence on the life history of salmon and steelhead (Brannon *et al.* 2002), performance of embryos exposed to different temperature and oxygen regimes may be an indication of the general amount of quantitative variation present within and between sample groups. We would suggest, therefore, that embryo condition and survival during incubation could potentially be a surrogate for the presence of adaptive variation influencing other life history phases. The Columbia Basin represents a microcosm of environments that have been most conducive to the elaboration of very different life history forms within and between species. These varying environmental conditions are the biological foundations that separate salmon stocks. Life histories are defined by the adult return, spawning, incubation, rearing, and marine phases that are expressed by the species in the river system, and can be roughly divided into spawning/incubation and the rearing/emigration profiles. The genetic diversity apparent within a population is considered an important component in their ability to adapt to new and changing environments within these profiles. If there is a correlation between genetic diversity and incubation performance, knowing the genotypic diversity of a stock may provide insight to overall survival success and genotypic diversity for other quantitative characters. We submit, therefore, that performance of

embryos during the incubation phase of their life history has the potential to be useful as an assessment of adaptive variation, and thus can be a predictive tool for the likelihood of success over the remainder of the life cycle. Likewise, heterozygosity has the potential for use as a corollary to fitness and to embryo performance in these experimental stocks.

This proposal will assess genetic diversity among chinook salmon hatchery populations that span the geographic and life history ranges among chinook within the Columbia River Basin. Incubation performance will be examined based on challenges under stress from temperature and oxygen level, using a suite of assessment criteria. Potential correlations between performance factors during incubation and genetic diversity will be examined. Differential success among family lines to environmental challenges will also be assessed by examining changes in offspring genotype from that of the parents. Equalized familial representation across treatments will allow for the removal of variance associated with familial lines and variance due to drift. These data will then be evaluated with respect to the hatchery stock success, based on the past history of smolt outmigrant and adult return performance, to determine if correlations exist between incubation performance (fitness) and survival to later life stages.

To put such assessment in a more practical timeframe, it is necessary to facilitate earlier predictive ability of hatchery fish performance. We propose that monitoring and assessment of salmon embryo performance through the incubation period can provide such a mechanism. Liskauskas and Ferguson (1991) demonstrated that genotypic heterozygosity of protein alleles was a measure of fitness in brook trout (*Salvelinus fontinalis*). In their work, survival and growth were shown superior in heterozygous individuals. Hutchings (1991) looked at other parameters of fitness in brook trout and made similar conclusions about fitness and size. Danzmann *et al.* (1988) also demonstrated that multilocus heterozygosity in rainbow trout (*Oncorhynchus mykiss*) resulted in increased metabolic efficiency. Moreover, other empirical evidence has also shown that heterozygosity of protein coding loci in other species correlates with increased fitness. Quattro and Vrijenhoek (1989) showed fitness differences in remnant populations of topminnows were based on heterozygosity. Heterosis has been correlated with allelic isozyme variation in several species. For example, Allendorf and Leary (1986) showed relationships between heterozygosity and fitness in natural populations of animals. Beacham (1987) suggested that genotype – temperature interactions could underlie phenotypic variations in pink and chum embryo development, while Hebert *et al.* (1998) demonstrated quantitative genetic variation in development rate of pink salmon, and suggested interaction between genotypes and environmental variation were pertinent to hatchery programs.

In short, this proposal will measure will accomplish three goals:

1. Quantifying the presence and degree of adaptive genetic differentiation expressed by the geographic and life history range of chinook salmon in the Columbia River Basin.
2. Assessing the degree to which heterozygosity at molecular (putatively neutral) loci can be used as a proxy for quantitative variation.
3. Examine the degree to which environmental stress affects genetic drift at putatively neutral molecular loci.

c. Rationale and significance to Regional Programs

Anadromous salmonids throughout the Columbia River Basin have been propagated at a large scale over an extended period of time. In many drainages, hatchery chinook return to spawn naturally, and conversely natural origin fish are included in the broodstock by design or occasionally by accident. In this proposal we are implicitly assuming that the effects of domestication and relaxed natural selection in the hatchery environment have not greatly decreased quantitative variation specific to geographic stock identity, and that hatchery stocks retain quantitative trait variation present among their naturally spawning conspecifics. However, we are not minimizing the potential for the loss of adaptive variation within hatcheries, rather we propose that a lack of quantitative differentiation between geographically distant groups, or between life history types would suggest that selection within the hatchery environment decreases adaptive diversity, or that stocks do not differ greatly in adaptive potential. The results of this study will therefore be valuable for measuring, quantifying, and evaluating the genetic and ecological consequences of outplanting hatchery origin fish in wild populations. Although the ultimate criterion in determining the response of a population to supplementation activities is adult return survival and reproductive viability under natural conditions, waiting 4 to 5 years to complete each phase of such work can take many years, and even then without any certainty of success.

The results of this study will address several of the priority research needs identified below by The Federal Columbia River Basinwide Fish Recovery Strategy (FCRPS).

Future Needs: Priorities for the Mainstem/System-wide Fish Wildlife Program Solicitation

Artificial Production Program	Future need identified
RPA 169, 182	Provide information on traits that are under selection in different environments
RPA 175, 184	Evaluate the effects of timing of freshwater transfer and rearing temperature on seasonal timing of spawning spring chinook to aid in understanding impacts of temperature on reproductive performance of either captively-reared fish or migration adults
RPA 184	Determine the rate of naturalization- i.e. the rate at which fitness increases when domesticated population spawns and rears naturally

Why are Genotype-Environment Interactions Important?

The strength of the GxE interaction is key in the development of a diversified aquaculture conservation program. In a typical program, fish managers create conditions that are consistent and maximize fish survival and productivity. Once such a program is established, it is perpetuated everywhere hatcheries are created. From an evolutionary perspective, environmental differences among hatcheries are minimal, and the same

genotypes may prevail. A good fish breeder will be very successful at optimizing fish production. Not only that, but good fish breeders will obtain seed from each other based on their proven productivity in the hatchery environment. The result of this is that hatcheries are potentially reducing the stock variability in any system. This is a problem in a conservation framework. Conservation hatcheries should be attempting to rear local broodstock, a different goal than what is typically assigned to the hatchery manager. For example, at high food input (such as typical of hatcheries), a particular genotype may be always superior (Figure 1). Such systems are not likely good replicates of natural systems. From Figure 1, it is clear that different genotypes may have superior performance under varying environmental conditions. The strength of this argument depends on the scale of the variance components. Under a local adaptation model widely acknowledged as critical of salmonid survival in a highly heterogeneous environment, it is argued that the GxE interaction is much stronger than the performance of a genotype across all environments (i.e. there are no genotypes that are superior in any salmonid environment). If GxE interaction is weak, then genotypes that perform better in all environments are possible. Knowledge of the strength of GxE interactions is critical in setting the geographic scale to local adaptation and the role of hatcheries in metapopulation health and recovery.

As described by Frankham *et al.* (2002), genotype-environment interactions are a major significance to the management of endangered species. The reproductive success of transplanted populations cannot be predicted if there is significant and strong genotype-environment interaction. Success of hatchery programs may be compromised by genetic adaptations to artificial environments since superior genotypes in hatcheries may not perform well once released into the wild. Mixing of genotypes from different fragmented populations may result in genotypes that do not perform well under the local conditions. Knowledge of the importance of genotype-environment interactions can assist in determining the choice of populations or population assemblages for restoration.

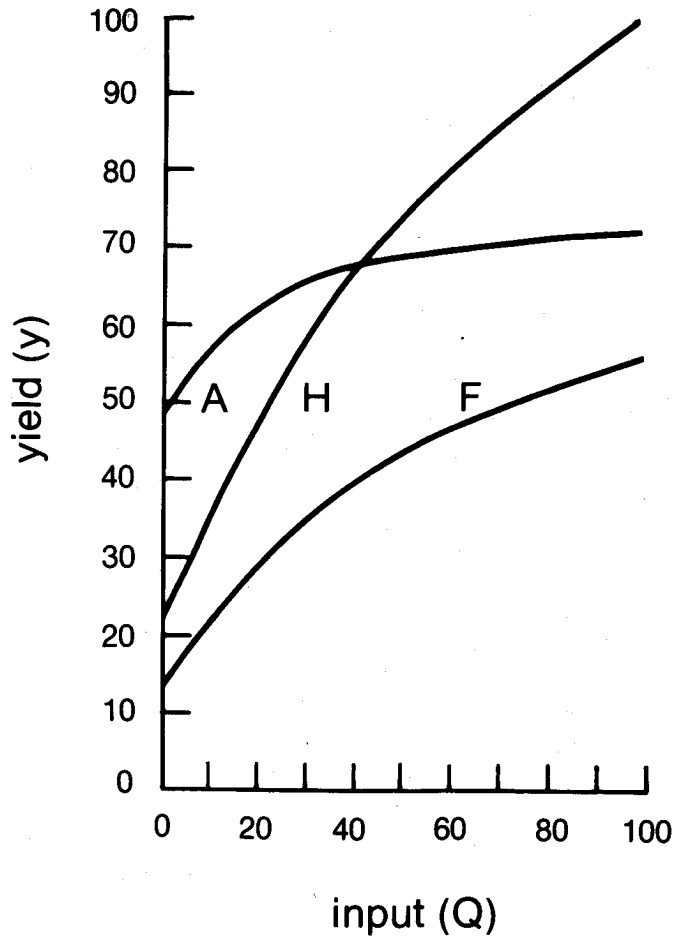


Figure 1 : Differences in yield as a function of input (in this proposal temperature) varies among genotypes. Knowledge of the importance of this interaction components is a building block of local adaptation, and is required to optimize hatchery designs. From Doyle *et al.* 1991.

EXPECTED BENEFITS TO F&W PROGRAM

Genotype-environment interactions may play an important role in determining the performance of fish transplanted into the wild from supplementation programs. This may be particularly the case where the stock has been reared in hatcheries for several generations. If GxE interactions are of a small magnitude, we predict that the superior genotypes would perform better in all geographic areas. If, on the other hand, GxE interactions are important in determining reproductive value, then hatchery programs may have a small, local role in population enhancement. The source populations selected for this study were made in order to maximize opportunity for a GxE interaction variance component. If a significant interaction term is estimated, we intend to draft a second proposal that will measure magnitude of GxE interactions over a smaller geographic range (e.g., populations inhabiting a common ESU).

d. Relationships to other projects

The results from this study will support the 3 projects identified below

Table 1. Current projects addressing Artificial Production Program research needs that are complemented by this proposal.(Mainstem/systemwide province artificial program summary – table 1)

Project Id.	Title	Sponsor
198909600	Monitor and evaluate genetic characteristics of supplemented salmon and steelhead	National Marine Fisheries Service, Conservation Biology Division
199005200	Performance/stock productivity impacts of hatchery supplementation	Biological Resources Division, U. S. Geological Survey
199009300	Genetic analysis of <i>Oncorhynchus nerka</i> (modified to include chinook salmon)	University of Idaho

Monitor and evaluate genetic characteristics of supplemented salmon and steelhead- The genetic diversity apparent within a population is considered an important component in their ability to adapt to new and changing environments. If our work shows that there is a correlation between genetic diversity and incubation performance, knowing the genotypic diversity of a stock may provide insight to overall survival success.

By combining the information from both of these studies, genetic diversity can be maintained and even promoted for maximum fitness under natural stream conditions. In our study we will be evaluating the performance of embryos during the incubation phase of their life history. The results of this study can be used as an assessment of population fitness, and thus can be a predictive tool for the likelihood of success over the remainder of their life history. Likewise, we will test whether or not heterozygosity can also be used as a corollary to fitness and to embryo performance in these experimental stocks.

This proposal will assess genetic diversity among chinook salmon hatchery populations that have had differing degrees of exposure to artificial propagation. Incubation performance will be examined based on challenges under stress from temperature and oxygen level, using a suite of assessment criteria. Potential correlations between performance factors during incubation and genetic diversity will be examined. Differential success among family lines to environmental challenges will also be assessed by examining for changes in offspring genotype from that of the parents.

Performance/stock productivity impacts of hatchery supplementation

This study has focused on the productivity of hatchery and wild fish in the wild. Our study will provide additional information on the effect of domestication of hatchery chinook salmon over a range environmental stressors. If we find that the tested hatchery stocks perform well under the stress treatments, it would suggest that hatchery reared fish

potentially maintain the quantitative variation that would allow successful reintroduction to natural environments.

Genetic analysis of chinook salmon- The information gathered in this study will expand our knowledge of hatchery chinook salmon genetics. This proposal will assess genetic diversity among chinook salmon hatchery populations that have had differing degrees of exposure to artificial propagation. Potential correlations between performance factors during incubation and genetic diversity will be examined. Differential success among family lines to environmental challenges will also be assessed by examining for changes in offspring genotype from that of the parents.

e. Project history (for ongoing projects)

N/A

f. Proposal objectives, tasks and methods

Objectives

1. Evaluate performance factors of chinook salmon embryos challenged with temperature and oxygen stress during incubation.
The H_0 is that no relationships exist between incubation performance and stress from high temperature and low dissolved oxygen. The H_1 corollary is that such relationships do exist, and that such factors can be used to evaluate ability of embryos to tolerate physical stressors associated with natural environmental variation.
2. Determine the relationship between incubation performance and individual multilocus heterozygosity.
The H_0 is that no relationships exist between incubation performance and genetic heterozygosity. The H_1 corollary is that such relationships do exist, and that performance is a function of diversity.
3. Assess the impact of incubation challenges on genetic drift between parents and progeny.
The H_0 is that performance under incubation challenges result in non-significant genotypic differences between parents and progeny. The H_1 corollary is that such response to stress is selective, and selection may be detectable as increased genetic drift observed between parents and their progeny surviving challenge exposure.

Task 1a. Test Hatcheries

Collect five female and five male prespawn chinook salmon from each of four chinook salmon hatchery facilities including; Priest Rapids (fall chinook), Cle Elum (spring chinook), Spring Creek Hatchery (fall chinook), and Cowlitz River (spring chinook).

Task 1c. Test Treatments

Setup 3 incubation systems at HFCES to accommodate incubators exposed to three treatments:

1. Incubation at 10°C as control lots.
2. Incubation at 14°C as temperature stress lots.
3. Incubation at 10° C at 4 ppm dissolved oxygen as oxygen stress lots.

Use the ambient spring water to provide 14°C water for treatment 2. Operate HFCES water cooling system to provide constant 10°C water for treatment 1 and 3. Build a nitrogen gas stripping column system to provide deoxygenated water for treatment 3. Arrange for thermographs at each hatchery and 3 units at HFCES for monitoring temperature.

Task 1b. Experimental Lots

The eggs of five females from each hatchery will be divided into 5 groups and each group will be fertilized with a separate male from the same hatchery. Each individual family will be subdivided into 8 allotments. Assuming 5,000 eggs per female, this would result in roughly 125 eggs per allotment. Two allotments from every cross will remain at the original hatchery site, to be incubated in ambient hatchery conditions. The remaining 6 lots from each female will be transferred to the Collaborative Center for Applied Fish Science (CCAFS) in Hagerman, Idaho to be incubated under three treatments.

Task 1c. Data recorded.

The data recorded over the incubation period will include:

1. Daily mortality
2. Percent unfertilized
3. Egg diameter (0.1 mm) of 30 eggs/lot at eyeing
4. Total volume of each separate allotment (0.1 ml) at eyeing
5. Dates when the 25%, 50%, 75% of embryos have hatched
6. Date when 50% of the alevins reach yolk absorption
7. Length and weight at yolk absorption (30 individuals per family)
8. Condition index at yolk absorption (30 individuals per family)
9. Incidence of disease
10. Number of deformities
11. Number of scale rows above the lateral line (30 individuals per family)
12. Vertebrae count (30 individuals per family)

During the incubation period, each lot will be checked daily to remove and record mortality. The number of unfertilized eggs will be assessed by the absence of embryo development in mortalities cleared for observation prior to eyeing, and by blanks after eyeing takes place. Length of 30 alevins (0.1 mm) at yolk absorption will be taken as total length. Yolk absorption will be determined by visually inspecting embryos to determine if chromatophores cover yolk sac i.e. “buttoned up” (Beachman 1988). Sixty alevins from each family will be will be preserved for genetic analysis.

Task 1.d. Incubation Data Summarization

The data from each egg allotment (HFCES and each hatchery) will be summarized. The means in egg diameter and volume at eyeing; alevin weight, length and condition factor at yolk absorption; standard deviations; range of temperature units to hatching and yolk absorption; percent hatch; percent mortality, and the incidence of disease and deformity will be analyzed as performance or performance related factors. The number of scale rows above the lateral line as well as vertebrae count are suspected to be highly heritable, and have been used as a method to determine stock structure (Schreck *et al.* 1986).

Traits that are strongly associated with developmental physiology (such as degree-days from fertilization to hatching) generally have very high heritabilities and are essentially fixed for specific temperatures. Egg survival has no heritability (one cannot select for survival), but survival probability is related to trait performance. In this study, traits that affect survival rate and have a genetic basis will be monitored, and are expected to show variance due to treatment. Those traits are well described in the physiological and developmental literature, and heritabilities have been estimated for most of them for various species of salmonids. As the fish grow, genetic variance becomes a smaller fraction of total phenotypic variance for most polygenic fitness-related traits. We will be measuring juvenile morphological characters and growth rates well into the post-hatch period.

This proposal, if funded, will be testing an important mechanism of local adaptation. This study, the first of its kind in the Columbia Basin, will produce a first estimate of the scale of genotype by environment interaction across life history type and geography. It will be a very important element of the relationship between quantitative genetics (trait characteristics and performance in different environments) and molecular genetics (genetic distance among populations, life history types and ESUs). Genetic correlations between life-history and behavioral traits can cause reproductive isolation (Miyatake and Shimizu 1999). Thus, establishing a genetic component to the geographic and evolutionary history of chinook stock can help us understand the functional ecology of the species and the mechanisms for isolation within and among ESUs. Synchronization of emergence of the fry in the spring with favorable environmental conditions implies potential genetic differences in development rate and timing of spawning among populations when streams differ in water temperature profiles. Review of the literature indicated that GxE interactions for traits related to incubation and development is a significant component to total genetic variance.

Task 2a – Genetic Analyses

Partitioning Variance

We know from first principles that the total phenotypic variance can be decomposed as follows:

$$V_p = V_g + V_e + 2 Cov_{g,e}$$

In this proposal, several variance components have been identified: stock, dam(female), maternal, sire(male), treatment (temperature/oxygen), and random error.

The data will be analyzed using an analysis of variance design. Variation in embryo and juvenile survival rates and fish characters will be analyzed with the model:

$$Y_{ijklm} = \mu + S_i + M_{ij} + F_{ik} + MF_{ijk} + R_{ijkl} + e_{ijklm}$$

where Y are the variables identified in task 1c, μ is an overall mean, S_i is the effect of the i^{th} stock (stock=1, 2... 4), M_{ij} is the effect of the j^{th} male (sire) within the i^{th} stock, F_{ik} is the effect of the k^{th} female (dam) within the i^{th} stock, MF_{ijk} is the interaction of the j^{th} male and k^{th} female within the i^{th} stock, R_{ijkl} is the effect of the l^{th} replicate within family jk in the i^{th} stock, and e_{ijklm} is the error term of the m^{th} observation in the subgroup $ijkl$. The mean and stock effects are considered fixed, with all other effects random. Variance components will be evaluated for each trait using standard methods (e.g. Henderson 1953).

From this model, heritability can be estimated as $\frac{4V_m}{V_p}$, where V_m is the sire component of

variance and V_p the total phenotypic variance. **Maternal effects** are calculated as

$$\frac{|V_m - V_f|}{V_p}, \text{ where } V_f \text{ is the female component of variance. Dominance is estimated as } \frac{4V_{mf}}{V_p}$$

where V_{mf} is the Male/Female interaction component of variance. Environmental effects

within families can be estimated as $\frac{MS_e - \frac{1}{2}V_a - \frac{3}{4}V_d}{V_p}$ the where MS_e is the error mean

square, V_a is the additive genetic variance and V_d the dominance variance divided by the total phenotypic variance. In calculating covariances and other parameters, negative variance components will be set to zero.

Genotype by Environment interactions (GxE) will be determined using the following model:

$$Y_{ijklmn} = \mu + S_i + T_m + M_{ij} + F_{ik} + MF_{ijk} + TM_{mij} + TF_{mik} + TMF_{mijk} + R_{ijklm} + e_{ijklmn}$$

where T_m is the effect of the m^{th} treatment (temperature/oxygen, $m=1,2\dots 4$), TM_{mij} is the interaction of the m^{th} treatment with the j^{th} male in the i^{th} stock, TF_{mik} is the interaction of the m^{th} treatment with the k^{th} female in the i^{th} stock, TMF_{mijk} is the interaction of the m^{th} treatment with the j^{th} male and the k^{th} female in the i^{th} stock, R_{ijklm} is the effect of the l^{th} replicate with family jk in the i^{th} stock in the m^{th} treatment, e_{ijklmn} is the error term for the n^{th} observation in subgroup $ijklm$, with the other terms defined as in the previous model. The mean, stock and treatment effects were considered fixed with the other effects random. The magnitude of the GxE (genotype

by treatment) interaction was estimated by the formula $\frac{V_t^2 + V_{tm}^2 + V_{tmf}^2}{V_p}$, where V_t , V_{tm}

and V_{tmf} are the variance components of the treatment, treatment/male interaction, and treatment/male/female interaction respectively.

The design permits the estimation of GxE interactions. At the highest level, an replicated ANOVA of performance indicators on treatment will allow the estimation of a temperature variance component, a ‘‘population’’ or sample effect, and a temperature by population interaction term as shown above.

Analysis of variance for hierarchical classification of variance components.

Source	d. f.	Variance Component
Total	$n-1$	\mathbf{s}_p^2
Among stocks	$s-1$	$\mathbf{s}_e^2 + i\mathbf{s}_r^2 + ir\mathbf{s}_f^2 + irf\mathbf{s}_m^2 + irfm\mathbf{s}_t^2 + irfms_s^2$
Among treatment within stock	$s(t-1)$	$\mathbf{s}_e^2 + i\mathbf{s}_r^2 + ir\mathbf{s}_f^2 + irf\mathbf{s}_m^2 + irfm\mathbf{s}_t^2$
Among sires within treatment	$st(m-1)$	$\mathbf{s}_e^2 + i\mathbf{s}_r^2 + ir\mathbf{s}_f^2 + irf\mathbf{s}_m^2$
Among dams within sire	$stm(f-1)$	$\mathbf{s}_e^2 + i\mathbf{s}_r^2 + ir\mathbf{s}_f^2$
Among replicates within dams	$stmf(r-1)$	$\mathbf{s}_e^2 + i\mathbf{s}_r^2$
Among individuals	$stmfr(i-1)$	\mathbf{s}_e^2

This approach has been used before successfully to identify GxE interactions in variable aquaculture environments (for example, Beacham 1988)

This design will allow us to detect main effects as well as interaction terms. When there is an interaction between the genotype of an individual and the environment (as would be hypothesized in local adaptation), the phenotype of an individual is not the simple sum of the genetic and environmental components of variance ($P=G+E$) but includes an interaction term ($P=G+E+I_{GE}$). Under such circumstances, selection for trait performance in a particular environment will not necessarily lead to increased trait performance under novel environments.

Variance components are implicitly recognized in the proposal and variance within groups as well as main effects and their interaction terms will be estimated. We assume that different populations from broadly different environments will be composed at least in part of different genotypes (as a result of local adaptation). In this study, “environment” represents different temperature incubation and dissolved oxygen concentrations as well as incubation at the hatchery of origin. Differences in life history among chinook populations are primarily attributable to differences in stream incubation temperatures (this is particularly the case between spring- and fall-run chinook), and the need to spawn at different times of the year in order to meet the required degree-days for incubation (Brannon *et al.* 2002).

Maternal effects are pre- and post-natal effects of the mother on the performance of progeny. They are often related to nutrition. Maternal effects, if not estimated directly, are an environmental variance component. Thus, if not estimated, the main effects will be underestimated and the dam effect overestimated. Our full factorial spawning matrix with replication within families that will greatly decrease the variance of the maternal variance estimates. We predict however that maternal effects will be relatively small in comparison to additive variance components. Maternal effects are often large in mammals (because of extended gestation) but smaller in broadcast spawners. We do not expect maternal effects to alter the direction of the genotype by environment interaction predicted under the local adaptation hypothesis and controlled experimental conditions.

Population Genetic analyses

As stated in Task 1, five females and males randomly sampled from each hatchery program will be spawned in a full factorial design. All parents will be fin clipped for later genotyping. Sixty progeny per treatment per hatchery (960 total) will be also be genotyped.

Tissue taken from adults and juveniles will be digested and DNA extracted using standard manufacturer's protocols from Qiagen® DNeasy™ in conjunction with a Qiagen® 3000 robot. Genomic DNA will be quantified and arrayed into 96 well plates for high throughput analysis. PCR will be used to amplify seven microsatellite loci designed from *O. tshawytscha* (*Ots1*, *Ots2*, *Ots3*, *Ots9*, *Ots10*; Banks et al. 1999) and *O. mykiss* (*OMM1020*; GenBank Accession AF346679 and *Omy77*; Morris et al. 1996) from genomic DNA. PCR amplifications will be performed using the AmpliTaq Reagent System (Applied Biosystems®) in an MJ® PTC-100 thermal cycler following manufacturer's protocols, with approximately 50 ng template genomic DNA in 15 µl total volume. Typical cycling conditions included an initial denaturation of 5 min at 96°C, followed by 30 cycles of 30 sec at 94°C, 30 sec at 50°-62°C, and 30 sec at 72°C. Final extension is carried out for 10 min at 72°C. Annealing temperature is adjusted to optimize PCR conditions (*Ots1* = 58°C, *Ots2* = 58°C, *Ots3* = 50°C, *Ots9* = 58°C, *Ots10* = 58°C, *OMM1020* = 62°C, *Omy77* = 58°C). Forward primers are fluorescently labeled (Applied Biosystems®), and PCR products will be genotyped using manufacturer's protocols with an Applied Biosystems® model 3100 genetic analyzer.

To estimate the level of within-population genetic diversity, expected heterozygosity (H_E ; eq 8.4 Nei 1987) will be calculated for all microsatellite loci. Private alleles are defined as alleles existing in two or less of the four sample populations and calculated for each sample population over all loci. Significant differences in heterozygosity are evaluated between sample populations using the Wilcoxin signed ranks test, implemented in SyStat (SPSS Inc.).

Exact-significance testing methods are used to evaluate conformance to Hardy-Weinberg and linkage equilibria, and homogeneity of spatial distributions of genetic variance. Unbiased estimators of exact significance probabilities will be obtained using the Markov-chain algorithm described in Guo and Thompson (1993), as implemented in GENEPOP, using 500,000 steps. To test for allele frequency homogeneity, the null hypothesis that alleles were randomly distributed among samples will be evaluated (Raymond & Rousset 1995b). Corrections will be made against Type I error using the Bonferroni method (Rice 1989). Probabilities over loci will be combined using Fisher's method (Sokal and Rohlf 1995).

The Weir and Cockerham (1984) method for generating unbiased F -statistics has been chosen to analyze patterns of genetic diversity within and among samples. F_{IS} refers to the local inbreeding coefficient, and will be calculated for each of the four sample populations. Because co-dominance is occasionally compromised as an artifact of PCR amplification (Hare et al. 1996), when considering a single, randomly mating population, this test may serve as a quality control to assure markers are co-dominant. If, however, individual collections are in equilibrium, but combined collections are out of equilibrium,

a heterozygote deficiency may indicate there is non-random mating among individuals in the combined collection (Wahlund effect). F_{ST} estimates will be obtained using GENEPOP v. 3.3 (Raymond & Rousset 1995a). We will measure pairwise genetic divergence within and between populations with corrections made against Type I error using the Bonferroni method (Rice 1989).

To further investigate the distinctiveness of each individual, and the success of crosses, Parentage assignments (maximum likelihood tests, Sancristobal and Chevalet 1997) will be performed as outlined in Weir (1996) and Bernatchez and Duchesne (2000). Exclusion tests calculate the probability that an individual's multi-locus genotype is derived from a single cross via the exclusion of other possible allele combinations from potential parents. These methods have been used successfully with several aquatic species of both hatchery and wild origin including most recently turbot and rainbow trout (Estoup et al. 1998) and Atlantic salmon (Letcher and King 2001).

Task 2b – Covariance of Treatment and Heterozygosity

Progeny from each treatment will be assigned back to parental crosses. The relative success of each cross, on each treatment from each group (hatchery) can then be assessed. Variance associated with poor crosses, poor females or poor males can be examined and removed from the results leaving treatment effects and levels of heterozygosity as remaining variables. Covariate analysis will thus permit association of performance with genetic diversity. This technique is commonly employed in animal breeding and has been demonstrated in aquatic species (Anang et al. 2001; Dodenhoff et al. 1999; Kinghorn 1983)

Task 2c - Independence.

To examine for correlation between incubation success and diversity as measured by performance factors, it will be necessary to first test for independence of the variables. Individual assignments will be tested for independence from treatments using Haber's *Chi Square* corrected for continuity (Zar 1996).

Task 2d - Correlations.

With performance not independent of the indicators, correlations will be measured. The level of correlation between non-independent variables will be assessed using a two-tailed correlation (ϕ_2 and r_n) analyses. The data will be summarized in double dichotomous tables (a 2x2 contingency table with no set rows or columns), testing performance against the indicator.

Two measures will be used because they offer advantages over usual one-tailed tests. First, they are two-tailed so that negative correlations can be evaluated and second, they are amenable to hypothesis testing for significance (as done above using Chi square analysis). A table will be set up with all the variables (*a priori*) to also evaluate the probability of the correlation. An executable Excel spreadsheet will be used to calculate the various measures of significance and correlation. If the values are extrapolated a range of probability, as covered in: Zar (1996).

Based on the evaluation of the suite of performance factors under the different treatments, genetic diversity will thus be evaluated as a potential mechanism for use in assessing stock fitness. The presence of a correlation between genetic diversity and incubation survival will be considered evidence that genetic diversity can be used as a fitness factor for application in supplementation and rehabilitation programs.

g. Facilities and equipment

The research will be conducted at the Collaborative Center for Applied Fish Science, located at the Hagerman Fish Culture experimental Station, Hagerman, Idaho.

h. References

Reference (include web address if available online)	Submitted w/form (y/n)
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Section 10 of 10. Key personnel

Dr. Ernie Brannon, Co-Principal Investigator Salmonid life history	0.2
Dr. André Talbot, Co-Principal Investigator, CRITFC coordinator	0.2
Mr. Chris Beasley, Fisheries Scientist, Benefit/Risk Analyst	0.8
Dr. Ron Hardy, Director for HFCES, facilities management and nutrition	0.1
Dr. Madison Powell, lead geneticist	0.4
Biologist	0.5
UI technician	0.5
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CRITFC technician	1.0
CRITFC technician	1.0
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POST-GRADUATE SCHOLARSHIPS:

Ph.D.: Natural Science and Engineering Research Council (NSERC). 1986

M.Sc.: Natural Science and Engineering Research Council (NSERC), 1981.

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McGill University Travel Fellowship, 1982, 1983.

RELEVANT EXPERIENCE

<i>Position</i>	<i>Dates</i>	<i>Institution and description</i>
Ecologist/geneticist and Senior Scientist	1997-present	Columbia River Inter-Tribal Fish Commission
Fisheries Scientist and Regional Unit Manager	1994-1997	Caribbean Fisheries Resource Assessment and Management Program/Canadian International Development Agency
Managing Partner & Consultant	1989-1994	Talbot and Associates (biostatistics, population dynamics, genetics, development)
Research Associate	1990-1994	Département des Sciences Fondamentales, Université du Québec à Chicoutimi, Québec, Canada
Research Associate & Project Manager	1983-1989	Biology Department, Dalhousie University, Halifax, Nova Scotia
Project Director	1982	National Museums of Natural History, Ottawa, Canada

RELEVANT PROJECTS

<i>Project description</i>	<i>Institution or Client</i>
Resource Assessment & Conservation Biology	
Development of a conceptual framework for ecological genetics of Pacific salmon conservation	CRITFC
ESA Project Leader	CRITFC
Co-ordinator for Collaborative Center for Applied Fish Science	CRITFC
Development of research strategies and co-ordinating project implementation for South American and Caribbean Shrimp/Groundfish Research Program	Canadian International Development Agency

<i>Project description</i>	<i>Institution or Client</i>
Development of a management plan and stock assessment capabilities, and technical assistance in artisanal and industrial groundfish and shrimp fisheries in Benin (Africa).	International Centre for Ocean Development / Canadian International Development Agency
Technical support for pelagic, groundfish and octopus fisheries research (Mauritania, Africa).	International Centre for Ocean Development.
Evaluation of management requirements for the Saguenay Marine Park groundfish fisheries and population dynamics of the turbot (<i>Reinhardtius hippoglossoides</i>), cod (<i>Gadus morhua</i>) and redfish (<i>Sebastes mentella</i>).	Environment Canada / Fisheries and Oceans
Sport and commercial fishing activities in the Saguenay fjord and its potential effects on the groundfish population.	Environment Canada / Fisheries and Oceans
Development of a population estimation method based on the Bayesian principle of simultaneous analysis of removal data from many sites.	Fisheries and Oceans Canada
Development of a monitoring methodology for the evaluation of the exploitation and fishing activities of landlocked Atlantic salmon (<i>Salmo salar ouananiche</i>) in Lac St-Jean	Ministry of the Environment and Fauna, Québec
Analysis of the effect of fishing pressure on the burbot (<i>Lota lota</i>) populations in Lac St-Jean	Ministry of the Environment and Fauna, Québec
Fisheries & Aquaculture	
Genetic improvement of common carp (<i>Cyprinus carpio</i>) and Tilapia (<i>Oreochromis spp.</i>) stocks. (Several individual projects, including cryopreservation of sperm)	International Development Research Centre
Genetics of growth and productivity of fish in aquaculture and the inter-relationship of life history strategies in relation to intraspecific competitive ability in fish.	International Development Research Centre
Dynamics of habitat use in relation to population abundance in Atlantic salmon parr: Test of ecological principles.	Fisheries and Oceans Canada
Study of the fecundity of Atlantic salmon (<i>Salmo salar</i>) in relation to growth at time at sea and its impact on production in rivers.	Ministry of the Environment and Fauna, Québec
Analysis of the effect of fishing pressure on landlocked salmon (<i>Salmo salar ouananiche</i>) populations in Lac St-Jean.	Ministry of the Environment and Fauna, Québec
Determination of productive capacity of habitat for juvenile Atlantic salmon and the application of juvenile density and production models in assessing the status of salmon stocks.	Fisheries and Oceans Canada

Project description

Determination of a classification method for the productive capacity of juvenile salmon habitat.
Prediction of the productivity of juvenile salmon in relation to physical and biological stream parameters.

Institution or Client

Ministry of the Environment and Fauna, Québec
Fisheries and Oceans Canada

RELEVANT PUBLICATIONS AND REPORTS

BRANNON, E., D. CAMPTON, M. POWELL, A.J. TALBOT, and T. QUINN. 2002. Population structure of Columbia River chinook salmon and steelhead trout and application to existing populations. BPA Contract No. 98BI08319.

PHILLIPS, J.L., J. ORY and A.J. TALBOT. 2000. Anadromous Salmonid Recovery in the Umatilla River Basin, Oregon: A Case Study. p. xxx-xxx in "*Watershed management for endangered species*". Journal of American Water Resources Association Vol XXX. In press.

BEASLEY, C.A., A.J. TALBOT, D.R. HATCH, and A. RITCHIE. 2000. Johnson Creek artificial propagation and enhancement project (JCAPE) benefit risk analysis. Prepared for the Nez Perce Tribe.

BEASLEY, C.A., A.J. TALBOT, D.R. HATCH, and M. WISHNIE. 1999. Nez Perce tribal hatchery benefit risk analysis. Prepared for the Nez Perce Tribe.

TALBOT, A.J. and R. A. MYERS. 200X. Density-dependent habitat use and population expansion in juvenile Atlantic salmon. Can. J. Fish. Aquat. Sci. In press.

TALBOT, A.J. and J.-M. SÉVIGNY. 1994. Caractéristiques de la population du flétan du Groënland (*Reinhardtius hippoglossoides*) du fjord du Saguenay. in Sévigny, J.M. et C. Couillard (eds.) 1994. Le Fjord du Saguenay: un milieu exceptionnel de recherche. Rapp. tech. can. sci. halieut. xxxx : xx + xx p.

TALBOT, A.J., A. BOURGEOIS and J.-M. SÉVIGNY. 1994. Évaluation de l'exploitation du Sébaste atlantique (*Sebastes mentella*) par la pêche sportive hivernale sur le Saguenay. in Sévigny, J.M. et C. Couillard (eds.) 1994. Le Fjord du Saguenay: un milieu exceptionnel de recherche. Rapp. tech. can. sci. halieut. xxxx: xx + xx p.

TALBOT, A. 1994. Habitat-dependence of population abundance and variability in juvenile Atlantic Salmon (*Salmo salar*). Ph.D. Thesis, Dalhousie Univ., Halifax, Nova Scotia, Canada B3H 4J1. 214 pp.

CARON, F. and A. TALBOT. 1993. Re-evaluation of habitat classification criteria for juvenile salmon, p. 139-148. In R.J. Gibson and R.E. Cutting [ed.] Production of juvenile Atlantic salmon, *Salmo Salar*, in natural waters. Can. Spec. Publ. Fish.

Aquat. Sci. 118.

TALBOT, A.J. and R.W. DOYLE. 1992. Statistical interrelation of length, growth, and scale circulus spacing: II. Use of marginal ossification to detect non-growing fish. *Can. J. Fish. Aquat. Sci.* 49(4):701-707.

TALBOT, A.J. and R.J. GIBSON. 1992. Habitat utilization by juvenile Atlantic salmon in Newfoundland rivers. p. 163-184 *In* J.B. Dempson [ed.] *Collected papers on fish habitat with emphasis on Salmonids*. Canadian Atlantic Fisheries Scientific Advisory Committee. CAFSAC Res. Doc. 90/77. 423 pp.

DOYLE, R.W., N.L. SHACKELL, Z. BASIAO, S. URAIWAN, T. MATRICIA and A.J. TALBOT. 1991. Selective diversification of aquaculture stocks: a proposal for economically sustainable genetic conservation. *Can. J. Fish. Aquat. Sci.* 48(1):148-154.

TALBOT, A.J. and R.W. DOYLE. 1990. Statistical properties and power of growth estimation using scale microstructure. p. 421-424 in R. Hirano and I. Hanyu (eds.). *The Second Asian Fishery Forum*. 991 pp. Asian Fisheries Society, Manila, Philippines.

MATRICIA, T., A.J. TALBOT and R.W. DOYLE. 1989. Instantaneous growth rate of Tilapia genotypes in undisturbed aquaculture systems. I. "Red" and "Gray" morphs in Indonesia. *AQUACULTURE*. 77:295-306. (also internal report to IDRC)

DOYLE, R.W. and A.J. TALBOT. 1989. Repeatability of relative size-specific growth in Tilapia. p. 451-456 in R.S.V. Pullin, T. Bhukasawan, K. Tonguthai and J.L. MacLean (eds.) *The Second International Symposium on Tilapia in Aquaculture*. ICLARM Conference Proceedings 15. 623 pp. Department of Fisheries, Bangkok, Thailand, and International Centre for Living Aquatic Resources Management, Manila, Philippines.

TALBOT, A.J., T.K. HAY, R.W. DOYLE and A.E.L. McNAUGHTON. 1989. "Current growth" estimators in Tilapia. p. 509-513 in R.S.V. Pullin, T. Bhukasawan, K. Tonguthai and J.L. MacLean (eds.) *The Second International Symposium on Tilapia in Aquaculture*. ICLARM Conference Proceedings 15. 623 pp. Department of Fisheries, Bangkok, Thailand, and ICLARM, Manila, Philippines.

DOYLE, R.W., A.J. TALBOT and R.R. NICHOLAS. 1987. Statistical interrelation of length, growth and scale circulus spacing: appraisal of a growth-rate estimator for fish. *Can. J. Fish. Aquat. Sci.* 44(9):1520-1528.

DOYLE, R.W. and A.J. TALBOT. 1986. Effective population size and selection in variable aquaculture stocks. *Aquaculture*. 57:27-36.

DOYLE, R.W. and A.J. TALBOT. 1986. Artificial selection on growth and correlated selection on competitive behaviour in fish. *Can. J. Fish. Aquat. Sci.* 43(5):1059-1064.

TALBOT, A., M. BENFIELD, and L. MORTON. 1996. Biology of selected shrimp and Groundfish species on the Guiana/Brazil Continental Shelf. *The Joint CFRAMP Sub-Project Specification Workshop and Fourth Meeting of the WECAFC Ad Hoc Shrimp and Groundfish Working Group on the Guiana/Brazil Continental Shelf*, Hotel Normandie, St. Ann's, Trinidad. 8-12 January 1996, Vol. Technical Papers. CFRAMP, Shrimp and Groundfish Resource Assessment Unit, PO Box 3150, Carenage Post Office, Carenage, Trinidad.

TALBOT, A.J. 1991. Simultaneous analysis of removal data from many sites. Presented to the Salmon Association of Eastern Newfoundland, St-John's, NFLD A1C 5N8 as part of the Habitat Modelling Project. 33 pp.

TALBOT, A.J. 1990. Production of juvenile Atlantic salmon in Newfoundland rivers: Test of habitat models using a structural approach. Final report of the habitat modelling project, Phase I, presented to the Salmon Association of Eastern Newfoundland, St-John's, NFLD, A1C 5N8. 84 pp. et 1 app.

TALBOT, A.J. and R. J. GIBSON. 1990. Habitat utilization by juvenile Atlantic salmon in Newfoundland rivers. Report to the members of the Canadian Atlantic Fisheries Scientific Advisory Council (CAFSAC, #90/118).

TALBOT, A.J. 1989. Improved abundance estimation of Atlantic salmon parr. Contract with Fisheries and Oceans (Number XAQ88-00132-(021)).

Ernest Leroy Brannon
December 2000

EDUCATION:

B.S., Fisheries, University of Washington, 1959
Ph.D., Fisheries, University of Washington, 1973

EXPERIENCE:

1988-pres: Director of the Aquaculture Research Institute, Professor of Fisheries Resources and Animal Science, and State Aquaculture Extension Specialist, University of Idaho, Moscow
1973-1988: Assistant/Associate/Full Professor, School of Fisheries, College of Ocean and Fisheries Sciences, Univ. of Washington, Seattle
1974-1983: Director, Finfish Aquaculture Prog., College of Fisheries, Univ. of Washington, Seattle
1971-1972: Chief Biologist, Int'l Pacific Salmon Fisheries Comm. (IPSFC), New Westminster, B.C., Can.
1969-1971: Supervisor, Sockeye Management Research, IPSFC, New Westminster, B.C., Can.
1962-1969 Habitat, incubation, and rearing habitat assessment, IPFSC, New Westminster B.C., Can.
1959-1969: Research Biologist, Fisheries Management, Artificial Propagation, Spawning Channel Development and Fish Culture, IPSFC, New Westminster, B.C., Can.

CURRENT RESEARCH:

2000-2003 Partnership for Innovation: Feeds and Generics, NSF
1999-2000 Columbia Basin White Sturgeon Genetic Variation, BPA.
1998-1999 Columbia River Chinook Salmon and Steelhead Population Structure, BPA.
1998-1999 Wastewater Treatment for Aquaculture and Confined, EPA.

RECENT PUBLICATIONS:

Brannon, E., D. Campton, M. Powell, A. Talbot, and T. Quinn. 2000. Population structure of Columbia River chinook salmon and steelhead trout and application to existing populations. BPA Contract No. 98BI08319.
Collins K., E. Brannon, L. Moulton, M. Cronin, and K. Parker. 2000. Hydraulic sampling protocol to estimate natural embryo mortality of pink salmon. Trans. Am. Fish Soc. 129:627-834.
Brannon E.L., K. Collins, L. Moulton, and K. Parker. Accepted. Resolving allegations of oil damage to incubating pink salmon eggs in Prince William Sound. Canadian Journal of Fisheries and Aquatic Sciences.
Brannon, E.L., D. Campton, M. Powell, A. Talbot, and T. Quinn. 2000. Population structure of Columbia River chinook salmon and steelhead trout and applications to existing populations. BPA Contract No. 98BI08319.
Brannon, E.L., K. Currens, D. Goodman, J. Lichatovich, B. Riddell, R. Williams. April, 1999. Review of artificial production of anadromous and resident fish in the Columbia River Basin Part 1 - A scientific basis for Columbia River production

- programs. Council Document 99-4, Program Evaluation and Analysis Section, Portland, Oregon. 132 pp
- Brannon, E.L. 1998. Columbia River downstream migrant passage and habitat recovery. *Pages 193 - 199 in* E.L. Brannon and W.C. Kinsel, editors. Proceedings of the Columbia River anadromous salmonid rehabilitation and passage symposium (June 5-7, 1995, Richland, WA). Sponsored by the University of Idaho and Washington State University. Aquaculture Research Institute, Moscow, Idaho.
- Cummings, S. A., E. L. Brannon, K. Adams, and G. H. Thorgaard. 1997. Genetic analyses to establish captive breeding priorities for endangered Snake River sockeye salmon. *Conservation Biology* 11(3):662-669.
- Brannon, E.L. and A.W. Maki. 1996. The *Exxon Valdez* oil spill: Analysis of impacts on the Prince William Sound pink salmon. *Reviews in Fisheries Science* 4(4):289-337.
- Brannon, E. L. 1993. The perpetual oversight of hatchery programs. *J. Fish Res.* 18:19-27.
- Brannon, E. L. and T.P. Quinn. 1990. Field test of the pheromone hypothesis for homing Pacific Salmon. *J. Chem. Ecol.* 16(2):603-609.

MADISON S. POWELL, Ph.D.
Senior Personnel

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3059F National Fish Hatchery Road
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Hagerman, ID 83332

Professional Preparation

1995 Ph.D., Zoology, Texas Tech University
1990 M.S., Zoology, University of Idaho
1985 B.S., Zoology/Biology, University of Idaho

Appointments

1997-present	Research Scientist, Hagerman Fish Culture Experiment Station, University of Idaho, Hagerman, Idaho
1996-1997	Research Scientist, Aquaculture Research Institute, University of Idaho, Moscow, Idaho
1995-1996	Postdoctoral Fellow, Aquaculture Research Institute, University of Idaho, Moscow, Idaho

Five Closely Related Publications

Powell, M.S. and P. Anders. (*submitted*). Karyotypic analysis of Kootenai River white sturgeon (*Acipenser transmontanus*). *Journal of Fish Biology*.

Paragamian, V.L., M.S. Powell, J.C. Faler, and S. Snelson. 1999 Mitochondrial DNA analysis of burbot *Lota lota* stocks in the Kootenai River Basin of British Columbia, Montana, and Idaho. *Trans. Am. Fish. Soc.* 128:868-874

Powell, M.S., V.L. Paragamian, and J.C. Faler 1998. Genetic characteristics of burbot in the Kootenai River drainage of Montana, Idaho, and British Columbia. *Proceedings of the International Congress on the Biology of Fish.* Burbot Symposium. pp1-4.

Anders, P. and M. Powell. 1998. Comprehensive management and conservation of Columbia Basin white sturgeon (*Acipenser transmontanus*): A zoogeographic

approach. *Proceedings of Ecosystem Based Management in the Upper Columbia River Basin*. Castlegar, British Columbia, Canada. pp53-54.

Powell. M.S. 1998. Quantitative genetics in aquaculture. *Extension Focus*, 12:6-8.

Synergistic Activities

American Society of Ichthyologists and Herpetologists

American Fisheries Society (Genetics Section)

Society for Conservation Biology

World Aquaculture Society

Phi Sigma (non-active)

Sigma Xi (non-active)

1999 (July) Grant Review Panel (Strengthening), U.S. Dept. of Agriculture.

1999-present Fisheries Genetics Consultant (Montgomery Watson Inc.)

1998-present White Sturgeon Genetics Workgroup. (Bonneville Power Admin.)

1996-present Technical Oversight Committee for Threatened Snake River Chinook Salmon. (Bonneville Power Admin.)

1995-present Technical Oversight Committee for Endangered Snake River Sockeye Salmon. (Bonneville Power Admin.)

Collaborators & Other Affiliations

- Idaho Department of Fish and Game (current)
- Montana Department of Fish, Wildlife and Parks (current)
- Oregon Department of Fish and Wildlife (current)
- Washington Department of Fish and Wildlife
- Nevada Department of Wildlife
- Kootenai Tribe of Idaho (current)
- Columbia River Inter-Tribal Fish Commission (current)
- Nez Perce Tribe (current)
- Confederated Tribes of the Warm Springs Reservation (current)
- Makah Tribe (current)
- Confederated Tribes of the Umatilla Indian Reservation (current)
- Henry's Fork Foundation (current)
- Nature Conservancy (current)
- Montgomery Watson Inc. (current)
- Utah State University
- U.S. Fish and Wildlife Service (current)
- Bureau of Land Management (current)
- U.S. Forest Service (current)
- National Marine Fisheries Service

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Professional Preparation

B.S., Zoology, University of Washington, 1969
M.S., Nutrition, Washington State University, 1973
Ph.D., Fisheries, University of Washington, 1978

Appointments

1978-1984	Research Associate Professor, School of Fisheries, University of Washington, Seattle, Washington
1984-1996	Supervisory Research Chemist, Utilization Research Division, NWFSC, NMFS, Seattle, Washington
1984-Present	Affiliate Professor, School of Fisheries, University of Washington, Seattle, Washington
1996-Present	Professor, Animal & Vet. Sciences, University of Idaho, Moscow, Idaho, and Director, Hagerman Fish Culture Experiment Station

Five Closely Related Publications

- Hardy, R. W. 1998. Feeding Salmon and Trout. Pp. 175-197 *In* Nutrition and Feeding of Fish, 2nd Edition, R. T. Lovell (ed). Kluwer Academic Press, Dordrecht, The Netherlands.
- Sugiura, S. H., Dong, F. M., Rathbone, C. K. and Hardy, R. W., 1998. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture*, 159: 177-202.
- Hardy, R. W., 1999. Collaborative opportunities between fish nutrition and other disciplines in aquaculture: an overview. *Aquaculture*, 177: 217-230.
- Hardy, R.W., 1999. Aquaculture's rapid growth requirements for alternate protein sources. *Feed Management*, 50(1): 25-28.

Sugiura, S.H., Dong, F.M., and Hardy, R.W., 2000. A new approach to estimating the minimum dietary requirement of phosphorus for large rainbow trout based on nonfecal excretions of phosphorus and nitrogen. *J. Nutrition*, 130: 865-872.

Synergistic Activities

World Aquaculture Society (Secretary, 1997 to 2001)
American Institute of Nutrition
Fish Nutrition Expert, Food and Agriculture Organization of the United Nations (FAO), Bangkok, Thailand. 1990-1991
Subcommittee on Warmwater Fish Nutrition, National Resource Council, National Academy of Science. 1981-1984.
Committee on Animal Nutrition, National Resource Council, National Academy of Science. 2000-2002.
Research Subcommittee of Technical Committee, Western Regional Aquaculture Consortium, USDA. Seattle, WA. 1987-2000.
Scientific Editor of *FISHERY BULLETIN* and *NOAA TECHNICAL REPORTS*, 1992-1995.
Member, Editorial Advisory Board, *AQUACULTURE*, Elsevier Publications, 1991-Present, and *REVIEWS IN FISHERIES SCIENCE*, CRC Press, 1992-present, *AQUACULTURE NUTRITION*, 1994-present.
Co-Editor of *AQUACULTURE RESEARCH*, Blackwell Science, Ltd., 1999 to present.

Collaborators & Other Affiliations

Arndt, R. (Utah F&G)
Babbitt, J.K. (U of Alaska)
Barrows, F.T. (USFWS/Bozeman)
Brannon, E.L. (UI)
Casten, M. (UI)
Collins, K. (UI)
Dominy, W. (Oceanic Inst.)
Dong, F.M. (UW)
Erickson, J.D.
Flagg, T. (NMFS)
Fornshell, G. (UI)
Forster, I.P.
Gabaudon, J.
Gatlin, D. (Texas A&M)
Haard, N.F. (UC-Davis)
Halver, J.E. (UW)
Hatch, C.R. (UI)
Hendry, A. (Vancouver)
Higgs, D.A. (Vancouver)
Kissil, G.Wm. (Israel)
Lupatsch, I. (Israel)
Majack, T. (NMFS)
Masse, K.C. (NMFS)
Nelson, C.
Overturf, K.E. (USDA/ARS)
Peterson, M. (NMFS)
Powell, M.S. (UI)
Pruder, G. (Oceanic Inst.)
Raboy, V. (USDA/ARS)
Rasco, B.A. (WSU)
Rathbone, C.K. (NMFS)
Roberts, R.J.
Rust, M.B. (NMFS)
Satoh, S. (Japan)
Schelling, G.T. (UI)
Scott, T.M. (NMFS)
Shearer, K.D. (NMFS)
Skonberg, D.I. (U of Maine)
Smiley, S. (U of Alaska)
Stickney, R.R. (Texas A&M)
Sugiura, S. (UI)
Swanson, P. (NMFS)
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Young, K. (USDA/ARS)

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Professional Contact

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Interests: Applied Conservation Biology; Ecological Genetics; Statistics; Fisheries Management

Objective: Apply ecological, genetic, and statistical theory towards conservation of endangered species, with an emphasis on maintaining evolutionary and demographic processes, in the context of real-time applied management.

Education: **M.S. Zoology, Minor Statistics**
North Carolina State University
Used ultrasonic telemetry and historical time series harvest data to quantify the effects of a low-head dam on production and sustainability of striped bass and American shad spawning aggregates. Individual spawning observations were used to non-parametrically assess aspects of preferred spawning habitat and assign metric values to currently available and potentially available spawning habitat.

B.S. Systematics and Ecology, Minor Aquatic Ecology
University of Kansas
Curriculum focused on ecology, fish physiology, genetics, and limnology. Independent study included assay and analysis of genetic samples to infer the phylogeny of Percomorph fishes; foraging and population dynamics of bluegill sunfish in a natural monoculture; and inferring limiting factors for re-establishment of *Ctenophora* spp. in Jamaica.

Academic Awards: 1995 recipient of the Frank Cross-Otto Tiemeier student scholarship for achievement in fisheries research.

Professional Experience:

Columbia River Inter-Tribal Fish Commission 1998-Present

Fisheries Scientist

Responsibilities include analysis of genetic and ecological data; risk analysis of management alternatives; and proposal writing. My goal at the Columbia River Inter-Tribal Fish Commission is to use a multidisciplinary approach to make informed management recommendations for applied conservation of anadromous Pacific salmon, lamprey, and sturgeon. Of particular interest to me is the application of statistical and genetic theory in the management of declining populations.

FishPro Incorporated 2000-Present

Consulting Geneticist

Responsibilities included biological assessment of the Coleman National Fish Hatchery chinook program and Russian River coho, steelhead, and chinook salmon hatchery programs; formulation of alternative propagation programs; and formulation of risk metrics for alternative management actions. My goal for these projects was to convert hatchery programs designed for mitigation purposes into conservation oriented programs.

Kansas Department of Wildlife and Parks 1995

Stream Biologist

Responsibilities included fish and invertebrate (insect and mussel) collection and identification; stream habitat surveys; collection of water quality data; and development of aquatic habitat health metrics. My goal at the Kansas Department of Wildlife and Parks was the development of rapid stream health assessments to aid in the identification and protection of unique aquatic habitats and species assemblages.

Recent Publications:

Beasley, C.A., S.R. Narum, A. Talbot, J. Whiteaker, D. Hatch, and M. Powell. 2002. Evaluating admixture of ESA listed and non-listed spring chinook salmon in the upper-Columbia River ESU. *In Review*. International Congress on the Biology of Fish.

Beasley, C.A., R. Sharma, and A. Talbot. *In Preparation*. Maintenance of family lineages in endangered salmonids: relative effectiveness of propagation alternatives. Targeted for the Canadian Journal of Fisheries and Aquatic Sciences.

Beasley, C.A. and J.E. Hightower. 2000. Effects of a low-head dam on the distribution and characteristics of spawning habitat used by striped bass and American shad. Transactions of the American Fisheries Society. 129:1316-1330.

Congratulations!