An Evaluation of the Efficacy of Steelhead Kelt Reconditioning to Address Biological Opinion Action 184b: The reproductive success of hatchery-origin and wild-origin repeat spawners.

By

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PROJECT SUMMARY

The goal of this project is to evaluate the reproductive performance of both hatcheryand wild-origin adult steelhead (*Oncorhynchus mykiss*) that are reconditioned to spawn a second time. Empirical evidence suggests that the process of reconditioning can alter both the timing of gonad development and the survival of progeny relative to natural returning individuals. Therefore an evaluation of the impacts of reconditioning on reproductive performance that compares virgin or first time spawners to artificially reconditioned spawners is needed before reconditioning can be considered a viable management option.

We propose a two-phase study to directly and rigorously evaluate the reproductive success of reconditioned summer-run steelhead kelts and determine the potential for use of kelt reconditioning as a management tool contributing to recovery of listed steelhead populations in the Columbia Basin. The study is to be fully integrated into the existing wild broodstock steelhead production and research program in the Kalama River, Washington. In this three and a half year study, we are proposing objectives that constitute Phase I.

In Phase I wild steelhead collected as broodstock in the ongoing Kalama River program and hatchery steelhead from earlier plants from that program will be reconditioned based on established protocols. The reproductive potential of the reconditioned hatchery (H) and wild (W) fish (and H x W variants) will be established by determining the quantity and quality of gametes, and survival to eyed-egg and swim-up stages. These variables will be compared to those of the same fish when they were first time (virgin) spawners, as well as between the three cross variants (HxH, WxH, WxW).

Phase II will begin during the third year of research if (1) during Phase I there is adequate survival and recovery of kelts, and (2) reconditioned kelts produce gametes qualitatively equal to or nearly equal to those of virgin spawners, and (3) work proposed here and elsewhere indicates manageable risk to the existing research program and the extant wild Kalama steelhead population.

Phase II will involve the release of reconditioned kelts into natural spawning waters of the Kalama drainage to spawn among themselves and with wild and hatchery fish. Reproductive success of the kelts will be established by capturing offspring and unambiguously establishing their parentage using an existing WDFW msDNA-based pedigree approach, modified as necessary for the work proposed here. Phase II will become an integral component of the ongoing reproductive success evaluations of hatchery and wild summer-run steelhead in the Kalama watershed.

PROJECT DESCRIPTION

Introduction

The impact of hatchery-origin fish spawning with wild-origin fish is a subject of great debate and controversy in the Pacific Northwest (Busack and Currens 1995, Campton 1995, Ford and Pearsons 2002-BPA proposal). The goal of many hatchery facilities is to not only provide fish for harvest and recreation but also to augment wild runs by allowing hatchery fish to spawn with wild cohorts. As a component of steelhead (*Oncorhynchus mykiss*) recovery measures in the region, the National Marine Fisheries Service requires scientific

research "to determine whether hatchery reforms reduce the risk of extinction for Columbia River basin salmonids and whether conservation hatcheries contribute to recovery" (NMFS, FCRPS BiOp Action 184, 2000). The reconditioning of post-spawned steelhead ("kelts") in aquaculture/hatchery facilities may bolster depleted populations of steelhead throughout the Columbia River Basin by increasing the number of repeat spawners. In this fashion, reconditioning may constitute a novel approach to hatchery reforms For many populations in the upper Columbia River, kelt reconditioning could also maintain the iteroparous life history strategy, a trait that sets steelhead apart from the Pacific salmon species. Iteroparity is currently being selected against by the almost complete mortality (*ca.* > 90%) of downstream migrating kelts associated with migration and passage issues imposed by the hydrosystem (Evans 2002). Reconditioning has the additional advantage of allowing fisheries managers to select which fish (e.g., hatchery or wild) are reconditioned, providing the means to intentionally supplement with the most appropriate stocks or components of the run.

Reconditioning is the process of culturing post-spawned fish so that they survive, grow and undergo gonad recrudescence for a second or even third spawning. Reconditioning of iteroparous species has been achieved with Arctic charr Salvelinus alpinus (Boyer and Toever 1993), Atlantic salmon Salmo salar (Johnston et al. 1990, Crim et al. 1992, Moffett et al. 1996) and more recently with summer-run steelhead from the Columbia River Basin (Evans et al. 2001). Feasibility studies conducted by the Columbia River Inter-Tribal Fish Commission (CRITFC) and the Yakama Nation (YN) using wild origin Yakima R. steelhead have demonstrated that kelts can resume feeding following spawning without reentering the salt-water environment. Upwards of 25% of the steelhead kelts collected from the Yakima R. were determined to have developing gonads within 6-8 months following capture. The vast majority (ca. > 90%) of both the collected kelts and reconditioned fish from this study were also female (Evans et al. 2001, Anders et al. 2002), suggesting that reconditioning may substantially increase egg production and that the contribution of repeat spawning males is limited in nature. A trend toward higher female survival and rematuration relative to males is consistent with kelt observations at Lower Snake River dams (Evans and Beaty 2000, Evans and Beaty 2001, Evans 2002) and with iteroparous salmonid species throughout the Pacific Northwest (Wither 1966, Ledier et al. 1986, Fleming 1998).

Despite the successful development of feeding regimens and rematuration of steelhead kelts in aquaculture facilities, some fundamental questions regarding the reproductive success of reconditioned kelts remains unanswered. For example, the viability of reconditioned fertilized eggs is unknown because kelt reconditioning efforts conducted thus far have released repeat spawners prior to evaluating reproductive potential. The timing of oogensis and spermatogenesis in salmonids is regulated not only genetically but also by environmental factors (e.g., food availability/quality, photoperiod, and water temperatures; Johnston et al. 1987, Crim et al. 1992, Johnston 1992). Thus, reconditioning may not only impact the viability of oocytes and spermatids but also the time at which reconditioned fish reach maturity relative to natural-ascending first time (virgin) spawners. Furthermore, reconditioning studies to date have focused only on wild migrants, whereas the efficacy of reconditioning hatchery fish is also a fundamental unknown. If it can be demonstrated that hatchery fish have similar survival, rematuration, and reproductive success rates to that of wild spawners (both virgin and repeat spawners), then studies aimed at evaluating the full life-cycle influence of repeat spawners are justified. Conversely, if the process of reconditioning alters the viability and/or maturation progression of reconditioned fish relative to natural spawners, the actual reproductive contribution of reconditioning may be significantly less than that implied by initial results from on-going studies.

Concern regarding the reproductive development rate and viability of reconditioned kelt gametes is supported by empirical data generated from Atlantic salmon reconditioning studies. Johnston et al. (1992) demonstrated that reconditioned kelts had lower progeny survival to the eyed-egg stage and that shifts in temperature regimens manipulated the normal spawning period. Crim et al. (1992) demonstrated that reconditioned female kelts produced eggs of high quality – as determined by fertilization rates and survival to the eyed-egg stage – but that emerging alevins had higher than normal mortalities rates. In a study comparing the reproductive performance of virgin spawners to reconditioned spawners, Moffett et al. (1996) illustrated that mean fecundity in reconditioned fish was 23% lower than that of controls (first time spawners) and progeny survival to swim-up stage was reduced by 7.8% in reconditioned fish. However, reconditioned fish produced larger ova relative to virgin broodstock, which may ultimately convey a competitive advantage to reconditioned progeny. Whether similar trends exist in reconditioned steelhead is, however, unknown and whether fish origin (i.e., hatchery or wild) is an important factor also warrants evaluation.

To address these key uncertainties – as outlined by BiOp Action 184b – we propose to evaluate the efficacy of kelt reconditioning on hatchery- and wild-origin adult steelhead from the Kalama River, WA. Tests will be conducted to evaluate the gamete quantity, quality and viability, and survival of progeny to the eyed-egg stage and swim-up stage. Comparison of all three variants (HxH, HxW, WxW) will be made for natural returning virgin spawners, reconditioned repeat spawners, and a limited number of natural returning repeat spawners¹-iteroparity is still a common life history among Kalama R steelhead (Figure 1). . Because iteroparity is most common among females, focus will be on hatchery and wild-origin females, however, some males will be retained to ensure synchronization of the maturation process (Johnston et al. 1987). Although the source population of fish proposed for this study lies outside the ESU's (Mid-Columbia, Upper-Columbia, and Snake R.) targeted by the request for kelt reconditions studies, the collection system and ongoing study of hatcherywild steelhead interactions make the Kalama River a unique research opportunity. Moreover, the results of this study will be directly applicable to summer-run steelhead populations from throughout the Columbia, because the Kalama River wild broodstock hatchery program uses husbandry protocols that are used or suggested- conservation hatcheries throughout the region. Furthermore, since iteroparity in Kalama steelhead has not been significantly suppressed by anthropogenic changes to the migration corridor (i.e., mainstem dams), reconditioning of Kalama steelhead has the added advantage of providing base-line data to evaluate reconditioning success rates generated from impounded, upriver steelhead populations.

Other peripherally important aspects of the current Kalama River research program include (1) annual operation of two rotary screw traps in the watershed to sample outmigrating juveniles, (2) existence of long-term (> 25yr) life history and genetic (allozyme-based) databases for wild and hatchery Kalama steelhead, (3) a productive, professional relationship with the WDFW Genetics Unit staff in Olympia and with fish cultural staff at Kalama facilities, and (4) a history of successful collaborative relationships

¹ Scales will be removed from adults to determine both the age and number of spawning episodes prior to reconditioning. We anticipate that ~ 8% of natural returnees will be repeat spawners (Figure 1).

with other research entities in the region including NMFS (Sharpe et al. 2002, Fuss et al. 2001) and the USGS-BRD Columbia River Research Laboratory (Connolly et al. 2002).

Research associated with this proposal will not only utilize existing fish production, monitoring and research facilities but will compliment the following ongoing and/or proposed kelt reconditioning studies.

- 1) CRITFC/YN wild kelt reconditioning study, funded by BPA (project ID 2000017000.
- 2) Okanogan wild kelt reconditioning study, proposed for BPA funding (project ID 29007)
- 3) Lower Granite Dam kelt enumeration and transportation study, funded by U.S. Army Corps
- 4) Reproductive Success of Natural-Origin, Endemic Hatchery Origin, and Reconditioned Kelt Summer Steelhead in the Tucannon River. Proposal to BPA

Objectives and Null Hypotheses

- 1) Determine the gamete quantity, quality and viability of first time (virgin) spawning and reconditioned steelhead.
- **HO:** Measures of gamete quantity, quality and viability of virgin steelhead spawners do not differ from those of reconditioned steelhead spawners.
- 2) Determine the gamete quantity, quality and viability of first time (virgin) hatchery- and wild-origin steelhead spawners.
- **HO:** Measures of gamete quantity, quality and viability of virgin hatchery-origin steelhead spawners do not differ from those of virgin wild-origin steelhead spawners.
- 3) Determine the gamete quantity, quality and viability of reconditioned hatchery- and wildorigin steelhead spawners.
- **HO:** Measures of gamete quantity, quality and viability of reconditioned hatcheryorigin steelhead spawners do not differ from those of reconditioned wild-origin steelhead spawners.
- 4) Provide preliminary data on the gamete quantity, quality and viability of naturally returning steelhead repeat spawners relative to reconditioned steelhead spawners. (Because of expected low sample size, there is no HO associated with this objective.)

Methods

Source Population and Facilities— An experimental hatchery program using natural origin (wild) summer-run steelhead is ongoing on the Kalama River, Washington (Sharpe et al. 1999, 2000). The objectives of the project are to evaluate in-hatchery performance, migratory behavior, smolt to adult returns, and, especially, determine the reproductive competence of first generation hatchery fish allowed to spawn naturally among themselves and with fish in the wild population from which they were derived. Three broods have been

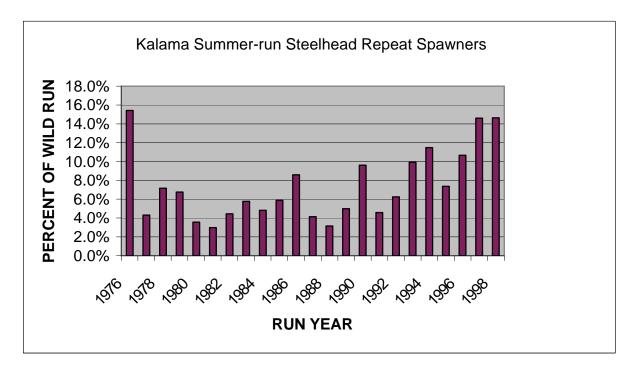
released as yearling smolts. The fourth is to be released this spring. Adult returns from the first (1999) brood returned last summer (2002) and a representative portion were passed upstream of a barrier falls in the Kalama watershed with an equal number of wild fish. Controlled passage of hatchery-origin spawners will occur for at least three consecutive broods. DNA samples were and will be obtained from all the adults passed upstream. Their natural reproductive performance will be established by sampling the naturally produced offspring and establishing their pedigrees (identifying and enumerating HxH, HxW, and WxW variants) using a microsatellite DNA-based protocol. That protocol has already been developed and tested by the Washington Department of Fish and Wildlife's Genetics Unit DNA lab. The protocol has been used to successfully pedigree residual (non-migrant) juvenile steelhead from our hatchery releases (Sharpe et al., in prep.). Given the successes of the Kalama Research Team (a collaborator on this proposal), successes with other species (e.g. Yakima spring chinook; Sewell Young, WDFW, Pers. Comm.) and outcomes of other ongoing work with steelhead (Bill Ardren, USFWS, Pers. Comm), we are confident our longer-term research goals can be accomplished using the approach.

Most of the wild fish sequestered as broodstock (approximately 50 fish/yr) survive spawning and are released into the river after a short recovery period. For Phase I of the work proposed here, we propose to alter this portion of our existing protocol by sequestering the females (up to 30) for an additional year or more, reconditioning them per details provided elsewhere in this document, and testing elements of the reproductive potential of the reconditioned kelts relative to virgin spawners. Further, we propose to sequester a similar or greater number of first generation adult hatchery returnees to allow a direct comparison of reproductive potential of hatchery vs. wild and virgin vs. reconditioned spawners.

Because the existing research program on the Kalama includes a near census of adults attempting to enter the spawning waters of the upper watershed, the opportunity exists in later years to directly evaluate the reproductive success of reconditioned kelts should it prove possible to enhance survival of an adequate number of them. First though, a measured, cautious approach to the management of the existing research program and the natural resources in the Kalama requires that we understand better (1) the potential for reconditioning the spawners, (2) the likelihood that reconditioned spawners produce gametes qualitatively equal or nearly equal to those of virgin spawners, and (3) substantive indications that integrating a kelt reconditioning study will not compromise the outcome of the existing research program or well-being of the existing wild population. Concerns (1) and (2) are explicitly addressed as research objectives of this proposal. We are already addressing the final concern as part of the ongoing project by reviewing and contributing to existing literature on genetic changes of wild salmonid populations, especially in the context of anthropogenic alteration of rates of inbreeding via supplementation in general. Increasing rates of iteroparity will increase variance in family size and, consequently, increase rates of inbreeding. However, the effect will be balanced somewhat by the temporal separation of at least one year between potential spawning interactions between the majority of half-sibs and, in the Kalama system, the likelihood that relatively small numbers of reconditioned kelts will be released. Further, it appears possible in the lower Columbia and certain in the upper Columbia that rates of iteroparity are lower now than historically. An increase to normative rates might well represent a beneficial recovery of a nearly extirpated life history strategy. However, no reconditioned kelts will be passed into the upper watershed until a thorough review of the potential advantages and, especially, consequences, is accomplished.

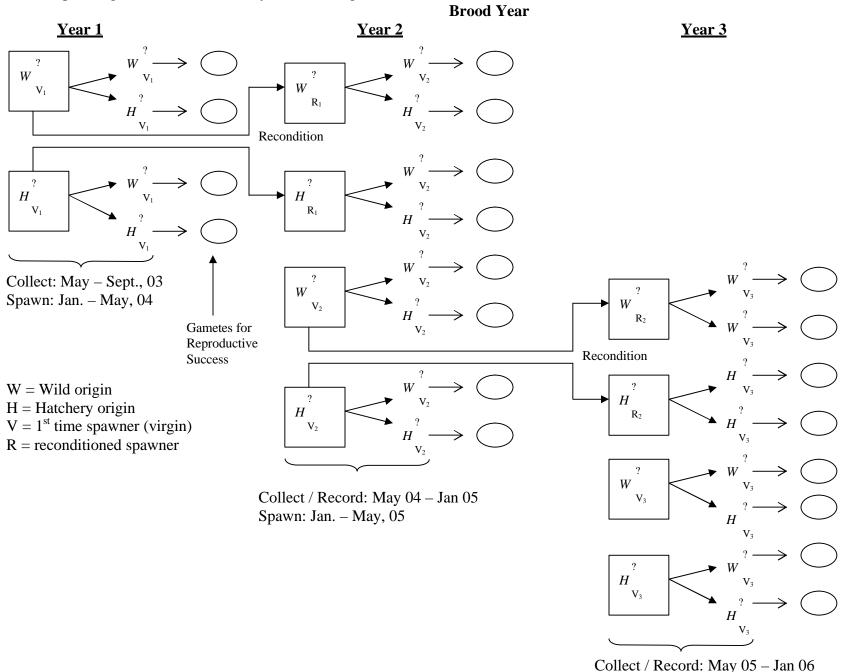
Importantly, the existing research program will directly measure the reproductive success of hatchery and wild origin repeat spawners occurring naturally in the system. Recent life history data from wild Kalama summer-run steelhead show natural repeat spawner rates approaching 15% of the total wild summer-run return (Figure 1).

Figure 1. Percent of repeat summer-run steelhead spawners returning to the Kalama River, Washington from 1976 through 1998 (Unpublished WDFW data, Kalama Research Team, 2003).



Viability Trials—The sequence and timing of the viability trials is illustrated in Figure 2. Reproductive success of reconditioned females will be measured by comparing variability in measures of egg quantity and quality, including absolute fecundity, relative fecundity, egg size (mass and diameter), fertilization rate in a sperm dilution series, and survival of embryos to the hatch and swimup stages of development. We have used this system for assessing reproductive success in chinook salmon exposed to high gas supersaturated water (Gale et al., 2001; Gale et al., in review). In the first year of the project, up to 30 wild-origin and 30 or more hatchery-origin females will be collected and held until they are ready to spawn. All females will be returned to the holding tanks after spawning for long-term reconditioning.

Figure 2. Diagram of the collection and mating strategies that will be used to determine reproductive success of virgin and repeat spawning steelhead by examining gamete quality and viability. Eggs from each female will be fertilized with milt pooled from virgin spawning males of both hatchery and wild origin.



Spawn: Jan. – May, 06

The volume and mass of eggs from each female will be determined and a subsample will be weighed and counted, and that number will be extrapolated to estimate absolute (eggs/female) and relative (eggs/kg body weight) fecundity. Mass of about 30 eggs per female will be determined to the nearest 0.001 g as a measure of egg size. Heath et al. (2003) have documented a differences in egg mass and fecundity between hatchery and wild females and related these to differences in maternal reproductive success. Earlier studies have documented that fertilizing fish eggs with too much or too little sperm can result in less than maximal fertilization (Levanduski and Cloud, 1988; Rurangwa et al., 1998; Liley et al., 2002). We also know that in a river the concentration of sperm and the length of time the eggs are exposed to sperm are considerably less than in a typical aquaculture spawning. This variability can result in a significantly lower fertilization rate in the wild as compared to a hatchery. For example, Liley et al. (2002) reported that exposing rainbow trout eggs to sperm for 5 sec resulted in just 27% fertilization. For that reason we will conduct fertilization using a sperm dilution series in order to determine the maximum fertilization rate and to estimate the sperm concentration that results in 30% fertilization. About 1000 to 1500 eggs from each female will be divided into 10 equal lots (isolates) and each isolate will be fertilized with sperm from one of two pools of milt collected from about 10 wild- or hatchery-origin males collected at the same time as the females. A series of four or five dilutions will be made from each pool of sperm, and duplicate groups of about 100 eggs will be fertilized and kept in separate sections of egg-hatching trays. The concentrations in the sperm dilution series will be determined empirically before the experiments. After the fertilization rates have been determined, all fertilized eggs from a female will be combined in trays to determine survival to the hatch and swimup life history stages

Differences in absolute and relative fecundity, and egg mass will be assessed using ANOVA, with $p \le 0.05$ for all tests of significance. Fertilization rate, and cumulative mortalities at hatch and swimup will be expressed as proportions and arcsin transformed prior to statistical analyses by nonparametric Kruskal-Wallis test. Based on results from previous studies with salmonids (Carragher et al., 1989; Campbell et al., 1994; Contreras-Sánchez, 1995), we believe that sample sizes of 12 adequate to detect differences in reproductive variables. In an earlier study (Gale et al. in review) we conducted after-the-fact power analyses [(1- β), β = probability of accepting a false null hypothesis—a Type II error]. We found that, with n = 30 for a pooled control group and n = 9 for treatment groups, power was between 70 and 99%.

Reconditioning Protocol— The general methods proposed here are similar to those developed by CRITFC/YN Kelt Reconditioning Study (Evans et al. 2001, Anders et al. 2002), providing further replication and scientific evaluation of long-term kelt reconditioning techniques. A detailed description of long-term kelt reconditioning methods can be viewed in Evans et al. (2001) and are only briefly summarized here. Two or more 20-ft circular reconditioning tanks will be used to culture fish throughout the study period. Pre-spawn adult steelhead will receive passive integrated transponder tags (PIT-tags) in the pelvic girdle and be randomly allocated to one of the two reconditioning tanks. Each tank will receive water directly from the Kalama R. (temperatures ranging from 5 to 16 ° C) and tanks will be drained at 340 to 380 L·min⁻¹. Tanks will be covered with nylon mesh to prevent jumping and to expose the fish to the natural photoperiod. Fungal infections will be controlled with formalin drips (ca. 1: 6,000 for 1 hour, as needed), outbreaks of Salmincola spp. with

injections of ivermectin, and other pathological treatments as needed. As has been previously shown, kelts will be fed krill twice daily for an 8-week period and then weaned to a more nutritionally-balanced pellet. Based on the success of these treatments and diet regimes on wild steelhead from the Yakima R., we anticipate an annual rematuration rate (percent of first time spawners that remature to spawn the following year) between 25 and 40% (Evans et al. 2001, Anders et al. 2002). Reproductive maturity will be evaluated based on the ultrasound methods of Evans (2003). Necropsies will be conducted on all steelhead that die during the reconditioning process and the stage of reproductive development noted. To ensure that reconditioning data collected from this study is comparable to on-going studies, data on fish length, weight, and morphological condition will also be recorded and the success of reconditioning based on the proportion of fish that survive, gain weight, and undergo gonad recrudescence.

Genetic Evaluations—A preliminary evaluation of the genetic impacts of increasing iteroparity rates via kelt reconditioning will be needed before reconditioning can be considered a management or research option in the Kalama watershed. The contribution of repeat spawners towards maintaining genetic diversity, influencing gene-flow (both within and among spawning cohorts), and maintaining life history traits is dependent upon numerous factors including: iteroparity rates, probability of inbreeding, domestication selection that propagates maladaptive genes, and effective population size. Evaluation of these factors will likely depend upon the demographics of the population (s) receiving additional repeat spawners (above and beyond the populations current number) and will likely require consideration on a case-by-case basis. Furthermore, an evaluation of the genetic impacts of reconditioning implies that iteroparity is regulated by quantitative traits (s) that can vary from one individual (or population) to the next. Although we recognize the importance of addressing genetic issues – and have incorporated DNA sampling and the collection of life history variables into the studies design – we believe it is imperative to first evaluate the reproductive viability of reconditioned gametes and progeny. A comprehensive assessment of the genetic impacts of reconditioning will require years of replicated study, potentially millions of dollars, and the sampling/handling of nearly 100% of an endangered population. We have not had the time to scope out either the opportunities or the risks involved, and believe an immediate incorporation and execution of such studies in the Kalama may be premature. In the first two years of this proposed Phase I research, we will fully explore those opportunities and risks as part of our contractual agreements with BPA.

Permitting— The bulk of the existing Kalama research and production program is funded by the National Marine Fisheries Service through the Mitchell Act. Steelhead in the Kalama are listed as threatened under the ESA and use of the wild fish in the hatchery program is permitted through use of the Hatchery Genetic Management Plan (HGMP) process. The Kalama research program is fully integrated into the existing steelhead HGMPs in the watershed and extending the use of the wild fish for the Phase I research proposed here will require only minor modifications of the existing documents. More substantial changes may be necessary for conducting the Phase II research but there are provisions in the HGMP process for altering agreements.

Additional Research Endeavors—After completion of Phase I and Phase II of this study, the Kalama system for collecting, spawning, and reconditioning returning adult steelhead will be available to examine other questions such as:

- 1) Comparing the reproductive viability of gametes and progeny from reconditioned spawners (i.e., those retained in a hatchery environment) relative to naturally returning repeat spawners. Because repeat spawners naturally exist in the Kalama R., such a study provides a unique opportunity evaluate artificially augmented iteroparity versus naturally occurring iteroparity.
- 2) Comparing smolt-to-adult returns from progeny generated from virgin spawners and progeny generated from reconditioned spawner.
- 3) Comparing spawning behavior of virgin spawners relative to reconditioned spawners in a semi-natural spawning channel.
- 4) Improve reconditioning protocols by such things as optimizing nutrition and determining the value of saltwater rearing.

Table 1. Phase I schedule of research activities. Following each fiscal year, a research report summarizing activities and results will be submitted to BPA.

Activity	Timeframe (initiation to completion)
Retention of pre-spawners and construction of reconditioning facilities	May 03 to Sept. 03
Viability trails (year 1)	Jan. 04 to May 04
Kelt reconditioning	Jan. 04 to Jan 05
Retention of pre-spawners (year 2 replicate)	May 04 to Sept. 04
Viability trails (year 2)	Jan. 05 to May 05
Kelt reconditioning (year 2 replicate)	Jan. 05 to Jan. 06
Viability trails (year 3)	Jan. 06 to May 06
Data analysis and Final Report	May 06 to Oct. 06
Completion of Phase 1	Oct. 06

Estimated Annual and Total Costs

FY 2003	\$162,335
FY 2004	\$281,593
FY 2005	\$286,566
FY 2006	\$296,238

Grand total \$1,026,732

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- Rurangwa, E., I. Roelants, G. Huyskens, M. Ebrahimi, D.E. Kime, and F. Ollevier. 1998. The minimum effective spermatozoa: egg ration for artificial insemination and the effects of mercury on sperm motility and fertilization ability in *Clarias gariepinus*. Journal of Fish Biology 53: 402-413.
- Sharpe, C.S., P.L Hulett, C.W. Wagemann, and M.J. Johnson. 1999. The Kalama River wild broodstock steelhead program: Comparisons of contemporary and traditional hatchery practices. Proc. 50th Ann. PNW Fish Cult. Conf. 43-52.
- Sharpe, C.S., P.L Hulett, C.W. Wagemann. 2000. Studies of hatchery and wild steelhead in the lower Columbia Basin. Progress Report for FY 1998, WDFW FPA 00-10. 51 pp.
- Sharpe, C.S., B. Beckman, P.L. Hulett. 2001. Residualism in wild broodstock steelhead trout. Annual report to Hatchery Scientific Review Group. IAC Project No. 01-1003N. 11pp.
- Withler I. L. 1966. Variability in life history characteristics of steelhead trout (Salmo Gairdneri) along the Pacific Coast of North America. Journal of the Fisheries Research Board of Canada, 23: 365-393.

Qualifications of Participants

Alec G. Maule, Ph.D.

Maule is a Supervisory Research Physiologist and Section Leader for Physiological Ecology Section of the USGS, WFRC Columbia River Research Laboratory. Maule has conducted research on Columbia River salmon since 1979. His work spans many levels of organization, from the cellular to the ecosystem. Current work focuses on the effects of poor water quality (e.g., gas supersaturation, chemical contaminants) on physiological functioning. In the current project, Maule will be the lead investigator and will oversee all phases of the work and will insure adequate experimental design, execution, analyses and reporting.

Maule's past experience with adult salmon includes an examination of immune-endocrine function during spawning migration and maturation of chinook salmon (Project contact: Dr. Carl Schreck, Department of Fisheries & Wildlife, Oregon State University, 104 Nash Hall, Corvallis, OR 97331. Phone: 541-737-1961, FAX 541-737-3590). Maule was also project leader for an examination of the effects of gas supersaturation on reproductive success of maturing spring chinook salmon. (Project contact: John Skidmore, BPA, Portland, OR; phone 503-230-5494) and was a co-PI on a project to examine possible reproductive dysfunction in fishes in Lake Mead, NV (Project contact: Dr. Steve Goodbred, USGS, Placer Hall, Sacramento State University, Sacramento, CA 95819-6129. Phone: 916-379-3715, FAX: 916-379-3774).

Maule currently serves as Adjunct Associate Professor of Biology at Portland State University and of Fisheries at Oregon State University. Maule has been active in professional activities: Past President, Physiology Section American Fisheries Society; Chair, Organizing Committee, 1996 International Congress on the Biology of Fishes; Past President, Oregon Chapter, American Fisheries Society.

ALEC G. MAULE, Ph.D.

Supervisory Research Physiologist USGS-BRD, Columbia River Research Laboratory. 5501A Cook-Underwood Rd. (509) 538-2299 x 239; (509) 538-2843 (FAX); alec_maule@usgs.gov.

Education:

B.A., University of California, Riverside (Psychology) 1969

B.S., California Polytechnic University, San Luis Obispo (Natural Resource Management) 1979

M.S., Oregon State Univ. (Fisheries Science) 1982

Ph.D., Oregon State Univ. (Fisheries Science) 1989

Other Professional Experience:

Associate Professor of Fisheries (Courtesy), OSU (1991-present)

Adjunct Associate Professor of Biology, Portland State University (1992-present).

Publications (most relevant of 41)

- Maule, A.G., C.B. Schreck, C.S. Bradford, and B.A. Barton. 1988. The physiological effects of the collection and transportation of emigrating juvenile chinook salmon past dams on the Columbia River. Transactions of the American Fisheries Society 117:245-261.
- Maule, A.G., R.A. Tripp, S.L. Kaattari, and C.B. Schreck. 1989. Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*). Journal of Endocrinology 120:135-142.
- Maule, A.G., D. Rondorf, J. Beeman, and P. Haner. 1996. Incidence and severity of *Renibacterium salmoninarum* in spring chinook salmon in the Snake and Columbia rivers. Journal of Aquatic Animal Health 8: 37-46. (Finalist for Best Paper in the journal for 1996).
- Maule, A.G., R. M. Schrock, C. Slater, M. S. Fitzpatrick, and C. B. Schreck. 1996. Immune and endocrine responses of adult spring chinook salmon during freshwater migration and sexual maturation. Fish and Shellfish Immunology 6:221-233.
- Schrock, R.M., S.D. Smith, and <u>A.G. Maule</u>. 2001. Profiles of mucus lysozyme during smoltification of coho salmon and spring chinook salmon. Aquaculture 198:169-177.
- Balfry, S.K., <u>A.G. Maule</u>, and G.K. Iwama. 2001. Coho salmon *Oncorhynchus kisutch* strain differences in disease resistance and non-specific immunity, following immersion challenges with *Vibrio anguillarum*. Diseases of Aquatic Organisms 47:39-48.
- Jørgensen, E.E., M.M. Vijayan, N. Aluru, and <u>A. G. Maule</u>. 2002. Fasting modifies Aroclor 1254 impact on plasma cortisol, glucose and lactate responses to a handling disturbance in Arctic charr. Comparative Biochemistry and Physiology, Part C 132: 235-245.
- Gale, W.L., <u>A.G. Maule</u>, A. Postera, and M Peters. In review. Acute exposure to gas supersaturated water does not affect reproductive success of female adult chinook salmon late in maturation. Regulated Rivers.

Current Projects:

- Gas bubble disease in resident fish below Grand Coulee Dam. Funded by USGS and Bureau of Reclamation. Dates: FY99 FY03.
- Gas bubble disease and monitoring of juvenile salmonids. Funded by BPA. Dates: April 96 to March 04.
- Endocrine disrupting chemicals and reproduction in white sturgeon. Collaborative study with Martin Fitzpatrick, Oregon State University and Eugene Foster, Oregon Department of Environmental Quality. Funding agency: EPA/USGS. Dates: July 99 to March 03.
- Xenobiotic impact on Arctic char: Nutritional modulation and physiological consequences. Collaborative study with Mathilakath Vijayan, University of Waterloo, Canada and Even Jørgensen, Norwegian Institute of Nature Research. Funding agency: National Science Foundation. Dates: Jan 00 to Dec 03.
- Water quality and viable salmon populations: A GIS-based recovery planning tool. Funding agency: NMFS. Dates: June 2001 April 2004.
- Sex reversal in Hanford Reach fall chinook salmon. Funding (Pending): BPA.

Professional Service:

Associate Editor for the Journal of Aquatic Animal Health 1997-present;

American Fisheries Society

Fish Health Section, Snieszko Graduate Award Committee (Chair) 1989-91;

Physiology Section (Charter member) - Vice Pres., Pres.-elect, Pres., Past-Pres. 1993-97; Awards Committee (Chair) 1997-98;

Oregon Chapter- AFS Legislative Committee 1983-84; AFS Oregon Annual Meeting, Program Committee 1985-93;

Director of Internal Committees 1989-90;

Pres.-elect/Pres./Past Pres. 1990-93.

Regional or National Committees:

NMFS' Willamette and Lower Columbia Rivers Technical Recovery Team (Apr 2000-Nov 2002

Water Quality Team (Dissolved Gas Team)

Grand Coulee Dam Dissolved Gas Committee (Chair)

USGS Endocrine Disrupting Chemical Work Group (ad hoc).

Matthew G. Mesa, Ph.D.

Ph.D., 1999, Fisheries Science, Oregon State University

Dr. Mesa will be responsible for overseeing and participating in the collection of gametes and the successful completion of fertilization trials. He will also assist in data analyses and report preparation. Mesa is a Research Fisheries Biologist within the Physiological Ecology Section at the Columbia River Research Lab. He has conducted research on Columbia River fishes since 1984, covering physiological, organismal, and ecosystem levels of organization. His current work focuses on evaluating energy expenditure in upstream migrating adult salmon, documenting sex steroid and other physiological profiles of Pacific lampreys, and assessing swimming performance and exhaustive stress in bull trout. He is currently an associate editor for *Transactions of the American Fisheries Society*.

Representative Publications:

- Mesa, M. G., T. P. Poe, D. M. Gadomski, and J. H. Petersen. 1994. Are all prey created equal? A review and synthesis of differential predation on prey in substandard condition. Journal of Fish Biology 45 (supplement A):81-96.
- Mesa, M. G., A. G. Maule, T. P. Poe, and C. B. Schreck. 1999. Influence of bacterial kidney disease on smoltification in salmonids: is it a case of double jeopardy? Aquaculture 174:25-41.
- Mesa, M. G., J. M. Bayer, and J. G. Seelye. *In press*. Swimming performance and physiological responses to exhaustive exercise in radio-tagged and untagged Pacific lampreys. Transactions of the American Fisheries Society 132.

Cameron S. Sharpe

Cameron Sharpe is a Fish Research Biologist with the Washington Department of Fish and Wildlife, Fish Program, Science Division, Hatchery/Wild Interactions Unit, Kalama Research Team. He received his Master's degree from Oregon State University in 1992, worked at Oregon State University as a Faculty Research Assistant until 1995 when he accepted his current position with WDFW. His primary area of expertise is fish genetics, especially population genetics and genetic effects of hatchery steelhead trout on wild populations. Other recent research interests include examination of stress physiology, behavior and physiology of wild salmonid juveniles, and life history variation in hatchery and wild steelhead.

The proposal requires individuals with expertise in steelhead genetics and development of novel hatchery protocols. Mr. Sharpe's primary responsibilities with WDFW in the Kalama Research Program are to design and conduct experiments to evaluate genetic and ecological interactions between hatchery and wild salmonids, especially steelhead, examine temporal and spatial genetic variation among steelhead populations, and develop new and innovative hatchery protocols to reduce risks posed by hatchery programs on wild populations. Other responsibilities include creation and maintenance of a long-term (20 yr) genetic and life history relational database, preparation of reports, professional presentations, and journal articles.

Past and ongoing collaborators for work cited in the proposal include: Dr. Brian Beckman: NMFS Research Biologist, 2725 Montlake Blvd E., Seattle WA 98112 ph:206.860.3461 fax:206.860.3467 email: brian.beckman@noaa.gov

Dr. Walton Dickhoff: UW/NMFS Program Manager/Professor, 2725 Montlake Blvd E., Seattle WA 98112 ph:206.860.3234 fax:206.860.3467 email: dickhoff@noaa.gov

Dr. Patrick Connolly: USGS-BRD Research Fisheries Biologist, CRRL 5501-A Cook-Underwood Rd, Cook, WA 98605 ph:509.538.2299 X269 fax:509.538.2843 email: Patrick_Connolly@usgs.gov

Mr. Howard Fuss: WDFW Research Scientist, 600 Capitol Way N., Olympia WA 98501 ph:360.902.2664 fax:360.902.2944 email: fusshjf@dfw.wa.gov

The later stages of the work in the proposal will require significant collaboration with the WDFW fish Genetics Unit staff in the Conservation Biology unit. Useful contacts in that respect would be:

Dr. Craig Busack: WDFW Research Scientist, 600 Capitol Way N., Olympia WA 98501 ph:360.902.2765 fax:360.902.2944 email: busaccsb@dfw.wa.gov

Dr Jim Shacklee: : WDFW Research Scientist, 600 Capitol Way N., Olympia WA 98501 ph:360.902.2752 fax:360.902.2944 email: shakljbs@dfw.wa.gov

Mr. Sharpe himself can be reached at WDFW/Kalama Research Team, 804 Allen St. #3 Kelso WA 98626 ph:360.577.0197 fax:360.577.0387 email: sharpess@dfw.wa.gov

Experience

1995-Present Fish Research Biologist III. Washington Department of Fish and Wildlife. Fish Program/Science Division/Kalama Research Team. Kelso, WA.

<u>Current responsibilities:</u> Design and conduct experiments to evaluate genetic interactions between hatchery and wild salmonids and examine temporal and spatial genetic variation among steelhead populations. Create and maintain long term (20 yr) genetic and life history relational database. Prepare reports, professional presentations, journal articles.

1992-1995 Faculty Research Assistant, OCFRU, Oregon State University, Corvallis.
1988-1992 Graduate Research Assistant, OCFRU, Oregon State University, Corvallis.
1985-1988 Research Assistant, OCFRU, Oregon State University, Corvallis.

Education

<u>School</u> <u>Degree</u>

Oregon State University, Corvallis M.S. Fisheries Science, 1992

University of Oregon, Eugene B.S. Biology, 1985

Expertise

Primary areas of expertise include fish genetics, especially population genetics and an examination of genetic effects of hatchery fish on wild populations using microsatellite-DNA based analyses. Other recent research interests include developing protocols for hatchery reform and use of wild fish in hatchery programs.

Publications and Reports

- Connolly, P.J., C.S. Sharpe and S. Sauter. 2002. Evaluate status of coastal cutthroat trout in the Columbia River basin above Bonneville Dam. Report to BPA. Project No. 2001-007-00. 22 pp.
- Sharpe, C.S., B. Beckman, P.L. Hulett. 2001. Residualism in wild broodstock steelhead trout. Annual report to Hatchery Scientific Review Group. IAC Project No. 01-1003N. 11pp.
- Fuss, H.J., C.S. Sharpe, P. Hulett, M. Ford, J. Hard and B. Berejikian. 2001. Differences in natural production between hatchery and wild coho salmon in Minter Creek, Washington. Annual Report to the Hatchery Science Review Group.
- Sharpe, C.S., P.L Hulett, C.W. Wagemann. 2000. Studies of hatchery and wild steelhead in the lower Columbia Basin. Progress Report for FY 1998, WDFW FPA 00-10. 51 pp.
- Beckman, B.R., Larsen, D. A., Sharpe, C., Lee-Pawlak, B., and W.W. Dickhoff. 2000. Physiological status of naturally-rearing juvenile chinook salmon in the Yakima River:

- seasonal dynamics and changes associated with the parr-smolt transformation. Transactions of the American Fisheries Society. 129:727-753.
- Sharpe, C.S., P.L Hulett, C.W. Wagemann, and M.J. Johnson. 1999. The Kalama River wild broodstock steelhead program: Comparisons of contemporary and traditional hatchery practices. Proc. 50th Ann. PNW Fish Cult. Conf. 43-52.
- Beckman, B.R., Dickhoff, W.W., Zaugg, W.S., Sharpe, C., Hirtzel, S., Schrock, R., Larsen, D.A., Ewing, R.D., Palmisano, A., Schreck, C.B., and Mahnken C.V.W. 1999. Growth, smoltification, and smolt-to-adult return of spring chinook salmon (Oncorhynchus tshawytscha) from hatcheries on the Deschutes River, Oregon. Transactions of the American Fisheries Society 128:1125-1150.
- Sharpe, C.S., D.A. Thompson, H.L. Blankenship, C.B. Schreck. 1998. Effects of routine handling and tagging procedures on physiological stress responses in juvenile chinook salmon. Prog. Fish-Cult. 60:81-87.
- McMichael, G.A., C.S. Sharpe, and T.N. Pearsons. 1997. Effects of hatchery-reared steelhead on growth of wild rainbow trout and spring chinook salmon. Trans. Amer. Fish. Soc. 126:230-239.
- Hulett, P.L., C.W. Wagemann, C.S. Sharpe, and S.L. Leider. 1995. Studies of hatchery and wild steelhead in the lower Columbia Basin. Annual Report to WDFW. RAD 95-03. 44 pp.
- Sharpe, C.B. Schreck, W.W. Dickhoff. 1994. Smoltification strategies in wild spring chinook salmon: Implications for aquaculture. Proceedings of the International High Performance Fish Symposium. Vancouver, B.C. Canada. p. 68-70.
- Maule, A.G., C.B. Schreck and C.S. Sharpe. 1993. Seasonal changes in cortisol sensitivity and glucocorticoid receptor affinity and number of leukocytes of coho salmon. Fish Phys. and Biochem. 10:497-506.
- Currens, K.P., C.S. Sharpe, R. Hjort, C.B. Schreck and H.W. Li. 1989. Effects of different feeding regimes on the morphometrics of chinook salmon (*Oncorhynchus tshawytscha*) and Rainbow trout (*O. mykiss*). Copeia (1989)3:689-695.
- Schreck C.B., H.W. Li, R. Hjort, C.S. Sharpe. 1986. Stock Identification of Columbia River chinook salmon and steelhead trout. Completion Report to BPA. Project 83-451. 184 pp.

Patrick L. Hulett

Patrick Hulett is the Project Leader for the Kalama Research Team, located in Kelso, Washington, within the Fish Program / Science Division of the Washington Department of Fish and Wildlife. Under Pat's direction since 1991, the Kalama Research Team has been conducting studies with major emphasis in areas of natural reproductive success, population genetics, hatchery-wild interactions, life history and population dynamics of salmonids, primarily on steelhead. Through most of Pat's career (1986-present), he has been a principal researcher on studies of the relative natural reproductive success of hatchery and wild steelhead in the Kalama River. Recent work has focused on efficacy, benefits, and risks of the use of wild steelhead as broodstock for hatchery production programs. That work (ongoing) involves extensive evaluation of performance traits in the hatchery, as migrant smolts, and as returning adults, including their reproductive success spawning in the wild. Importantly, that work has (since 1998), and will continue to involve the holding and non-lethal spawning of wild steelhead at Kalama River hatchery facilities to create and evaluate steelhead wild broodstock hatchery programs (summer-run and winter-run).

Patrick's responsibility in the project proposed in this response will center on all aspects of carrying out the successful holding, spawning, and kelt reconditioning of the steelhead in this proposal. Additionally, Pat would have a major role in the reproductive success evaluations planned for the second phase of this proposed work. These roles match very well with Pat's experience and expertise, and dovetail efficiently with the ongoing reproductive success and hatchery evaluations ongoing in the Kalama basin. Additionally, the long-term data base of steelhead life history and genetic profiles that the Kalama Research team has generated (since 1976) will be invaluable to the success of the proposed project.

Contact information on past and ongoing collaborators relevant to this project (e.g., Howard Fuss, Craig Busack, Jim Shaklee) are identical to those listed by my colleague, Cameron Sharpe.

Partrick Hulett can be reached at WDFW/Kalama Research Team, 804 Allen St., #3, Kelso WA 98626. phone: 360-577-0197, fax: 360-577-0387, e-mail: huletplh@dfw.wa.gov

Experience

1986-Present Fish Research Project Leader. Washington Department of Fish and Wildlife. Fish Program, Science Division, Kalama Research Team. Kelso, WA.

<u>Current responsibilities:</u> Principle researcher and project leader for Kalama Research Team, conducting studies in areas of fish genetics, reproductive success, hatchery-wild interactions, life history and population dynamics: develop and implement experimental designs, supervise project staff, develop and administer budget, prepare reports, journal articles, and oral presentations, coordinate research activities with appropriate parties within and outside WDFW, provide input and consultation to agency staff on fish genetics and hatchery-wild interaction issues.

1983-1986 Graduate Research Assistant, OCFRU, Oregon State University, Corvallis. 1981-1983 Research Assistant, OCFRU, Oregon State University, Corvallis.

Education

<u>School</u> <u>Degree</u>

Oregon State University, Corvallis M.S. Fisheries Science, 1991 Oregon State University, Corvallis B.S. Fisheries Science, 1981

Expertise

Primary areas of expertise include salmonid population genetics, especially genetic interactions and reproductive success of hatchery and wild fish, as well as life history attributes and ecological interactions of hatchery and wild steelhead. Recent work has focused on efficacy, benefits, and risks of the use of wild steelhead as broodstock for hatchery production programs. That work involves extensive evaluation of performance traits in the hatchery, as migrant smolts, and as returning adults, including their reproductive success spawning in the wild.

Publications and Reports

- Hulett, P.L., C.S. Sharpe, and C.W. Wagemann. 2002. Review of Hatchery Programs Using Wild Steelhead Broodstocks in Washington. In: Summary of the Eighth Pacific Coast Steelhead Management Meeting, Corbett, OR. Pp. 10-16.
- Sharpe, C.S., P.L. Hulett, and C.W. Wagemann. 2000. Studies of hatchery and wild steelhead in the lower Columbia region. Progress report for fiscal year 1998. Wash. Dept. Fish and Wildlife, Fish Prog. Rep. FPA 00-10. 38 pp + appendices.
- Hulett. P.L., C.S. Sharpe, and C.W. Wagemann. 1998. Evaluations of broodstock performance including natural reproductive success for non-local and local wild broodstock hatchery steelhead stocks in the Kalama River, Washington. In: Proceedings of the 49th Annual Pacific Northwest Fish Culture Conference, Boise, ID. pp. 125-130.
- Phelps, S.R., S.A. Leider, P.L. Hulett, B.M. Baker, and T.H. Johnson. 1997. Genetic analyses of Washington steelhead: preliminary results incorporating 36 new collections from 1995 and 1996. Wash. Dept. Fish and Wildlife, Fish Mgmt. Program Unnumbered Progress Report. 29pp. + figures.
- Hulett, P.L., C.W. Wagemann, and S.A. Leider. 1996. Studies of hatchery and wild steelhead in the lower Columbia region. Progress report for fiscal year 1995. Wash. Dept. Fish and Wildlife, Fish Mgmt. Program. Report RAD 96-01. 22pp.
- Bradford, R.H., S.A. Leider, P.L. Hulett, and C.W. Wagemann. 1996. Differential leaping success by adult summer and winter steelhead at Kalama Falls: implications for estimation of steelhead spawner escapement. Wash. Dept. Fish and Wildlife, Fish Mgmt. Program Report RAD 96-02. 56pp.
- Leider, S.A., P.L. Hulett, and T.H. Johnson. 1994. Preliminary assessment of genetic conservation management units for Washington steelhead: implications for WDFW's

draft steelhead management plan and the federal ESA. Wash. Dept. Fish and Wildlife, Fish. Mgmt. Program. Report 94-15. 42pp.

Hulett, P. L. and S. A. Leider. 1993. Genetic conservation of wild steelhead in Washington streams: a genetically-based conservation and management model to integrate hatchery and wild production. Wash. Dept. Wildlife, Fish. Mgmt. Division. Report 93-17. 58pp.

Leider, S.A., P.L. Hulett, J.J. Loch, and M.W. Chilcote. 1990. Electrophoretic comparison of the reproductive success of naturally spawning transplanted and wild steelhead trout through the returning adult stage. Aquaculture 88:239-252.

Allen F. Evans, Fisheries Scientist

aevans@realtimeresearch.org

Education:

B.A.. Biology. The College of Wooster. 1995

M.S.. Fisheries Science. Oregon State University. Commencement June 2003

Work Experience:

Fisheries Scientist, Real Time Research. 2003 - Current

Fisheries Scientist, Columbia River Inter-Tribal Fish Commission. 2000 to 2002.

Fisheries Biologist, Columbia River Inter-Tribal Fish Commission. 1996 to 2000.

Fishery Technician, Biological Resources Division. 1995

Fisheries Researcher, J.L.B. Smith Institute of Ichthyology. 1994

Relevant Professional Experience:

Allen has been working with both juvenile and adult salmonid species for the last 10 years and is currently a researcher with Real Time Research (RTR), an environmental consulting firm located in Bend, OR. Allen helped pioneer and implement much of the kelt related research that is currently being funding in the Columbia River basin (see Relevant Publications below), including BPA's funded kelt reconditioning project and the U.S. Army Corps funded kelt research at Lower Granite Dam. Allen's experience includes a thorough understanding (and contribution) to the kelt literature, expertise in kelt identification and maturation assessment techniques (via ultrasound imaging, determining reproductive hormones via radioimmunoassay procedures, and gonad histology), knowledge of kelt reconditioning methods, and experience with rearing *O. mykiss* embryos and fry. As a Fisheries Scientist, Allen's duties include grant writing, study design, statistical analysis, report/publication writing, scientific presentations, and managing/training staff for field research endeavors.

Some Relevant Publications:

Evans, A.F. 2003. Development and application of steelhead (*Oncorhynchus mykiss*) kelt identification techniques. MS Thesis. Oregon State University. Corvallis, OR.

- Evans, A.F. 2002. Steelhead (*Oncorhynchus mykiss*) kelt outmigration from Lower Granite Dam to Bonneville Dam: Abundance, downstream conversion rates, routes of passage, and travel times. Ann. Rep. To US Army Corps of Engineers, Walla Walla District, for Contract No. DACW68-02-M-3102.
- T.W. Backman and A.F. Evans. 2002. Gas Bubble Trauma Incidence in Adult Salmonids in the Columbia River Basin. Journal of North American Fisheries Management Vol.22 (2) pp.579-584.
- Evans, A.F., R.E. Beaty, and D.R. Hatch. 2001. Kelt Reconditioning: A Research Project to Enhance Iteroparity in Columbia Basin Steelhead (*Oncorhynchus mykiss*). Annual Project 2000-017 Report to U.S. Department of Energy, Bonneville Power Administration Division of Fish and Wildlife, Portland, OR.
- Evans, A.F. and R.E. Beaty 2001. Identification and Enumeration of Steelhead (*Oncorhynchus mykiss*) Kelts in the Juvenile Collection Systems of Lower Granite and Little Goose dams, 2000. To US Army Corps of Engineers, Walla Walla District, for Contract No. DACW68-99-M-3102.
- Davies M.T, and A.F. Evans. 1996. The possible significance of egg size on post-hatched growth of rainbow trout (*Oncorhynchus mykiss*). Proceedings of the Aquaculture Association of Africa. No 5. Page(s) 137-143. 1996