

## Attachment A

### Umpqua Coho Pedigree Study: ODFW Component

Proposal to: Oregon Watershed Enhancement Board  
775 Summer Street NE, Ste 360  
Salem OR 97301-1290

Submitted by: Jim Muck  
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Umpqua Fish District  
Oregon Department of Fish and Wildlife  
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Total amount requested: \$103,387  
Proposed duration: August 1, 2006 to June 30, 2007 (16 months)  
Desired starting date: August 1, 2006

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- I. Project Title:** Conservation Hatchery Implementation Program: ODFW Component for Nonpareil Dam Adult Trap and Coho Salmon Genetic Pedigree Project
- II. Contact:** Jim Muck, Oregon Department of Fish and Wildlife  
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### III. Project Description

This ongoing project is investigating several areas of uncertainty about the use of hatchery fish to increase the abundance of wild populations. There is interest in using hatcheries to speed the recovery of wild populations but the value of such programs is untested and may pose risk to wild populations. New molecular genetics methods now allow us to explore the critical questions and risks associated with hatchery programs. DNA fingerprints can now be utilized to pedigree entire populations under some circumstances and develop lineages that continue for multiple generations under natural and hatchery supplemented spawning conditions. We are now able to produce direct evidence of the success or failure of hatchery supplementation programs and provide direct measurements of some of the risks predicted by genetic theory. We propose to continue our ongoing research and experimental supplementation program on the coho salmon in the Calapooya River, a tributary of the Umpqua River on the Oregon Coast. OSU is conducting the genetic analysis to evaluate the associated risk from these hatchery introductions (see OSU component of the grant application.)

This Conservation Hatchery Implementation Program study is the ongoing project to evaluate the supplementation project for coho salmon in the Calapooya Creek (Umpqua Basin) using the following hatchery scenarios: 1) hatchery stock for released smolts, 2) hatchery stock released as unfed fry, 3) first generation wild type hatchery stock released as smolts, and 4) first generation wild type hatchery stock released as unfed fry (see Table 1.) This project started in 2001 and will continue to 2009 to follow three complete generations of coho salmon replicated within three consecutive years. The Calapooya Coho Salmon Genetic Pedigree Project is a cooperative research project with Oregon State University and the Oregon Department of Fish and Wildlife.

### IV. Initial Achievements

The supplementation releases were completed in 2005 as follows:

<u>Year</u>	<u>Fry Releases</u>	<u>Smolt Releases</u>
2002	370,000	
2003	492,000	24,000
2004	456,000	22,000
2005		20,000

The supplementation design is shown in Table 1.

The Department of Fish and Wildlife has operated the adult trap on the Calapooya River since fall/winter 2002-03. We trapped 1,091 adult coho salmon in 2002-03, 825 adult coho salmon in 2003-04, 1,311 adult coho salmon in 2004-05, and a 1,415 adult coho salmon through December 12, 2005.

DNA samples were collected from all brood fish utilized for the supplementation program. Beginning in 2003-04, we took DNA samples from all jack coho salmon. These fish were the first to return from the supplementation program. In fall/winter 2004-05 and fall/winter 2005-06, all returning coho salmon (jacks and adults) were or will be sampled for DNA.

**Table 1**  
**Supplementation Program design for the CHIP program on the Calapooya Basin.**

Year	Broodstock captured at Winchester		Broodstock captured at Nonpariel (All wild)	Unfed fry releases (unmarked)	Smolt releases (2 mark groups)	Spawners sampled and passed at Nonpariel Dam*		
	Hatchery (Rock Cr stock)	Wild				Unmarked		Hatchery fish
						wild	hatchery	
2001	200	200						
2002	200		200	400,000		400		
2003	200		200	400,000	20,000	400	5	30
2004				400,000	20,000	400	75	350
2005					20,000	400	75	350
2006						400	70	320
2007						600		
2008						600		
2009						600		
2010								

**Parent Generation**

**Supplemented Generation**

**Offspring Generation**

**V. Future Objectives**

The Department is continuing the collection of DNA samples on all returning coho salmon at the Nonpareil site until the final F2 generation returns in fall/winter 2009-10. The initial samples for early coho salmon returns have been tested by OSU and they have successfully demonstrated the ability to pedigree the entire population using molecular markers (See OSU component of the grant application.) The pedigree analysis of the F1 generation will be completed with the final returns in 2006-07. This will give us three complete years of return data to develop correlations and evaluate hypotheses from the first F1 generation. The F2 generation fish will be returning in run years 2007-08, 2008-09 and 2009-10. The F2 generation will determine the survival of hatchery and wild mating combinations from the spawning grounds. This too will be evaluated from adult to adult survival conversions.

Substantial numbers of hatchery fish spawning naturally in a wild population, may in theory pose five major genetic risks to wild populations. These risks are as follows:

- Risk 1) Population Bottleneck: This risk occurs when a small number of parents (those taken into a hatchery) produce a substantial proportion of the fish in the supplementation population (those left in the wild). Since they share so few parents, the hatchery fish in

the supplementation population are more likely to be related to each other, thus increasing the incident of inbreeding.

- Risk 2) Increased Inbreeding: This risk occurs when only a small number of parents (those taken into the hatchery) produce a substantial proportion of the fish in the supplemented population. Since they share so few parents, the hatchery fish in the supplemented population are more likely to be related to each other, thus increasing the incidence of inbreeding.
- Risk 3) Increased Genetic Load: This risk results from the increased reproductive success and survival that occurs while fish are in the captive environment. Increase reproductive success and survival in captivity occurs because natural selection pressures are intensely relaxed which leads to an increase in the level of genetic load.
- Risk 4) Genetic Variation is Lost: When offspring population is smaller than its parent population genetic variation is lost. This is due to reproductive failure by some parents and the loss of the genetic material they carry. Additional random loss of genetic variation may occur when populations are very small.
- Risk 5) Accumulative Genetic Variation: If the hatchery program continues over multiple generations the impacts of the risks will accumulate in the wild populations due to the nature of the genetic mechanisms involved.

OSU will continue to evaluate genetic data from returning F1 and F2 generation coho salmon captured from the Nonpareil Adult Trap. ODFW and OSU will evaluate each of the five genetic risks.

## **VI. Conclusions**

The Department has supplemented the Calapooya River with four groups of hatchery fish for three consecutive years to evaluate the use of hatchery fish as a conservation tool for fish recovery. These hatchery fish were developed from wild X wild brood and hatchery X hatchery brood with both groups released as smolt and fry. We are now trapping the returning F1 generation fish from these releases. The initial samples from these F1 coho salmon returns have been tested by OSU and they have successfully demonstrated the ability to pedigree the entire population using molecular markers. Completing the field samples of the final F1 generation, and the following three run years to obtain the F2 generation will help develop conclusions about how hatchery and wild interactions occur. The return data will also be useful to evaluate the success or failure of different hatchery release strategies (such as fry releases), most often utilized by Salmon Trout Enhancement Program (STEP) volunteers.

## **VII. Requested Budget Proposal for 2005-2007**

The hatchery releases from 2001 to 2003 were conducted by the Oregon Department of Fish and Wildlife and paid for by the Oregon Wildlife Heritage Foundation. ODFW costs for the field collection proportion of the CHIP project were as follows: \$51,150 (OWEB 204-910) for

fiscal year 2003-2004; and a \$78,850 addendum (OWEB 204-910) for the current 2004-2005 fiscal year.

The Department has utilized the remaining addendum grant (OWEB 204-910) from 2004-2005 to operate the adult trap in fall 2005. The Department is requesting \$103,387 to complete the field sampling for the rest of the biennium (March 2006 – June 2007). The initial field operations were limited to brood collections. The program has now expanded to the collection of DNA samples from all returning fish. This increase in workload requires the Department to request funding for additional staff to assist with the field studies portion of the work.

## **Budget Justification**

### **1) Personnel**

Field personnel are needed to collect DNA samples at the Nonpareil Dam adult trap operated from October through January each year for returning coho salmon. Field staff needed for DNA collections includes four Experimental Biological Aides (EBA's) for the months of October 2006 through January 2007. The cost of an EBA is \$2,994 a month for a Project Cost of \$47,896 for 16 EBA months. The Natural Resource Specialist 2 (NRS 2) is required for reports, project leadership, data collection, and coordination with OSU staff. The NRS 2 salary is \$4,561 a month (6 months total) for a project cost of \$27,365. The personnel cost for the total project is \$75,261 (see Project Budget Form).

### **3) Materials and Supplies**

The field collection cost required for the project includes the following:

- Two vehicles for travel from the office and trapping locations. One vehicle is required from October 2006 to January 2007 (4 months), and the other vehicle is required from October 2006 to March 2007 (6 months) totaling 10 months. The cost per month for vehicles is estimated at \$600 a month for a project cost of \$6,000.
- Field supplies required for the project include waders, boots, floy tags and dispensing guns, vials, preservatives, dam boards, raingear, gloves, storage containers, shipping cost, nets, and other miscellaneous equipment. These supplies are estimated at \$10,000 for the project.

## **VIII. Time Line and Returnable**

The time line and returnable portion of this grant has not changed from ODFW's original CHIP proposal. Please refer to the ODFW Component for Nonpareil Dam Adult Trap and Genetic Pedigree grant for years 2004-2007.

**Umpqua Coho Pedigree Study:  
OSU Component**

Proposal to: Oregon Watershed Enhancement Board  
775 Summer Street NE, Ste 366  
Salem OR 97301-1290

Submitted by: Dr. Gregory R. Moyer  
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Total amount requested: \$143,948  
Proposed duration: One year  
Desired starting date: July 1, 2006

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**I. Project Title:** Conservation Hatchery Implementation Program: OSU Component for Nonpareil Dam Adult Trap and Genetic Pedigree

**II. Contact:** Dr. Gregory R. Moyer, Oregon State University  
Dr. Michael Banks, Oregon State University

### III. Project Description

We have investigated several areas of uncertainty about the use of hatcheries for increasing abundance of wild populations. There is considerable interest in using hatcheries to speed the recovery of wild populations; however, substantial literature indicates hatchery programs may pose risks to wild populations. If risks are apparent, supplemented hatchery fish may impede further recovery of endangered Oregon coast coho salmon populations. We employ genetic methods to explore critical questions and risks associated with hatchery programs. New molecular methods allow us to use DNA “fingerprints” to temporally track the fate (survival) of hatchery supplemented and naturally reproducing coho salmon. In doing so, we can produce direct evidence for successes or failures of hatchery supplementation programs, and provide direct measurements of some of the risks predicted by population genetic theory.

### IV. Initial Achievements

**DNA EXTRACTION.** Tissue samples ( $n = 1,200$ ) were obtained from ODFW (Roseburg Office) in October 2004. These samples represent 100 hatchery  $\times$  hatchery (H  $\times$  H) and 100 wild  $\times$  wild (W  $\times$  W) paired matings for three years (2001-2003). Tissue samples from the first return year of progeny spawned in 2001 ( $n = 1,177$  adults, 156 jacks, and 11 strays) were acquired from ODFW in March 2005. DNA was extracted from all 2,544 tissue samples obtained to date.

**CHOICE OF LOCI.** We screened 96 candidate loci known to amplify in salmon species. Of these loci, we successfully amplified 41 candidates for further evaluation. Due to inconsistent scoring, presence of null alleles and deviation from Hardy Weinberg expectations, 27 loci were eliminated. The remaining 14 were used to genotype the 2001 hatchery and wild broodstock ( $n = 384$ ). Genotype data were then subjected to simulations to address how many of loci are needed to obtain 95 and 99% accuracy for parentage analysis. Results indicate that at  $\alpha = 0.05$ , eight loci can accurately assign progeny to parental arrays, and at  $\alpha = 0.01$ , 12 loci are necessary for accurate parentage analysis.

**PARENTAGE ANALYSIS.** We received  $n = 1,344$  returning Calapooya River coho (1,177 adults, 156 jacks, and 11 strays), and successfully genotyped these fish for 10 microsatellite loci. Returning H  $\times$  H ( $n = 235$ ,) and W  $\times$  W ( $n = 163$ ) coho were distinguished by adipose and maxillary fin clips and subsequently confirmed via genetic parentage analysis. The remaining fish were assigned as naturally reproducing coho or unfed fry, via parentage analysis (i.e., matching 2001 brood genotypes to 2004 returns). Parentage analysis confirmed that  $n = 775$  were naturally reproducing coho,  $n = 27$  were H  $\times$  H unfed fry, and  $n = 24$  were W  $\times$  W unfed fry. Our verification of substantial returns from naturally reproducing coho demonstrates the importance of monitoring of hatchery  $\times$  wild introgression in subsequent years (see below).

**ANALYSIS OF REPRODUCTIVE SUCCESS.** The first phase of this project was to determine the relative reproductive success of H  $\times$  H and W  $\times$  W matings. Our null hypothesis states that there is no difference in reproductive success between H  $\times$  H and W  $\times$  W adults. Our findings indicate that reproductive success of H  $\times$  H and W  $\times$  W coho (reared until smolt release) was ca. 1.9 and 1.3%, respectively. This difference in reproductive success was significant ( $P = 0.0001$ ). In contrast, reproductive success of H  $\times$  H (0.007%) and W  $\times$  W (0.006%) coho released as unfed fry was not significant ( $P = 0.33$ ). The difference in reproductive success between H  $\times$  H and W  $\times$  W jacks ( $n = 39$  and 26, respectively) was also nonsignificant ( $P = 0.26$ ). These findings

indicate that reproductive success of hatchery and wild smolts is different, but whether these findings are consistent through time remains uncertain. Only after the 2005 and 2006 returns are analyzed will we have an adequate understanding of the temporal variance in reproductive success between hatchery and wild supplemented coho.

**POPULATION BOTTLENECK AND INBREEDING.** Hatchery supplementation programs should strive to minimize loss of genetic diversity between hatchery and naturally reproducing stocks. In accordance, ODFW exercised considerable effort to minimize any loss in genetic variation (due to bottlenecks or inbreeding) by randomly sampling hatchery fish for brood and by performing 1:1 paired matings. To evaluate the effectiveness of this strategy we assessed whether genotypic distributions of hatchery and wild progeny differed from the parental hatchery and wild broodstock. We found no differences ( $P = 0.97$ ) in genotypic distributions of hatchery progeny vs. hatchery brood as well as no differences ( $P = 0.58$ ) in wild progeny vs. wild brood comparison.

A quantitative assessment of the amount of inbreeding can not be obtained until we can determine the number of successful 2004 spawners (i.e., not until the 2007 returns).

**POPULATION GENETIC ANALYSIS.** We also addressed whether differences in allele frequencies exist between naturally reproducing and hatchery returns. Our null hypothesis was that the allelic distribution is identical between  $H \times H$  (supplemented) and naturally reproducing Calapooya coho. We tested for genotypic differentiation using eight microsatellite loci that conformed to Hardy-Weinberg expectations. We found that  $H \times H$  and naturally reproducing populations are significantly different ( $\chi^2 = 42.14, P = 0.001$ ).

These findings indicate that ODFW's mating strategy is preserving genetic variation between progeny and broodstock; thus eliminating the potential risk of loss of genetic variation or inbreeding from a bottleneck in genetic diversity. Although the mating strategy has preserved the genetic variation for a generation, the difference in allele frequencies among hatchery and naturally reproducing Calapooya coho still create a genetic risk to the population via outbreeding depression (see below). To assess the influence of outbreeding depression requires implementing the next phase of CHIP – monitoring 2007-2009 returns.

## **V. Future Objectives**

We have successfully demonstrated the ability to pedigree an entire population using molecular markers. This success will greatly increase our knowledge of hatchery supplementation programs by 1) evaluating differences in temporal estimates of reproductive success between hatchery and naturally reproducing coho, and 2) assessing major genetic risks to wild populations. Although ODFW has exercised considerable effort to minimize any loss in genetic variation, outbreeding depression and a decrease in effective population size are, nonetheless, two genetic risks that a hatchery supplementation program may incur. These risks are difficult to assess empirically; however, the CHIP project has a unique position to monitor these long term risks by estimating the reproductive success of 2007-2009 returns.

**OUTBREEDING DEPRESSION.** Although hatchery fish stocked into the Calapooya are from Rock Creek Hatchery (next drainage), we have demonstrated that significant allelic differences exist between fish of these basins. Population genetic theory predicts that mixing coadaptive gene complexes (i.e., wild × hatchery hybrids) could result in decreased survival of hybrids and ultimately hinder wild stock recruitment. Continuation of the CHIP project will help identify potential impacts of outbreeding depression when hatchery stocks are allowed to spawn with naturally reproducing coho. Assessing the consequence of outbreeding depression will be achievable when coho return to the Calapooya in 2007-2008.

**EFFECTIVE POPULATION SIZE AND THE RYMAN-LAIKRE EFFECT.** Supportive breeding typically results in a trade off -- there is a gain in the total production of offspring but there could be a reduction in effective population size ( $N_e$ ) of the supplemented population due to the excess loss of genetic variability. The reduction of  $N_e$  due to supplementation (i.e., Ryman-Laikre effect) is caused by a greater relative contribution of hatchery than naturally reproducing fish. In order to measure the relative contribution, hatchery fish must be taken from the same population as the naturally reproducing population. Although the CHIP project supplemented North Umpqua hatchery fish in Calapooya Creek in 2001 (i.e., we could not measure the effects of the Ryman-Laikre effect), in 2002 and 2003 the CHIP project sampled both wild and hatchery coho from the Calapooya and repatriated these fish in the Calapooya. Therefore, the 2005 and 2006 returns allow us to assess the trade-off between a gain in production and a reduction in  $N_e$  due to the Ryman Laikre effect.

**FRY VS SMOLT REPRODUCTIVE SUCCESS.** A primary objective of the CHIP project is to determine the reproductive success of unfed fry and smolts. Although, the difference in unfed fry vs. smolt reproductive success appears substantial (ca. 2% vs. 0.0007%), conclusions based on these data are presumably due to a fully seeded system in 2002 when these fry were released. We will have a better idea of the reproductive success of smolts vs. fry in the upcoming years 2005 and 2006 because these will be years when fry were supplemented in a partially seeded system. The result should be a much similar estimate of reproductive success between smolt and fry coho.

## **VI. Conclusions**

Management of fish stocks for optimum sustainable yields, where sustainability is the long-term survival of fish stocks, is a constant struggle for fisheries managers. The Nonpareil CHIP Project is a truly unique study system that allows us to evaluate reproductive success between hatchery and wild coho salmon through subsequent generations. We have evaluated ODFW's conservation hatchery implementation program and report that although the program is conserving the genetic diversity of the naturally reproducing stock, outbreeding depression and reduction in effective population size remain genetic risks that require further evaluation. Only by studying the long-term genetic and demographic consequences of hatchery supplementation and genome introgression will fisheries managers adequately understand the potential risks associated with hatchery supplementation.

## VII. Requested Budget Proposal for 2005-2007

Projected costs for the genetic proportion of the CHIP project are as follows: \$254,912 for fiscal year 2004-2005; \$130,711 for fiscal year 2005-2006; and \$134,377 for fiscal year 2006-2007. Allocation of funds for biennium 2003-2005 was \$385,623 and \$134,377 for the current 2005-2007 biennium. OWEB has already committed to \$134,377 for fiscal year 2006-2007; however, due to the enormous initial cost of screening microsatellite loci ( $n = 96$ ), greater than projected number of returning adults ( $n = 870$  vs 1,377), and unforeseen costs of two new automated machines (liquid handling robot and DNA genotype machine), we are requesting an additional \$143,948 to be amended to our existing budget for fiscal year 2006-2007. The justification of this budget is given below.

### Annual and Total Budget

<b>Hatchery/wild Reproductive Success 2006-7</b>						
<b>SALARIES &amp; WAGES</b>						
Name, Position, Title	SS#	Monthly Salary	OPE %	FTE	MM	Totals
Research Associate		\$584	55%	1	12	\$ 7,008
						\$ -
<b>A. TOTAL SALARIES &amp; WAGES</b>						\$ 7,008
<b>B. FRINGE BENEFITS</b>						\$ 3,854
<b>C. EXPENDABLE SUPPLIES &amp; EQUIPMENT - under \$5,000 per unit</b>						\$ 20,000
<b>D. TRAVEL</b>						
				Instate:		
				Out of state:		\$ -
<b>E. PUBLICATION COSTS</b>						
OTHER COSTS (subcontracts, consultants, computer time, etc.)						
1. Communications						\$ -
2. Publications						\$ -
<b>F. TOTAL OTHER COSTS</b>						\$ -
<b>G. GRADUATE STUDENT TUITION ( 1 students for 3 terms)</b>						\$ -
<b>H. PERMANENT EQUIPMENT</b>						
ABI 3730xl						\$ 100,000
<b>I. TOTAL PERMANENT EQUIPMENT - \$5000 or more per unit</b>						\$ 100,000
<b>J. GRAND TOTAL REQUESTED (sum Items G to J)</b>						\$ 130,862
<b>K. INDIRECT COSTS</b>						
Indirect Cost Rate						
ON-campus Cost at		0.1	% (multiply G x rate)			\$ 13,086
<b>L. GRAND TOTAL REQUESTED</b>						\$ 143,948

## **VIII. Budget Justification**

### **1) Personnel**

Dr. Moyer has been assigned leadership responsibilities at the Assistant Professor (research) level, thus requested salary is based on first year cost of such a position at OSU, and will be amended to his current contract [\$45,000 (Assistant Prof.) – \$38,000 (Research Associate) = \$7,000].

### **3) Materials and Supplies**

This research requires substantial genetic characterization of genetic loci in order to provide sufficient pedigree resolution. To process these samples in a timely manor requires numerous state-of-the-art, high-throughput machines that facilitate PCR and genotyping portions of this project. Recently, the Marine Fisheries Genetics Laboratory has experienced irreparable damage to both genotyping machines. Our damaged genotyping equipment has been replaced by a new ABI 3730xl machine that has been leased from ABI. We have the option to purchase this machine at cost; therefore, we request that 1/3 of the cost (\$100,000) be contributed by this proposal (there are two other major projects in the Laboratory that will fund the other 2/3 of the cost).

The initial microsatellite screening process and a greater than projected number of returning adults (*expected* = 870 vs. *observed* = 1,344) has depleted much of our Expendable Supplies and Equipment budget for fiscal year 2005-2006. We estimate  $\leq 1,000$  coho should return to Calapooya Creek in 2005-2006. Annual projected laboratory running costs for each year are \$3 per sample per microsatellite locus (12 loci  $\times$  1,000 fish  $\times$  \$3/fish = \$36,000). We are only asking for \$20,000 of this \$36,000 because our current budget has \$16,000 for supplies and equipment. This estimate is based on current commodities used for DNA extraction, PCR, and genotyping at the Marine Fisheries Genetics Laboratory at HMSC.

OWEB has already committed to \$134,377 for fiscal year 2006-2007; in addition to these funds, we are requesting an additional \$143,948 to be amended to our existing budget for fiscal year 2006-2007.

## **IX. Time Line and Returnables**

The time line and returnables portion of this grant has not changed from OSU's original CHIP proposal. Please refer to the OSU Component for Nonpareil Dam Adult Trap and Genetic Pedigree grant for years 2004-2007.