

Interim report to:

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

Submitting by:

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Coastal Oregon Marine Experiment Station  
Hatfield Marine Science Center  
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Newport, OREGON 97365

**OSU Component for Nonpareil Dam Adult Trap and Genetic Pedigree  
Progress Report and Scope of Work for 2007-2009**

Total amount requested:  
\$359,112

Proposed duration:  
Two years

Starting date:  
July 1, 2007

Principal Investigator:

Dr. Michael A. Banks

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*Progress Report  
&  
Budget proposal sent  
to Ken Beery 3/26/07*

## OSU Component for Nonpareil Dam Adult Trap and Genetic Pedigree 2007 – 2009 Scope of Work

The CHIP Project Proposal Narrative (see below) detailed the following 8 primary tasks:

**Task 1.** What is the relative success of using a first generation, wild-type broodstock in a supplementation program compared to a broodstock that has been captive for multiple generations?

**Task 2.** What is the relative success of unfed fry releases compared to smolt releases in producing returning adults?

**Task 3.** What is the reproductive success in the wild of adult fish from the following treatments:

- a. First-generation hatchery fish from unfed fry releases;
- b. First-generation hatchery fish from smolt releases;
- c. Multi-generation hatchery fish from unfed fry releases;
- d. Multi-generation hatchery fish from smolt releases; and
- e. Wild fish.

**Task 4:** How does the supplementation program modify the effective population size of the population in the Calapooya (termed the "Ryman-Laikre Effect" (Ryman and Laikre 1991, Ryman et al 1995)

**Task 5:** What is the level of inbreeding that results from the supplementation program?

**Task 6:** What is the incidence of natural crossing between adults from the different treatment groups while on the natural spawning grounds and the consequences of mate choice to the relative production of offspring by individuals;

**Task 7:** What differences in reproductive success occur by treatment by age (males), by gender, by adult run time, and by adult body size (length)?

**Task 8:** Does the size of the naturally-produced population increase due to successful natural reproduction by hatchery fish? Does the contribution to this increase vary by treatment group?

Initial data from 2004/5 and 2005/6 returns have enabled us to make a primary assessment of tasks 1 through 5. See attached manuscript currently in second review for publication in the *Canadian Journal of Fisheries and Aquatic Sciences* which focuses primarily on task 1, 4 and 5. Publication of findings for task 2 and 3 has been deferred until we have data for at least one more cohorts.

Ongoing funding is hereby requested for the following scope of work to provide additional data for tasks 2, 3 and 6 through 8.

## **Scope of Work**

### **2007/2008**

Pedigree analysis of 2006 returns.

Perform analysis to determine:

1. The relative success of unfed fry verses smolt releases at returning adult fish to the basin for 2004,2005 and 2006 cohorts, including comparisons to the adult production by wild fish naturally spawning **(tasks 2&3)**
2. Effective size for wild coho salmon inferred from demographic data: an evaluation of  $N_e$  estimators **(task 4 continued)**
3. The influence of mate choice on fitness of wild coho **(task 6)**

Prepare peer review scientific papers on these findings.

### **2008/2009**

Pedigree analysis of 2007 and 2008 returns.

Perform analysis to determine:

4. What differences in reproductive success occur by treatment by age (males), by gender, by adult run time, and by adult body size (length)? **(task 7)**
5. Does the size of the naturally-produced population increase due to successful natural reproduction of hatchery fish? Does contribution to this group vary by treatment? **(task 8)**

Prepare peer review scientific papers on these findings.

**Nonpariel Dam coho pedigree Genetics 2007-2008**

'SALARIES & WAGES		Monthly	OPE	FTE	MM	Totals
Name, Position, Title		Salary	%			
Assistant Prof (Greg Moyer – Veronique Theriault)		3,900	52%	1	12	\$ 46,800
Graduate Research Assistant (Marc Johnson)		\$1,800	0.03	0.49	9	\$ 16,200
Res. Asst:(Summer salaries for Marc)		\$3,600	0.05	1	3	\$ 10,800
<b>A. TOTAL SALARIES &amp; WAGES</b>						\$ 73,800
<b>B. FRINGE BENEFITS</b>						\$ 25,362
student medical benefit		\$ 523			3	\$ 1,569
<b>C. EXPENDABLE SUPPLIES &amp; EQUIPMENT - under \$5,000 per unit</b>						\$ 48,000
<b>D. TRAVEL</b>				Instate:	2,000	
Domestic				Outstate:	2,000	\$ 4,000
<b>E. PUBLICATION COSTS</b>						
OTHER COSTS (subcontracts, consultants, computer time, etc.)						
1. Communications						\$ 180
2. Publications						\$ 600
<b>F. TOTAL OTHER COSTS</b>						\$ 780
<b>G. GRADUATE STUDENT TUITION ( 1 students for 3 terms)</b>			\$3,085	3		\$ 9,255
<b>H. PERMANENT EQUIPMENT</b>						
<b>I. TOTAL PERMANENT EQUIPMENT - \$5000 or more per unit</b>						
<b>J. GRAND TOTAL REQUESTED (sum items G to J)</b>						\$ 161,197
<b>K. INDIRECT COSTS</b>						
		Indirect Cost Rate				
ON-campus Cost at		0.1	% (multiply G x rate)			\$ 16,120
<b>L. 2007-8 TOTAL</b>						\$ 177,317

<b>Nonpariel Dam coho pedigree</b>		<b>Genetics 2008-2009</b>				
<b>'SALARIES &amp; WAGES</b>		<b>Monthly</b>	<b>OPE</b>	<b>FTE</b>	<b>MM</b>	<b>Totals</b>
<b>Name, Position, Title</b>		<b>Salary</b>	<b>%</b>			
Assistant Prof (Greg Moyer-Veronique Theriault)		4,056	52%	1	12	\$ 48,672
Graduate Research Assistant (Marc Johnson)		\$1,872	0.03	0.49	9	\$ 16,848
Res. Asst:(Summer salaries for Marc)		\$3,744	0.05	1	3	\$ 11,232
<b>A. TOTAL SALARIES &amp; WAGES</b>						\$ 76,752
<b>B. FRINGE BENEFITS</b>						\$ 26,376
student medical benefit		\$ 550			3	\$ 1,650
<b>C. EXPENDABLE SUPPLIES &amp; EQUIPMENT - under \$5,000 per unit</b>						\$ 48,000
<b>D. TRAVEL</b>						
				Instate:	2,000	
Domestic				Outstate:	2,000	\$ 4,000
<b>E. PUBLICATION COSTS</b>						
OTHER COSTS (subcontracts, consultants, computer time, etc.)						
1. Communications						\$ 180
2. Publications						\$ 600
<b>F. TOTAL OTHER COSTS</b>						\$ 780
<b>G. GRADUATE STUDENT TUITION ( 1 students for 3 terms)</b>						
				\$3,120	3	\$ 9,360
<b>H. PERMANENT EQUIPMENT</b>						
<b>I. TOTAL PERMANENT EQUIPMENT - \$5000 or more per unit</b>						
<b>J. GRAND TOTAL REQUESTED (sum items G to J)</b>						\$ 165,268
<b>K. INDIRECT COSTS</b>						
		Indirect Cost Rate				
ON-campus Cost at		0.1	% (multiply G x rate)			\$ 16,527
<b>L. 2008-9 TOTAL</b>						\$ 181,795
<b>GRAND TOTAL (2007-9)</b>					<b>Total</b>	<b>\$359,112</b>

## Biographical Sketch *Michael A. Banks January, 2007*

### Professional Preparation

University of Cape Town	Zoology	BSc,	1981
University of Cape Town	Physics, Chemistry & Biology	HED,	1982
Louisiana Tech University	Zoology	MSc,	1988
University of California, Davis	Population Genetics	PhD,	1994

### Appointments

Director of the Cooperative Institute for Marine Resources Studies		2006 –
Assistant Professor	Marine Fisheries Genetics	2001 –
Assistant Geneticist	Bodega Marine Laboratory	1996 – 2000
Postdoctoral Fellow	Bodega Marine Laboratory	1994 – 1996
Research Assistant	Univ. of California, Davis	1989 – 1993
Research Assistant	Univ. Of Texas at Austin, MSI	1987 – 1988
Head of Dept. Science & Biology	Ngangelizwe Secondary School	1984 – 1986
Assistant Teacher	Umtata High School	1983

### Selected Publications

- Bucklin, K., M.A. Banks and Hedgecock D. 2007. Assessing genetic diversity of protected coho salmon populations in California. *Canadian Journal of Fisheries and Aquatic Science*. 63(1): 30-42
- Gomez-Uchida, D. and M.A. Banks. 2006. Integrating Temporal and Spatial Scales in Rockfish Population Genetics: Shaping Conservation and Management Goals. In press for: *Biology, Assessment and Management of Pacific Rockfishes*. 2005 Wakefield symposium.
- Gomez-Uchida, D. and M.A. Banks. 2006. Estimation of effective population size for the darkblotched rockfish *Sebastes crameri*. In press for *Journal of Heredity*. 97: 603-606.
- Wofford, J.E.B., R.E. Gresswell and M.A. Banks. 2005. Factors influencing within-watershed genetic variation of coastal cutthroat trout. *Ecological Applications*: 15(2):628-637.
- Banks, M.A. 2005. Stock identification for the conservation of threatened or endangered species. In: *Stock Identification Methods* Eds: Cadrin, S.X., K.D. Friedland and J.R. Waldman. Elsevier Press. pp609-629.
- Miller, J.A., M.A. Banks, D. Gomez-Uchida, and A.L. Shanks. 2005. Population structure in black rockfish (*Sebastes melanops*): a comparison between otolith microchemistry and DNA microsatellites. *Canadian Journal of Fisheries and Aquatic Science*. 62:2188-2198.
- Gomez-Uchida, D. and M.A. Banks. 2005. Microsatellite analysis of special genetic structure in darkblotched rockfish (*Sebastes crameri*): is binning safe? In Press for *Canadian Journal of Fisheries and Aquatic Sciences* 62:1874-1886.
- Banks, M.A., W. Eichert, J.B. Olsen. 2003. Which Genetic Loci have Greater Population Assignment Power? *Bioinformatics* 19(11):1436-1438.
- Gomez-Uchida, D., E.A. Hoffman, W.R. Ardren and M.A. Banks. 2003. Microsatellite Markers for the heavily exploited canary (*Sebastes pinniger*) and other rockfish species. *Molecular Ecology Notes* 3:387-389.
- Banks, M.A., V.K. Rashbrook, M.J. Calavetta, C.A. Dean, and D. Hedgecock. 2000. Analysis of microsatellite DNA resolves genetic structure and diversity of chinook salmon in California's Central Valley. *Canadian Journal of Fisheries and Aquatic Sciences* 57:915-927.

### Other Synergistic Activities

- Primary initiator of OSU research in the population genetics among over-fished rockfish stocks. We developed & published novel rockfish microsatellites, presented findings at 4 national meetings (including 2 invited talks) and published 6 papers in the peer review literature.
- Primary initiator of research into the genetic basis of life history diversity in Chinook salmon through investigation of clock genes and a genomic survey of gene expression profiles. We are the first to have isolated *clock*, *bmal*, *cry* and *period* from Chinook. Our findings of multiple copies have been presented at international meetings and are currently under peer review for publication.
- Primary initiator of research into the assessment of genetic & ecological diversity of Oregon's coastal coho

evolutionary significant unit – manuscript under peer review for publication.

Co-PI on an inter-laboratory standardization of coast-wide Chinook salmon genetic data for international harvest management – manuscripts under peer review for publication.

Primary initiator if development of computer applications for utilizing increased information content of microsatellite data. Programs developed include: WHICHRUN, WHICHLOCI, WHICHPARENTS, SIBLINGS & associated publications

### *Collaborators & Co-Editors*

Beecham, T.	Fish. & Oceans, Canada	Kyriacou, B.	Univ. of Leicester, UK
Bellinger, R.	OSU	Lacey, M.	CDFG
Boehlert, G.	OSU	Masuda, M.	NMFS, AKSC
Blouin M.	OSU	Buonaccorsi, V.	Juniata College
Camara, M.	USDA, HMSC	Meusnier, I.	OSU
Clarke, E.	NMFS, NWFSC	Miller, J.	OR Inst. of Mar. Biol./OS
Devlin, B.	Fish. & Oceans, Canada	Moran, P.	NMFS, NWFSC
Fleming, I.	Memorial Univ., Canada	Nuram, S.	CRITFC
Garza C.	NMFS,SWFSC	Palumbi, S.	Hopkins/Stanford
Greene, S.	CADWR	Riemer, S.	ODFW
Greg M.	USFWL	Seeb, L.	Alaska DF&G
Gresswell, B.	USGS	Shanks, A.	Univ. of Oregon
Hedgecock, D.	Univ. of S. California	Wofford, JEB	US Forest Serv.
Kvitrud, M.	Univ. of Minnesota	Young, S.	WDFW

### *Graduate & Postdoctoral Advisors*

Dennis Hedgecock, Paxson H. Offield Prof. in Fisheries Ecology and Biol. Sciences, Univ. of S. California  
Joan Holt, Associate Director for Mariculture, Marine Science Institute, The University of Texas at Austin

### *Thesis Advisor & Postgraduate-Scholar Sponsor*

<i>Post-doctoral Trainees (2)</i>		Where from	Completion Year
Gregory Moyer		Univ. New Mexico	2006
Isabelle Meusneir		Univ. of Laval, France	2006

### *Graduate Students (17)*

#### *Major Professor*

Rebecca Baldwin	PhD		est. 2010
Mattias Johansson	PhD	San Francisco State	est. 2009
Marc Johnson	PhD	University of Brasilia	est. 2008
Kathleen O'Malley	PhD	University of Guelph	est. 2007
Jeremiah Bernier	MS	University of Oregon	2006
Daniel Gomez-Uchida	PhD	University of Hull, UK	2006

#### *Co-advisor - Committee Member*

David Stick	PhD	Penn State University	(major Langdon, OSU)	2006
Molly Stephens	PhD	Sonoma State	(major May, UC Davis)	2006
Mark Christie	PhD		(major Hixon, OSU)	2008
Bret Gallanger	MS		(major Hepell, OSU)	2006
Paul Lang	PhD	Louisiana State Univ.	(major Langdon, OSU)	2007
Marion Mann	MS	HMSC	(major Fleming, OSU)	2007
Mike Wetz	MS	COAS	(major Wheeler, OSU)	2005
Jeb Wofford	MS	Oregon State University	Gresswell (OSU) & Banks	2003
Troy Guy	MS	Oregon State University	Gresswell (OSU) & Banks	2004
Jessica Miller	PhD	Univ. of Oregon	advisor (major Shanks, UO)	2004
Anja Liebert	MS		advisor (major Schreck, OSU)	2004

# CHIP Project Proposal Narrative

I. **Project Title:** Nonpariel Dam Adult Trap and Coho Genetic Pedigree

II. **Contact:** Dave Loomis, Oregon Department of Fish and Wildlife  
Dr. Michael Banks, Oregon State University

III. **Project Abstract:**

This proposal would investigate several areas of uncertainty about the use of hatcheries to increase the abundance of wild populations. There is a considerable interest in using hatcheries to speed the recovery of wild populations. However the value of such programs is untested. Substantial literature exists that indicates hatchery programs may pose high risks to wild populations, rather than aid them (see the following reviews: Hindar et al 1991, Waples 1991, Waples 1999, and Lichatowich 1999 and literature cited therein). If the risks are real, hatcheries may *interfere* with recovery, rather than speed it. Until recently, analytical methods to explore the critical questions and risks associated with hatchery programs were unavailable because we were not able to track lineages in streams once hatchery and wild fish were allowed to spawn together. New molecular genetics methods now allow us to use DNA fingerprints to pedigree entire populations under some circumstances and develop lineages that continue for multiple generations under natural spawning conditions. We can finally produce direct evidence of the success or failure of hatchery supplementation programs and provide direct measurements of some of the risks predicted by genetics theory. We propose to utilize these methods on an experimental supplementation program for coho salmon on the Calapooya River, a tributary of the Umpqua River on the Oregon Coast.

IV. Proposal:

A. **Project Need:**

1. **Intent:**

The effective use of hatchery fish to increase the size of an extant wild population has not been demonstrated. The concept is to take part of a small wild population into captivity, disproportionately increase the number of offspring produced by them, release those offspring into the wild, and then allow them to spawn naturally as adults thereby significantly increasing the total number of natural spawners. If this larger spawning population reproduces successfully in the stream it should produce a much larger naturally-produced ("wild") population in a small number of generations. The benefit of this larger population size may out-weigh the impact of genetic risks caused by the action (Figure 1).

However the success of this approach has not been evaluated or demonstrated. We know we are able to substantially increase the number of natural spawning fish by adding hatchery adults to a stream. But to date we have not been able to

demonstrate that this action increases the number of naturally-produced (“wild”) adults in the stream. We also expect, based on genetics theory, that substantial genetic risks to the wild population may occur as a result of this action, but we have not been able to directly measure the risks. Our biggest handicap to evaluating these efforts has been our inability to determine the parentage of naturally-produced offspring in a natural stream setting. New developments in molecular genetics now allow us to pedigree entire populations, provided we are able to handle the adults. These methods let us exactly match offspring to parents. The results are straightforward and unambiguous. We are able to follow lineages from parents to offspring to grand-offspring. We finally will have a clear answer as to whether hatchery fish breed as successfully in streams as wild fish do, which will measure the success of the hatchery program. We will also be able to directly measure several genetic risk factors.

Reproductive success by hatchery fish spawning in a stream is expected to be lower than that of wild fish. The lower fitness of hatchery-born adults manifests itself in two ways: First, hatchery-born adults do not compete for mates or build nests as successfully as wild fish (Fleming and Petersson, 2001, Chebanov and Riddell 1998). Second, the survival of their offspring is reduced owing to relaxed natural selection and to domestication selection that occurs during the egg-to-smolt stage in the hatchery (Lynch and O’Hely 2001, Reisenbichler and McIntyre 1977, Reisenbichler and Rubin 1999). Successful reproduction by the hatchery fish spawning in the stream – specifically production of adult offspring -- is required if the benefit of an increased wild population size is to occur. We will be able to directly measure the reproductive success of the hatchery fish relative to wild fish by knowing exactly how many adult offspring are produced by each natural spawning individual.

Hatchery programs, where substantial numbers of hatchery fish spawn naturally in a wild population, theoretically cause five major genetic risks to wild populations. The risks are demonstrated in Figure 1 and include the following:

- Risk 1. Population Bottleneck (Ryman and Laikre 1991):** This risk occurs when a small number of parents (those taken into the hatchery) contribute more offspring per parent to the supplemented population than the rest of the population (those left in the wild). This difference in family size causes a decrease in the effective population size of the total population.
- Risk 2: Increased Inbreeding (Ryman et al 1995):** 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 (Lynch and O’Hely 2001):** This risk results from the increased reproductive success and survival that occurs while fish are in the captive environment. Increased 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.

All of these risks are inevitable in any hatchery supplementation program. However, if the hatchery fish breed successfully, and the program succeeds in increasing the size of the wild population, and it stabilizes at the larger size, and the hatchery program stops removing further risk, a net benefit to the wild population may occur. If, on the other hand there is reproductive failure by the supplemented population, further genetic risks will occur:

**Risk 4: Genetic Variation is Lost (Nei et al, 1975):** When an offspring population is smaller than it's 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.

And finally, if the hatchery program continues over multiple generations the impacts of these risks will accumulate in the wild population due to the nature of the genetic mechanisms involved (**Risk 5**).

Direct measurements of effective population size, inbreeding coefficient, and reproductive success or failure can be made using pedigrees. Occurrence of increased genetic load and loss of genetic variation can be inferred from the measures of individual reproductive success.

Additional questions exist about the best protocols to use in implementing a supplementation program. For example, using single-generation hatchery broodstock (parents taken from the wild each generation) rather than old hatchery stocks should minimize the genetic effects, but there has never been a test of this hypothesis. Similarly, releasing unfed fry should reduce the extent to which selection is relaxed in the hatchery to only that experienced during the egg-to-fry stage, and to selection on any parental behaviors such as maternal nest building ability. Therefore, although survival from egg to adult of fish released as unfed fry is much lower than that of fish released as smolt, the hatchery adults that return from the unfed fry releases may be nearly as successful at natural reproduction as completely wild fish. This hypothesis has also never been tested. It is not possible to test all possible protocols in a single experiment. This study proposes to investigate the following strategies:

- a. Is a first-generation wild-type broodstock a better choice than an older, multi-generation broodstock? Theoretically, the first-generation broodstock should have less genetic load and domestication build-up than an older one and should succeed better. The existing Rock Creek Hatchery coho broodstock is an older and also partly mixed-origin broodstock. The success of these will be compared to wild fish collected at Winchester Dam in 2001 and at Nonpareil Dam in 2002-03 to form a first generation broodstock.

- b. Is a less invasive hatchery program better than a more invasive one? In a less invasive program, fish are held captive through a lesser portion of their life cycle, which should decrease genetic load build-up. The down-side of holding fish captive for a shorter period is that the survival benefits, and therefore the rapid increase in number of fish, are compromised. In our experiment we compare two options:
- i. Captivity during reproduction and rearing through hatching (release of unfed fry); and
  - ii. Captivity during reproduction and rearing through smoltification (release of smolts).
- c. The reproductive success of adults returning from all of the hatchery treatments will be compared to that of wild fish returning at the same time (in years 2004 through 2007, including both jacks and adults, with their offspring returning in 2007 through 2010, including both jacks and adults).

The potential benefits of a supplementation program also depend on the carrying capacity of the basin. The naturally-produced population can increase in size only if the basin is capable of producing more fish than are currently present. It is therefore important to evaluate the apparent carrying capacity of the supplemented basin at the beginning of the program.

2. **Basin, stock(s):** Umpqua River, coho

3. **Strategic goals:**

This project would be used to evaluate hatchery program effectiveness as required by the Oregon Plan for Coastal Salmonids and NMFS ESA Section 4(d) rulings, and by the ODFW Wild Fish Management Policy (OAR 635-07-525 through 529) and the ODFW Hatchery Fish Gene Resource Management Policy (OAR 635-07-540 through 541).

4. **Literature review:** Background material, theory, methodology and concepts are provided elsewhere in this document, based on the following references:

Blouin, M.S., M. Parsons, V. Lacaille, and S. Lotz. 1996. Use of microsatellite loci to classify individuals by relatedness. *Molecular Ecology* 5:393-401.

Chebanov, N.A. and B.E. Riddell. 1998. The spawning behavior, selection of mates and reproductive success of chinook salmon (*Oncorhynchus tshawytscha*) spawners of natural and hatchery origins under conditions of joint spawning. *Journal of Ichthyology*. 38: 517-526.

Fleming, I.A. and E. Petersson. 2001. The ability of released hatchery salmonids to breed and contribute to the natural productivity of wild populations. *Nordic Journal of Freshwater Research*.

## **A. Objectives:**

The objective of this study is to conduct an experimental supplementation project for coho salmon in the Calapooya, tributary of the Umpqua River, using the following hatchery scenarios:

- a. Rock Creek hatchery stock released as smolts (a “conventional hatchery program”);
- b. Rock Creek hatchery stock released as unfed fry (a low- intervention hatchery program);
- c. First-generation wild-type hatchery stock released as smolts; and
- d. First-generation wild-type hatchery stock released as unfed fry.

We will evaluate the success and genetic implications of these alternative hatchery scenarios using DNA pedigree reconstruction. We will establish the pedigree of fish from the hatchery and subsequently above Nonpareil Dam that is illustrated in Figure 2.

Three generation-lines will be developed to provide a replication of the study. The total sampling and release design is presented in Table 1. One limitation for this project is that the trap in Nonpareil Dam is not yet installed (as of October 2001). Installation of the trap is expected to occur in the summer of 2002. Although we will begin the pedigrees for the hatchery fish in 2001 we will not be able to begin sampling wild fish in the Calapooya until 2002. Also the first year of wild-type broodstock collection will occur (in 2001) at Winchester Dam, rather than at Nonpareil Dam. The subsequent two wild-type broods will be collected from the Calapooya. This limitation provides us with a unique opportunity to compare two generations of a true “local” wild-type brood to one that came from an adjacent basin. Theory predicts that the true local wild-type brood should be the superior one.

This project will specifically address the following tasks:

**Task 1.** What is the relative success of using a first generation, wild-type broodstock in a supplementation program compared to a broodstock that has been captive for multiple generations?

**Task 2.** What is the relative success of unfed fry releases compared to smolt releases in producing returning adults?

**Task 3.** What is the reproductive success in the wild of adult fish from the following treatments:

- f. First-generation hatchery fish from unfed fry releases;
- g. First-generation hatchery fish from smolt releases;
- h. Multi-generation hatchery fish from unfed fry releases;
- i. Multi-generation hatchery fish from smolt releases; and
- j. Wild fish.

**Task 4:** How does the supplementation program modify the effective population size of the population in the Calapooya (termed the “Ryman-Laikre Effect” (Ryman and Laikre 1991, Ryman et al 1995)

**Task 5:** What is the level of inbreeding that results from the supplementation

program?

**Task 6:** What is the incidence of natural crossing between adults from the different treatment groups while on the natural spawning grounds and the consequences of mate choice to the relative production of offspring by individuals;

**Task 7:** What differences in reproductive success occur by treatment by age (males), by gender, by adult run time, and by adult body size (length)?

**Task 8:** Does the size of the naturally-produced population increase due to successful natural reproduction by hatchery fish? Does the contribution to this increase vary by treatment group?

This study will use highly variable DNA markers to pedigree coho salmon in the Calapooya, tributary of the Umpqua. The study design will require the installation of a trap in Nonpareil Dam on the Calapooya so that the sampling and data collection can occur. The laboratory analysis will be done under contract to Dr. Michael Banks, OSU, Marine Fisheries Genetics Laboratory.

The results of the evaluation of unfed fry in this project will also be compared to results from other work currently underway in the Umpqua. This other work uses otolith marks to mark unfed fry. Marked fish are recaptured as adults, providing a measure of unfed fry to adult survival rates (Jackson and Loomis 2001). It was not possible to measure the reproductive success of the adults resulting from these releases of marked unfed fry. Final results from the otolith work will be reported in the 2002 annual report for this project.

The district will be initiating an evaluation of the productivity and carrying capacity of the upper Calapooya subbasin in 2002. This evaluation will address natural juvenile production in the subbasin. Existing information on habitat capacity will be compiled in 2002 and provided in the 2002 annual report for this project.

## **B. Methodology:**

Spawning of coho adults will occur at Rock Creek Hatchery and rearing of smolts will occur at Rock Creek Hatchery or Butte Falls Hatchery. Unfed fry will be released under the jurisdiction of the ODFW STEP program with assistance from ODFW volunteers. The Nonpareil trap will be staffed by ODFW district staff out of the Roseburg Fish District office.

In summer of 2002 an adult trap will be installed in the existing fishway in the Nonpareil Dam. The trap would be operated during the adult coho migration period. Fin clips and scales would be taken from each returning adult. Fish will be wanded for coded wire tag collection. Identity of hatchery and wild fish will be based on marks, with a back-up of scale pattern identification. The following information will also be collected at both Nonpareil Dam and at Winchester Dam during the initial broodstock collection:

- a. Run time at the respective dam;
- b. Gender;

- c. Adult size (body length);
- d. Age (applies to males only in coho: "Jacks" (age 2) and "Adults" (age 3));
- e. Total number of adult fish arriving at and passing Nonpareil Dam each year.
- f. Origin of all adult fish arriving at and passing Nonpareil Dam each year.
- g. Fecundity of individual females in the hatchery broodstocks (possibility of measuring this will be explored in 2001; if successful it will continue)

Microsatellite DNA markers that are sufficient to identify individuals in the population, and to match offspring to parents, will be used to trace genetic pedigrees. Microsatellite DNA markers are already available for coho salmon (M. Ford NMFS, personal communication; M. Blouin OSU, personal communication, Smith 2001) and the laboratory techniques are routinely used in Dr. Barks's lab. The statistical methods of parentage analysis that are to be used are well established (Blouin et al 1996, SanCristobal and Chevalet 1997, Marshall et al 1998, and Lynch and Ritland 1999). Laboratory staff will initially determine the heterozygosity and number of alleles per locus at each marker locus in the Umpqua population, and will choose the most informative subset of the available markers for use with this population. This screening and optimization of markers will occur in the first year of the contract (2002). The total sampling design for this project, including estimated fish sampled and run through the lab each year, is provided in Table 1. Laboratory work on the sampled fish in a year will begin following final collection of the samples.

Progress reports will be provided annually and major project reports will be developed as results become available. Since this study ultimately addresses the reproductive success of hatchery fish in the wild it is necessary to trace the lineages over three generations (parents, supplemented population, naturally-produced offspring) before the most interesting results become available. The first major project report will be completed in 2006 addressing the relative success of unfed fry releases, and smolt releases at returning adults to the dam. A second major report addressing the return of all hatchery adults and comparisons with returns from wild parents will be completed in 2008. A third major report will occur in 2009 to address the first results comparing reproductive success of hatchery and wild fish in the wild, with a final series of reports in 2011 that will include all results. A schedule of these papers is provided in Table 2. The laboratory will be expected to publish the results in peer-reviewed journals.

### **C. Research/Management Implications:**

This project will evaluate major areas of uncertainty about the use of hatchery programs to increase the abundance of wild populations. The project will be able to uniquely address important questions, listed in the objectives above, that currently limit the usefulness of hatchery supplementation in conservation and recovery. Although this project is specific to one hatchery program for coho in the Umpqua, the results will be of immense value in the design and application of supplementation programs throughout Oregon.

## **D. Evaluation**

### **1. Define success:**

Success in this project is clear information about the relative reproductive success of our various hatchery fish treatments and wild fish. This project can be uniquely implemented in the Calapooya/Umpqua for the following reasons:

1. The study can only be conducted on populations (including hatchery and wild parents) of a particular size. Populations that are too small introduce random errors, while populations that are too large (in the thousands) exceed the abilities of the methods. Populations between 100 and 1,000 adults are appropriate.
2. The study requires that the *entire* population can be sampled without error. We must be able to capture 100% of the fish passing into the population, handle them, sample and measure them and release them unharmed. All individuals must receive the same treatment. The trap must be effective over multiple years for the duration of the project.
3. Coho are particularly attractive as a study species because of their 3-year life history.
4. We must be able to collect other kinds of information on the fish, including abundance, origin, gender, and life history data. This information can also be collected using an effective adult trap.

### **2. Describe monitoring programs:**

The following samples and information must be collected:

- i) Monitoring of the hatchery broodstock:
  - (1) The experimental broodstocks will consist of exactly 200 wild fish and exactly 200 hatchery fish, Rock Creek stock. Each experimental fish should be marked upon capture and assigned a number so that subsequent individual information can be tracked. All data must be kept in a spread sheet or data base.
  - (2) Age, size (fork length), date of capture (aka run time at Winchester Dam), and date of spawning of each parent in the hatchery.
  - (3) Tissue clip from each parent, stored in ethanol. Scale sample from each parent.
  - (4) Gender of each hatchery parent; the sex ratio must be exactly 50% females and 50% males.
  - (5) Each parent will be paired with only one mate.
  - (6) Crosses will consist of W x W and H x H only.
  - (7) Identification of mates for each parent fish (which male is paired with which female).
  - (8) Individual family survivals (or small groups of families) must be tracked as long as possible. Generally this is through hatch or early fry stage.
  - (9) Any catastrophic loss or other incident that affects any family or groups of families.

- ii) Monitoring of the wild fish and supplemented population at Nonpareil Dam:
  - (1) Exact number of hatchery and wild fish passed above the dam each year of the study. Each adult should be given a number so that subsequent individual information can be tracked. All data must be kept in a spread sheet or data base.
  - (2) A fin clip from each fish passed above the dam, stored in ethanol. A scale sample from each fish.
  - (3) Age, size (fork length), date of passage, origin (marked or unmarked), and gender of each fish passed above the dam.
  - (4) Wild population size (number of naturally produced fish) returning to Nonpareil Dam should be monitored indefinitely into the future, but at least for 10 years after the supplementation program and this study are concluded.
  - (5) Average production of offspring per adult and wild fish survival should be monitored indefinitely into the future, but at least for 10 years after the supplementation program and this study are concluded. This information can be obtained from the adult data at the dam using number of fish passed, sex ratio, average fecundity, and number of naturally-produced fish returning to the dam in the next generation. It would also be useful to use smolt traps to estimate smolt production from the basin.

## **Overall Context**

### **1. Relationship to other projects**

This evaluation program can be implemented without interfering with natural production or any element of the hatchery program under evaluation or any other program. It will provide very important information that will be useful in our consideration of all supplementation programs implemented in Oregon. This study is being repeated in other locations and for other species in Oregon and elsewhere in the Northwest, however it will not be possible to do it in every location where hatchery programs occur. Therefore, it will be necessary to extrapolate the results of this project, and of several other similar projects that are underway elsewhere, to other supplementation programs.

In the Calapooya, this hatchery project will be coordinated with a study of the habitat and productivity of the upper Calapooya subbasin.

### **2. Adaptive management components**

This program will provide information useful for evaluating the Calapooya unfed fry program and comparing it to smolt programs. But equally important, this study will address critical questions that are hindering the effective use of supplementation in recovery throughout Oregon and elsewhere in the Northwest. The results of this study should confirm those elements of supplementation projects that are effective, provide factual data about risks, and pin-point some effective and ineffective actions and strategies.

**V. Annual and Total Project Budget**

**Capital Construction**

Adult Trap (2002 only)	\$ 10,000
Annual costs (2002):	<b>\$ 10,000</b>
Total costs (2002):	<b>\$ 10,000</b>

***Hatchery Operations (2002-04)***

Rearing 20,000 smolts @10/lb or 2,000 pounds	\$ 6,920
Adult holding facilities (2002 only):	\$ 1,000
Incubation tray partitions (2002 only):	\$ 2,500
2002 Annual cost:	<b>\$ 10,420</b>
Future annual costs (2002-3):	\$ 6,920
Total costs (2002-04):	<b>\$ 24,260</b>

***District Costs for field work (2002-2010)***

**Salaries and benefits**

EBA Seasonal 0.33 FTE	\$ 24,130
EBA Seasonal 0.33 FTE	\$ 24,130
NRS 4 Permanent 0.25 FTE	\$ 38,830
EBA (0.5 month) scale reading	\$ 2,500

**Field Supplies and equipment**

Glassware, Nets, Anesthetic Tanks, waders, CWT wand, etc.	\$ 10,000
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**Travel & per diem**

100 miles/day; 4 months/year; \$.325/mile	\$ 5,200
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Total annual costs:	<b>\$ 151,590</b>
Total costs (2002-10)(estimated*)	<b>\$1,364,310</b>

***Contract Services for DNA analysis***

(Contracted to Dr. Michael Banks, OSU)	\$130,000
Annual costs:	<b>\$130,000</b>
Total costs (2002-11)(estimated*):	<b>\$1,300,000</b>

**Total cost for 2002: \$302,010**

**Estimated annual costs through 2004: \$288,510**  
**Estimated annual costs 2005 through 2010 \$281,590**  
**Estimated cost in 2011 \$130,000**

\* Actual laboratory costs may vary depending on inflation levels, cost-of-living increases, and actual numbers of coho returning to the Nonpareil Dam. District costs will vary based on cost of living and inflation increases.

## Sampling and release design for the total study at Nonpareil Dam.

Year	Broodstock captured at Winchester		Broodstock captured at Nonpareil (All wild)	Unfed fry releases (unmarked)	Smolt releases (2 mark groups)	Adults sampled and passed at Nonpareil 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		
2004				400,000	20,000	400	5	30
2005					20,000	400	75	350
2006						400	75	350
2007						450	70	320
2008						500		
2009						500		
2010						500		

***Parent  
Generation***

**Supplemented  
Generation**

**Offspring  
Generation**

\*Estimated numbers (6375). We do not have historic counts at Nonpareil Dam. The estimated number of adult hatchery fish is based on anticipated average survivals.

Table 2. Schedule of delivery of major products.

Year	Product
2002	Annual progress report. Report on the final results of the otolith marking. Report on the compilation of existing information on the habitat condition in the Calapooya subbasin.
2003	Annual progress report
2004	Annual progress report
2005	Annual progress report
2006	First report on the relative success of unfed fry verses smolt releases at returning adult fish to the basin;
2007	Annual progress report
2008	Final report on the relative success of unfed fry verses smolt releases at returning adult fish to the basin, including comparisons to the adult production by wild fish naturally spawning. Measurements of effective population sizes as influenced by the hatchery program ( <i>RISK 1</i> , Bottleneck Risk); measurements of the degree of relatedness in the supplemented population (initial part of <i>RISK 3</i> , Inbreeding Risk).
2009	First report on the relative reproductive success in the natural environment of hatchery adults from the various treatment groups, as compared to wild fish.
2010	Second report on the same.
2011	<p>Final reports on the following topics:</p> <ul style="list-style-type: none"> <li>Relative reproductive success of hatchery fish from the various treatment groups and wild fish on the natural spawning grounds (<i>RISK 2</i>, potential for <i>BENEFITS</i>);</li> <li>Inbreeding coefficient (<i>RISK 3</i>);</li> <li>Level (if any) of reproductive failure (<i>RISK 4</i>);</li> <li>Relative reproductive success by the following phenotypes (jack vers adult males, run time, body size) and variations in these (if any) in hatchery verses wild fish;</li> <li>Mate selection on natural spawning grounds (potential of mixing of hatchery and wild fish) and implications for reproductive success.</li> <li>Abundance of naturally-produced fish in three offspring years, and contribution of hatchery fish to any increases in abundance (<i>BENEFIT</i>). Abundance would need to continue to be monitored for at least ten years after the conclusion of this study to determine whether any abundance increases are maintained.</li> </ul> <p>We anticipate additional analyses and products from this data.</p>

Figure 1. Genetic risks and benefits caused by supplementation programs.

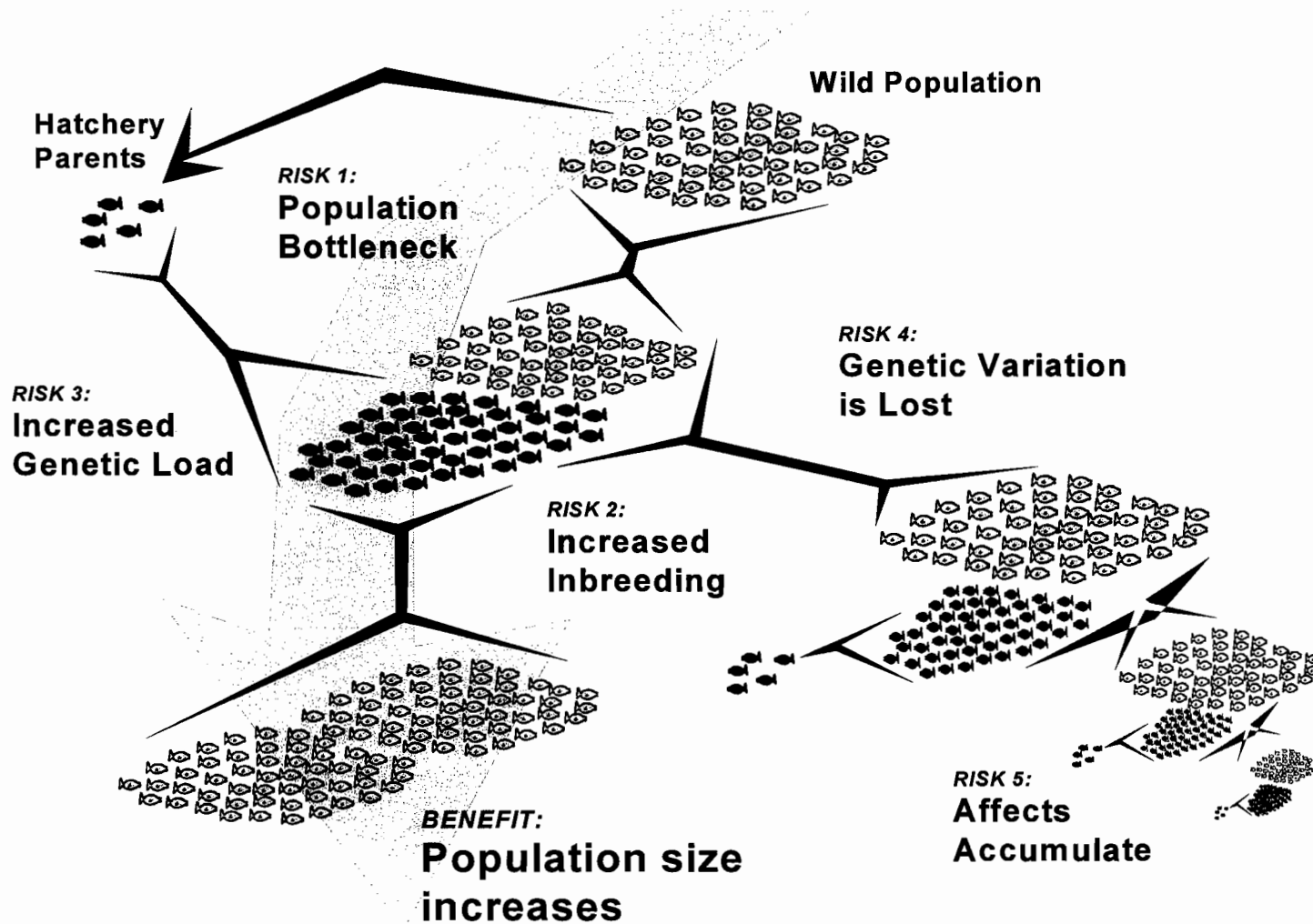
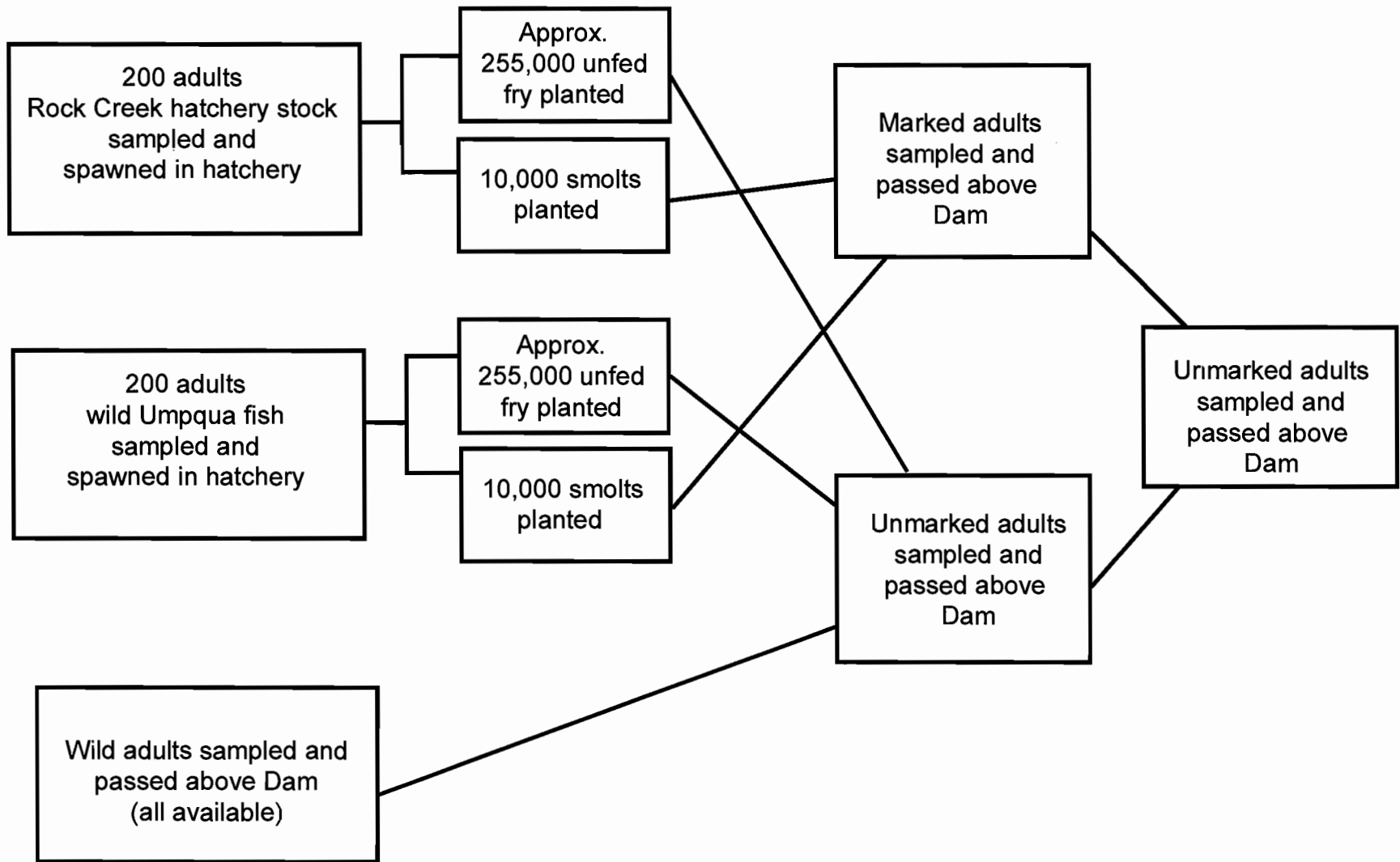


Figure 1. Pedigree reconstruction of coho salmon in the Calapooya subbasin of the Umpqua River, including hatchery fish used in supplementation.



**The influence of family-correlated survival on  $N_b/N$  for progeny from integrated multi- and single-generation hatchery stocks of coho salmon (*Oncorhynchus kisutch*).**

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Running Title: **family-correlated survival of supplemented coho**

## ABSTRACT

There exist surprisingly few data on the final variance and mean of family sizes for hatchery-born fish at the adult stage. Thus, it is difficult to predict, for a conservation hatchery operation that minimizes the variance in progeny number, how much lower the true  $N_e$  of a cohort of hatchery-born adults will be than  $N_e$  predicted simply by the number of parents that produced them. We used parentage analysis to estimate the survival and  $N_e$  for two integrated stocks of hatchery coho salmon (*Oncorhynchus kisutch*). One hatchery is a multi-generation stock obtained by spawning 70% hatchery with 30% naturally reproducing fish, while the second is a single-generation stock derived from naturally reproducing coho. There was no significant difference in average overall survival between stocks, but observed  $N_e$  was significantly less than expected for each stock. Family-correlated survival contributed to roughly a 20% reduction in  $N_e$  over the freshwater and marine life stages. This reduction is similar to previous estimates, and suggests a value that can be used when estimating effective number of hatchery parents (primarily when the program equalizes sex ratios and family sizes) in applications of the Ryman-Laikre formula.

**Key words:** conservation hatchery, effective population size, nonrandom survival, parentage analysis, Ryman-Laikre effect,

## Introduction

Fish hatcheries occupy various roles ranging from fish production for commercial harvest to augmentation for recreational purposes. Recently there has been a shift in hatchery programs from merely supplying fish for harvest to incorporating conservation objectives as a means to revive threatened wild populations (Hedrick et al. 2000; Miller and Kapuscinski 2003; Brannon et al. 2004).

The goal of a conservation hatchery is to boost the existing adult census size of a wild population by breeding a fraction of the wild population in captivity and releasing their offspring into the natural habitat (also known as supplementation). While there may be a gain in total production of offspring, one cost associated with such a gain may be a reduction in the effective population size ( $N_e$ ) of the total population (termed the Ryman-Laikre effect; Ryman and Laikre 1991). The simple formula

$$\frac{1}{N_e} = \frac{x^2}{N_h} + \frac{(1-x)^2}{N_w} \quad (1)$$

(Ryman and Laikre, 1991) describes the change in the inbreeding effective size of a population that results when a large fraction of the breeders in a population descend from a small number of founders. Here  $N_h$  is the effective size of the hatchery fish breeding in the wild and  $N_w$  is the effective size of the wild fish breeding in the wild. Thus, for application of the Ryman-Laikre equation, it is important to know the effective size of each group of fish. Estimating  $N_w$  for wild salmon populations can be quite difficult, and has been the subject of much study (Waples 1990; Waples 2002a; Shrimpton and Heath 2003). Here we focus on how accurately one can predict the effective size of the hatchery component of a supplemented population,  $N_h$ . It is often assumed that the effective size of the hatchery group can be estimated simply from the numbers of males and females used as broodstock to produce that hatchery group. However, few data exist on the realized effective

size of a cohort of hatchery fish relative to the effective size as predicted by the number of broodstock that produced them. For example, if two hundred male-female pairs are spawned in a hatchery, and if variance in family sizes is random through all stages of the life cycle of their offspring, then the inbreeding effective size of that cohort of offspring would equal the census size,  $N = 400$ . Non-random (family-correlated) survival during any stage of the life cycle would cause the  $N_e$  of that cohort of returning adults to be less than 400. How much less can be expected for typical conservation hatchery operations, which try to minimize variance in family size, remains an open question. Family-correlated survival can occur at any stage of the life cycle, but may be particularly high in captivity (Allendorf 1993). Thus, some estimates of the mean and variance in family sizes, and true realized  $N_e$  of hatchery cohorts, would be very useful for application of the Ryman-Laikre formula in practice.

Hatcheries have traditionally used broodfish that have been passed for many generations through a hatchery because such stocks perform well in a hatchery environment. In contrast, conservation hatcheries have opted to produce single-generation stocks using naturally-born broodstock or have integrated captive with naturally-born broodstock (Moberg et al 2005). It is unknown whether the mean ( $\bar{k}$ ) and variance ( $V$ ) in final family size differ between the two broodstock strategies adopted by conservation hatcheries. Differences, if large, might cause one to substantially under/over estimate the effective size of a cohort of single-generation stock, relative the expectation of an integrated stock.

Here we test whether there is a significant difference in the mean and variance in number of surviving adult progeny between a single-generation and an integrated multi-generation hatchery stock. We also estimate, for two cohorts of each stock, the reduction in realized  $N_e$  over that predicted by the number of parents that produced each cohort. We found no significant difference in

survival between single- and multi-generation stocks with both stocks experiencing similar reductions in  $N_e$ . This reduction was significantly less than expected indicating nonrandom survival occurred throughout the freshwater-marine life stage. Our data suggest that the hatchery coho used in this study experienced about a 20% reduction in  $N_e$  over that predicted throughout their freshwater and marine life stages, regardless of the type of hatchery stock.

## Materials and Methods

### Sampling design – year 2001

For at least the past decade, the North Umpqua River hatchery program has been managed as a harvest program, augmenting the recreational and commercial fisheries. The program integrates a random collection and spawning of adults throughout the run by mixing 70% hatchery fish (i.e., adipose clipped fish) with 30% natural fish. Each fish released from the hatchery is adipose clipped to ensure the designation “hatchery fish”. Therefore, we considered adipose clipped coho collected in the North Umpqua to be of multi-generation hatchery origin. In fall and early winter 2001, Oregon Department of Fish and Wildlife (ODFW) collected 100 female and 100 male coho salmon that had a marked adipose fin (our multi-generation hatchery stock; MGHS) from the North Umpqua River at Winchester Dam (Fig. 1). They also collected 94 females and 94 males having unmarked adipose fins (considered our single-generation hatchery stock, SGHS, because progeny were reared in a hatchery). Collections were performed randomly with respect to age, run-time, and length. Males and females from MGHS were randomly paired and spawned at ODFW’s Rock Creek hatchery facility using single-pair matings (i.e., each male and female was used only once). SGHS were spawned following the same protocol. Eggs from each mating pair were incubated separately until the eyed-egg stage. At this stage we attempted to equalize the variance in progeny number by randomly sampling 140-150 eyed-eggs/mating pair and rearing them to the smolt stage (MGHS and

SGHS were reared separately). While 140-150 eggs/mating pair was a goal, our observed mean in eyed-eggs/mating pair was less than expected (see Results) due to high egg mortality from a few mating pairs. As a result our variance in progeny number was also greater than expected.

#### Sampling design – year 2002

Sampling design for the 2002 brood stock was similar to 2001. However, the SGHS was collected from Calapooya Creek (Fig. 1), which is a tributary of the Umpqua River and considered part of the Umpqua Population Complex (Ford et al. 2004). Coho inhabiting Calapooya Creek are considered naturally reproducing (i.e., there is no recognized hatchery contribution to this system); however it is unclear if these fish are of hatchery origin or have introgressed with hatchery strays.

In fall and early winter 2002, Oregon Department of Fish and Wildlife (ODFW) collected 100 female and 100 male coho salmon having an unmarked adipose fin (SGHS) from Calapooya Creek at Nonpareil Dam (Fig. 1). The MGHS for 2002 consisted of 100 males and 100 females collected at Winchester Dam on the North Umpqua, as before. Collection, mating, and rearing of 2002 brood stock followed the 2001 sampling design protocol.

In spring 2003 and 2004, smolts from respective 2001 and 2002 brood years were released in Calapooya Creek above Nonpareil Dam (Fig. 1). Coho salmon smolts typically migrate to the Pacific Ocean a few weeks after release. Fins from released smolts were clipped adipose and left maxillary, or adipose and right maxillary, to designate progeny from MGHS or SGHS, respectively. Coho have a three year life cycle. Mature adults typically return to spawn at age III, but reproductively mature males called jacks can return at age II. Thus, coho salmon released as smolts in 2001 returned to the Calapooya Creek during the fall of 2003 (jacks) and 2004 (adult males and females), and smolts released in 2004 returned in the fall of 2004 and 2005. ODFW constructed a fish trap at the base of Nonpareil Dam (Fig. 1), allowing for the capture, fin-clipping, and above-dam release of all returning

adult coho salmon in this study.

#### Screening, optimization, and identification of microsatellite markers

DNA was extracted using QIAGEN DNA extraction kits. DNA concentration ( $\sim 4\text{-}24\text{ng}/\mu\text{l}$ ) was quantified using a Victor<sup>3</sup>V 1420 multilabel counter (Perkin Elmer).

Choosing appropriate markers for accurate parentage assignment is a function of population size, number of loci, and number and distribution of alleles per locus (Bernatchez and Duchesne 2000). We took the following strategy for choosing appropriate markers for this study. First, we screened 96 candidate loci known to amplify in salmon species. Of these loci, we successfully amplified 41 candidates for further evaluation. Next, loci were selected based on repeat motif (tetra-nucleotides were chosen over di-nucleotide repeats because of increased scoring error for di-nucleotides), allelic diversity, and allelic distribution (Blouin et al. 1996; O'Reilly et al. 1998; Bernatchez and Duchesne 2000). Using these criteria, we narrowed the pool of potential markers to 21 and screened the 2001 pair-matings ( $n = 388$ ) using these markers. Due to inconsistent scoring, presence of null alleles, and deviation from Hardy Weinberg expectations (HWE), 10 of these loci were excluded from subsequent analyses. Primer information, including range of allele sizes, repeat motif, annealing temperature and buffer pH, for the remaining 11 loci used in this study are listed in Table 1. Single-locus PCR amplifications were performed in 5  $\mu\text{L}$  reactions using 0.175 mM each of dNTP, 0.15  $\mu\text{M}$  each primer, and 0.025 U Taq polymerase (Promega) (see Table 1 for buffer and  $\text{MgCl}_2$  concentrations). PCR conditions were an initial denaturation at 94°C (3min), followed by a touchdown procedure involving four cycles of denaturing (94°C), annealing, and extension (74°C), where the initial annealing temperature was decreased by one °C $\cdot$ cycle<sup>-1</sup> (see Table 1 for initial and final annealing temperatures). After initial cycles, reactions were run for 30 cycles at the final annealing temperature.

Prior to electrophoresis, 0.8  $\mu$ L PCR product from 3-5 separate reactions were combined (Table 1) and mixed with a 4  $\mu$ L solution containing 62.5% formamide, 25% bromophenol blue, and 12.5% Genescan LIZ 500 size standard (Applied Biosystems). Microsatellite reactions were visualized with an ABI 3730xl Prism (Applied Biosystems) using fluorescently labeled forward primers and analyzed using GeneMapper Software v3.7 (Applied Biosystems). To minimize potential pipetting error (PCR and genotyping were performed using 384-welled plates), DNA and PCR products were transferred using a PlateMate Plus pipetting robot (Matrix Technologies).

Parentage analysis and estimating mean and variance in progeny number

We assessed the statistical power of our 11 loci for successful parentage analysis ( $\alpha = 0.20$  & 0.05) via simulations as implemented by CERVUS v2.0 (Marshall et al. 1998). CERVUS uses known allele frequency data to generate a pair of parental genotypes, plus a series of random genotypes representing unrelated candidate parents of one sex. Offspring are then produced by Mendelian sampling of the true parents' alleles. Once simulated parent and offspring genotypes were generated, we obtained an estimate of the number of loci needed for accurate parentage assignment assuming all candidate parents had been accurately genotyped. We estimated the proportion of loci typed (95%) from the known 2001 candidate parents and designated a genotyping error rate of 1.5%. For simulations, we based the genotyping error rate on previous published data sets (Bonin et al. 2004). We confirmed this value by matching known hatchery parents to hatchery returns ( $n = 384$ ) and assessing the proportion of mistyped loci for each correct assignment. CERVUS simulations were run for 10,000 cycles.

Parentage analyses were performed using exclusion and categorical allocation methods (Jones and Ardren 2003). Exclusion was implemented using WHICHPARENT v1.0 (W. Eichert, available at <http://www.bml.ucdavis.edu/facresearch/salmonsw.html> or by request) where the number of

mismatches was set to two. This setting was chosen because it produced the greatest number of true assignments based on preliminary runs that assigned known progeny to known hatchery brood stock. Categorical allocation, which involves calculating a logarithm of the likelihood ratio (LOD score) for any parentage relationship, was implemented using FAMOZ (Gerber et al. 2003). Simulations to calculate the LOD score threshold value for parentage assignment were implemented in FAMOZ as described by Gerber et al. (2003) (the intersection of the distributions was chosen as the threshold value). Simulations and actual parental assignments were conducted assuming a genotyping error rate of 1.5% per locus and an analysis error rate of 0.01% per locus (see Sancristobal and Chevalet 1997 and Gerber et al. 2000 for details about analysis error rate).

Once parent-offspring assignments were confirmed, we estimated the mean ( $\bar{k}$ ) and variance, ( $V$ ) in progeny number per family for MGHS and SGHS (separately for both brood years). We tested the hypothesis that there was no difference in mean progeny number/parent between SGHS and MGHS (each year analyzed separately) using a two tailed  $t$ -test (Sokal and Rohlf 1995). A test for homogeneity of variances between groups was performed using the  $F$ -test (Sokal and Rohlf 1995).

Assessment of nonrandom survival and realized vs. expected  $N_e$

Crow and Morton 1955) referenced  $V/\bar{k}$  as the index of variability ( $R$ ; Geiger et al. 1997; Waples 2002b). To assess whether survival is completely random from eyed-egg to adult life stage in hatchery coho salmon, we scaled  $R$  at the eyed-egg stage to the expected value assuming random survival in a population of constant size (i.e.,  $\bar{k} = 2$ ) using the equation

$$R^* \approx 1 + \bar{k}_2 \frac{(R-1)}{\bar{k}_1} \quad (2)$$

where  $R^*$  is the scaled index of variability,  $\bar{k}_1$  represents the observed mean progeny number per family, and  $\bar{k}_2$  is the expected mean progeny number per family (i.e., we assumed  $\bar{k}_2 = 2$ ). If survival were random from egg to adult stage with  $\bar{k}_2 = 2$ ,  $R^*$  at the eyed-egg stage ( $R_e^*$ ) should be similar to the observed value of  $R$  at the adult stage ( $R_a$ ). Therefore, a deviation between these two values ( $R_e^*$  and  $R_a$ ) is an indication of nonrandom survival among families.

An approximate expression for the ratio of expected  $N_e/N$  was calculated using the equation

$$\frac{N_e}{N} \approx \left[ \frac{\bar{k}_2}{1 + R^*} \right] \quad (3)$$

where  $\bar{k}_2$  is the expected mean progeny number per family (we assumed  $\bar{k}_2 = 2$ ) and  $N$  is the census size of the parent population (Waples 2002b). Note that  $N$  is not the census size of the cohort of adult offspring. Rather it is the census size of the parents that produced them (Waples 2005), and because the number of females equals the number of males, it is also the expected  $N_e$  of the offspring cohort given random family survival. Also note that estimates of  $N_e$  associated with family size data should be interpreted as estimates of the effective number of breeders ( $N_b$ ) per year (Waples and Teel 1990, Waples 2005). Depending on whether  $N_b/N$  was estimated for egg or adult stages, the parameter  $R^*$  was calculated as above using the respective value of  $\bar{k}_1$ . We assessed confidence in parameters  $R_e^*$ ,  $R_a$ ,  $R_a^*$ , and  $N_b/N$  by bootstrap resampling (Sokal and Rohlf 1995). Randomization tests (Sokal and Rohlf 1995) were used to investigate whether estimates of  $R_e^*$ ,  $R_a$ , and  $N_b/N$  were significantly different between SGHS and MGHS (each cohort analyzed separately).

## Results

Parentage simulations using CERVUS concluded that our predicted success rate for the 11 loci used in this study (when neither parent is known *a priori*) was 64% at  $\alpha = 0.05$  and 100% at  $\alpha = 0.20$ . We obtained similar estimates for the number of progeny/parent using exclusion and categorical assignment methods (the LOD threshold value for parentage assignment using FAMOZ was 8.0); therefore only results from WHICHPARENT are presented.

In 2001, the mean number of eggs/mating pair ( $\bar{k}_e$ ) for SGHS cohort collected for smolt production was 147 ( $V = 433.47$ ), and the mean number of eggs/mating pair sampled for smolt production for MGHS matings was 136 ( $V = 397.34$ ). In April 2003, 12 016 adipose left maxillary clipped (progeny from 2001 MGHS cohort) and 12 357 adipose and right maxillary clipped (progeny from 2001 SGHS cohort) smolts were released in the Calapooya Creek above Nonpareil Dam (Fig. 1).

The returning 2004 cohort was comprised of 62 marked jacks (one of which was dropped from subsequent analyses; see below) and 323 marked adults (seven of which were excluded from subsequent analyses) in 2004 (Table 2). Four of eight fish were excluded because WHICHPARENT assigned returns to a male and female that were not crossed in the hatchery. This discrepancy was probably due to spilling of gametes during hatchery spawning. One fish was excluded because it had <8 genotyped loci. Last, four fish, deemed unresolved, were deleted from further analyses because they could not be assigned to a specific mating pair, indicating that they may be age IV (but see below) or stray marked fish from another system.

The mean number of eggs/mating pair for 2002 SGHS cohort reared for smolt production was 138 ( $V = 276.66$ ) vs. 139 ( $V = 108.16$ ) for MGHS. In April 2004, 11 018 adipose left maxillary clipped (progeny from MGHS) and 10 979 adipose and right maxillary clipped (progeny from SGHS) smolts were released in the Calapooya Creek above Nonpareil Dam. The 2005 cohort was comprised of 68

marked jacks (two of which were eliminated from further analyses) and 687 marked adults (35 of which were excluded from subsequent analyses) in 2005 (Table 2). Seven returns were dropped from subsequent analyses – all a consequence of having mating strategies where WHICHPARENT assigned returns to a male of one mating pair with a female of the next mating pair. Twenty nine fish could not be assigned to a specific mating pair and were unassigned to the 2001 parental dataset indicating they were not age IV fish; therefore, we suspect that these fish were marked strays for reasons previously addressed.

The number of returning progeny assigned to the 2004 cohort was 214 MGHS and 163 SGHS. The 2005 cohort contained 391 and 328 assigned progeny, respectively. Calculated  $\bar{k}$ ,  $V$ ,  $R^*$ , and  $N_b/N$  for egg and adult stages are reported in Table 3. A statistically greater variance was found in MGHS than SGHS for the adult 2004 cohort, [ $V_{MGHS} = 3.62$ ,  $V_{SGHS} = 2.52$ ;  $F_{0.05(1)99,93} = 1.43$ ;  $P = 0.04$ ], but comparison between MGHS and SGHS were non-significant for the adult 2005 cohort [ $V_{MGHS} = 6.67$ ,  $V_{SGHS} = 5.58$ ;  $F_{0.05(1)99,99} = 1.39$ ;  $P = 0.18$ ]. Despite significant variances between MGHS and SGHS for the 2004 cohort, estimates of  $\bar{k}_a$  between groups were non-significant in both cohorts [2004 cohort  $t = 1.66$ ,  $t_{0.05(2),189} = 1.97$ ,  $P = 0.10$ ; 2005 cohort  $t = 1.80$ ,  $t_{0.05(2),198} = 1.97$ ,  $P = 0.07$ ]. Values of  $R_e^*$ ,  $R_a^*$  and  $N_b/N$  were not significantly different (all  $P > 0.05$ ) between MGHS and SGHS for each cohort; however, confidence intervals for  $R_e^*$  and  $R_a^*$  were non-overlapping for all within stock comparisons (Table 3) indicating non-random survival occurred between the egg and adult stages for the 2004 and 2005 cohorts. Likewise, confidence intervals were non-overlapping for all egg/adult  $N_e/N$  comparisons.

The expected  $N_b$  of the 2004 cohort, given random family survival, was 200 for MGHS and 188

for SGHS. The expected  $N_b$  for the 2005 cohort was 200 for both MGHS and SGHS. In contrast, the observed  $N_b$  for each cohort was always significantly (i.e., non-overlapping confidence intervals) less than expected (2004 cohort  $N_{b\text{MGHS}} = 152$ ,  $N_{b\text{SGHS}} = 148$ ; 2005 cohort  $N_{b\text{MGHS}} = 168$ ,  $N_{b\text{SGHS}} = 164$ ), indicating that on average a 20% reduction in  $N_b$  (caused by family-correlated survival) occurred in the life cycle of coho salmon during this study.

## Discussion

Our study found no difference in survival between two types of integrated (i.e., managed as a component of a natural population) hatchery programs – one that integrates 30% naturally reproducing fish with 70% hatchery fish vs. one that uses only naturally reproducing fish as brood. These findings seem to contradict previous studies (Fleming and Gross 1993; Berejikian et al. 1997; Berejikian et al. 2001) that fish maintained in a hatchery for multiple generations generally are less fit (the component of fitness being survival) than fish that have never experienced captive conditions (when environmental differences are eliminated). However, there is quite a distinction between present and previous studies. Previous studies have compared segregated hatchery brood fish (i.e., managed as if they are a distinct population relative to natural populations) to naturally reproducing ones; in contrast, our study compares two types of integrated hatchery programs. Furthermore, theoretical studies have indicated that hatchery programs with a one-way geneflow rate of 10-20% per generation quickly achieve the fitness-level of the donor population (Ford 2002; Lynch and O’Hely 2002). Our study provides empirical evidence indicating that relative survival of progeny from a multi-generation hatchery stock exposed to an integrated breeding program is similar to a naturally reproducing stock experiencing hatchery conditions for the first time. However, we urge caution when applying these findings to other such hatchery programs because findings are often context specific and depend on the type of brood stock used (integrated vs segregated), the breeding program (in this case single-matings and equalization of family

size), the amount of gene flow between stocks, and the past history of stocks.

Predictions regarding  $N_b$  are often calculated using sex-ratio information of the organism in question (Wright 1938; Waples and Teel 1990). Estimating  $N_b$  this way assumes that variance in reproductive success among males (or females) is random. Our data clearly show that this assumption is violated for hatchery-reared coho salmon. Both SGHS and MGHS groups had significantly greater variances in family size than would be expected based on random survival from egg to adult (i.e., confidence intervals for  $R_e^*$  do not overlie those of  $R_a$ ). Comparisons between values of  $R_a$  and  $R_e^*$  for MGHS and SGHS cohorts were not significantly different, suggesting that the extent of among-family selection, whether occurring in the hatchery or wild (see below), appears similar between MGHS and SGHS. These findings, which corroborate Waples (2002b), indicate that even in closely monitored hatchery operations, sex ratio information may be a poor indicator of  $N_b$  for hatchery reared coho salmon. Fortunately, non-random survival was not extreme enough to substantially reduce  $N_b$  – a finding similar to Waples (2002b) who also applied demographic data to predict  $N_b/N$  for coho salmon.

It is difficult to discern the exact stage where differential family-correlated survival occurred for both groups. Mortality can occur in the hatchery at the egg-presmolt stage, in freshwater as smolts migrate to estuarine habitat, or during estuarine/ocean phases. While this study was not intended to test where differential survival occurred, minimizing the variance in family size at the egg stage should reduce the effects of selection in captivity (Allendorf 1993). It is interesting that estimated survival from egg to released fry was approximately 89% and 80% for 2004 and 2005 cohorts, respectively (data not shown). As predicted, these estimates leave little room for family-correlated survival to occur at the hatchery stage (at least for the 2004 cohort) and suggest that differential survival among family groups transpired during smolt migration to estuarine habitat or during the

ocean stage of their life cycle – a finding similar to that of Hobday and Boehlert (2001) and Linely (2001).

Few studies have examined the demographic parameters necessary to compute  $N_b$  and  $N_b/N$  for hatchery salmon populations; therefore, the reduction in  $N_b$  below  $N$  is generally unknown. Summarizing  $\bar{k}$  and  $V$  in female families for five cohorts of hatchery reared coho salmon, Waples (2002b) showed that  $N_b/N$  ranged from 0.59-0.94 (mean = 0.76). These estimates may tend to underestimate the overall variance in  $\bar{k}$  because  $V$  was computed for only females; therefore,  $N$  may only approximate the expected  $N_b$  for the cohort. Nevertheless, our estimates of  $N_b/N$ , which ranged from 0.76-0.84 (mean = 0.8), are similar to those in Waples (2002b), suggesting that  $N_b$  for hatchery salmon might be generally predictable from knowledge of the number of parents used as broodstock, particularly when hatchery practices perform single-pair matings and equalize family size prior to release. In fact, when these data are averaged together (i.e, Waples 2002b and current study), a 22% ( $\pm 8\%$ ) reduction in  $N_b$  is predicted in the life cycle of hatchery coho salmon. Scaled adult estimates of  $N_b/N$  for other salmon species reared in captivity, which are  $>0.7$  for most cohorts (Hedrick et al. 2000; Waples 2002b), are similar to those for coho salmon, suggesting that this value (22%) may be used in conjunction with census data for calculating  $N_b$  of other salmon species.

While a 22% reduction in  $N_b$  might be predicted for coho salmon, this value, as a general predictor, should be used with caution. Any factor that causes selection among family groups can increase the variance in family size and subsequently reduce  $N_b$ . These factors can include (but are not limited to), artificial selection, size-selected predation, isolated disease outbreaks, and varying freshwater or ocean conditions – none of which are mutually exclusive. It should also be stressed that this study attempted to equalize/minimize the variance in family size at an early life stage and equalize sex ratios. Equalization of these parameters is promoted by conservation hatchery programs

as a means to maintain higher  $N_e$ ; therefore hatchery programs not attempting to equalize sex ratios or family size would expect on average a >22% reduction; how much greater depends on the level of variation among family groups and mating design (e.g., full factorial vs. 1:1 pair-matings)

In conclusion, our data and data in Waples (2002b) suggest a simple calculation for estimating one of three necessary parameters for predicting the Ryman-Laikre effect,  $N_h$ . More importantly, this parameter appears stable regardless of the type of integrated hatchery breeding program used by hatchery managers. Predicting the  $N_e$  of wild populations of salmon from census data has also been the subject of much study, and a rough rule of thumb for that parameter is also emerging (e.g., Waples 2002a). Much fewer data exist on the third parameter, the relative reproductive success of hatchery vs. wild fish when breeding in the wild (but see Araki et al. 2006). Reliable estimates of these parameters will allow fisheries professionals to predict the loss of genetic diversity associated with supportive breeding.

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Table 1. Description and PCR conditions of microsatellite loci used for this study. The abbreviation  $k$  represents the number of alleles per locus, and  $H$  is the observed heterozygosity. The exclusion probability is the average probability of excluding a single unrelated candidate parent from parentage of a given offspring. Loci with the same letter designate combined genotyping runs. The first annealing temperature number is the initial annealing temperature and the latter is the final annealing temperature (see text for details).

Marker	$k$	$H$	Range of alleles	Exclusion probability	Annealing temperature	MgCl <sub>2</sub>	Buffer pH	Reference
<i>OTS519</i> <sup>A</sup>	8	0.72	286-310	0.35	58-54	1.0	9.0*	Naish and Park 2002
<i>OTS520</i> <sup>A</sup>	24	0.87	190-252	0.72	58-54	1.0	8.5 <sup>†</sup>	Naish and Park 2002
<i>ONE111</i> <sup>A</sup>	6	0.84	180-190	0.32	52-48	2.5	9.0*	Olsen <i>et al.</i> 2000
<i>P53</i> <sup>A</sup>	19	0.91	165-197	0.73	58-54	1.0	9.0*	de Fromentel <i>et al.</i> 1992
<i>OTS3</i> <sup>B</sup>	10	0.89	142-162	0.53	58-54	1.0	9.0*	Banks <i>et al.</i> 1999
<i>ONEμ2</i> <sup>B</sup>	25	0.89	196-257	0.72	63-59	1.0	9.0*	Currens <i>et al.</i> 1997
<i>OCL8</i> <sup>B</sup>	20	0.88	90-121	0.65	50-46	1.5	9.0*	Condrey and Bentzen 1998
<i>OTS215</i> <sup>C</sup>	8	0.72	155-160	0.35	60-56	1.0	9.0*	M. Banks (unpublished)
<i>ONEμ13</i> <sup>C</sup>	15	0.81	194-236	0.57	60-56	1.5	9.0*	Scribner <i>et al.</i> 1996
<i>OMY1011</i> <sup>C</sup>	11	0.84	178-212	0.58	50-46	1.5	9.0*	P. Bentzen (unpublished)
<i>OKI23</i> <sup>D</sup>	20	0.80	120-180	0.74	60-56	1.5	8.5 <sup>†</sup>	A. Spidle (unpublished)

\* 1× Taq Reaction Buffer (Promega)

<sup>†</sup> 20 mM Tris, pH 8.5 and 50 mM KCl (Williamson *et al.* 2002)

Table 2. Count of hatchery coho passed above Nonpareil Dam (Calapooya Creek) for 2004-2005 cohorts. Comparisons are between returns from single- and multi-generation hatchery stocks (SGHS and MGHS, respectively). Individuals excluded from analyses are as follows: progeny that were unassigned to a known parental array (Unresolved), and progeny having fewer than eight genotyped loci or were returns from incorrect hatchery matings (see text for details).

	Male	Jack	Female	Unresolved	Dropped	Total
2004 cohort						
SGHS	69	23	71	2 (1M, 1J)	2 (2M)	167
MGHS	98	38	78	2 (1M, 1F)	2 (2M)	218
2005 cohort						
SGHS	121	38	169	20 (4M, 1J, 15F)	0	348
MGHS	174	28	189	9 (2M, 1J, 6F)	7 (2M, 5F)	407

Table 3. Estimated demographic parameters for juvenile and adult coho salmon from 2001 and 2002 hatchery stocks. Comparisons are between single- and multi-generation brood stocks (SGHS and MGHS, respectively). Subscripts represent the egg (e) and adult (a) life stages. Mean and variance in progeny number estimated from parentage analysis are designated as  $k$  and  $V$ , respectively. The parameter  $R$  is the observed index of variability (Crow and Morton 1955) and is calculated as  $V/k$ . The asterisk denotes scaled values of  $R$  and effective size to census size ( $N_b/N$ ) obtained from equations 2 and 3, respectively. Confidence intervals are reported in parenthesis. The designation n/a indicates confidence values that were unascertainable via bootstrapping due to the small variance among replicates.

	Egg					Adult				
	$k_e$	$V_e$	$R_e$	$R_e^*$	$N_b/N^*$	$k_a$	$V_a$	$R_a$	$R_a^*$	$N_b/N^*$
MGHS-2001	126.20	424.56	3.36	1.03 (1.00-1.08)	0.98 (0.96-1.00)	2.14	3.62	1.68 (1.25-2.00)	1.64 (1.27-1.97)	0.76 (0.66-0.86)
SGHS-2001	137.80	469.85	3.40	1.03 (1.00-1.09)	0.98 (0.95-1.00)	1.72	2.52	1.46 (1.11-1.79)	1.53 (1.15-1.90)	0.79 (0.69-0.92)
MGHS-2002	138.96	108.16	0.79	0.99 (n/a-1.02)	1.00 (0.99-n/a)	3.84	6.67	1.73 (1.25-2.13)	1.38 (1.16-1.57)	0.84 (0.77-0.93)
SGHS-2002	137.66	273.92	2.00	1.01 (n/a-1.05)	0.99 (0.97-n/a)	3.13	5.58	1.67 (1.30-2.05)	1.43 (1.17-1.63)	0.82 (0.76-0.92)

Figure 1. Map of the Umpqua basin, Oregon. Right caption depicts evolutionary significant units for coastal coho salmon, comprising Northern California, Oregon (shaded black), and Washington. Our study involved sampling natural and hatchery coho in the North Umpqua at Winchester Dam, spawning and rearing offspring at Oregon's Department of Fish and Wildlife Rock Creek Hatchery facility, and releasing smolts above Nonpareil Dam on Calapooya Creek. All returning progeny were sampled at a fish trap located at Nonpareil Dam, genotyped using 11 microsatellite loci, and assigned to most likely broodstock through parentage analysis.

