Completion Report to:

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OSU Component for Nonpareil Dam Adult Trap and Genetic Pedigree

COMPLETION REPORT for Contract 209-904

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OSU Component for Nonpareil Dam Adult Trap and Genetic Pedigree

This writing concerns a genetic pedigree study of hatchery and wild spawning coho salmon of the Umpqua River in southern Oregon. Graduation of two post docs, one PhD and one MS student, a total of eight peer-review writings already published, and a ninth manuscript in final preparation, present primary products from this research. Efforts were focused towards eight tasks originally detailed in the full CHIP (Conservation Hatchery Incentive Project) proposal:

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?*

We found: No significant difference in overall survival was observed between progeny from single-generation hatchery spawning and rearing of naturally produced coho (*vis a vis* supplementation programs) and progeny from a multiple-generation integrated hatchery stock. But see below under task 3 for evidence on the relative fitness of these two progeny types when they later spawned along with wild fish in a natural context.

Edited from published abstract.

For peer-review see attached below:

Moyer, G. R., M. S. Blouin, and M. A. Banks. 2007. The influence of family-correlated survival on *Nb/N* for progeny from integrated multi- and single-generation hatchery stocks of coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Fisheries and Aquatic Science. 64: 1258-1265.*

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

We Found: Mean adult survival of individuals released as unfed fry was less than individuals released as smolts (0.03% vs. 2.39%). The relative reproductive success (RRS) of the fry release strategy to wild spawning was significantly greater for one of two cohorts while the smolt release strategy to wild RRS was significantly greater for both cohorts. Fish released as smolts were significantly smaller upon returning as adults than unfed fry or wild returns. Mean run timing was also significantly biased towards an earlier run time for hatchery released fish when compared to the wild component. The incidence of jacking (males maturing at the age of 2) was greater among fish stocked as smolts than for fish stocked as fry. Differences in survival, RRS, and life history appeared the result of hatchery practices and indicated that a fry stocking strategy produced fish more similar to the wild component of the population than that of fish released as smolts.

Edited from published abstract.

For peer-review see attached below:

Thériault, V., G. R. Moyer, and M.A. Banks. 2010. Survival and life history characteristics among wild and hatchery coho salmon (*Oncorhynchus kisutch*) returns: how do unfed fry differ from smolt releases? *Canadian Journal of Fisheries and Aquatic Sciences*. 67:486-497.

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.

We Found: That hatchery coho salmon had lower reproductive success than wild fish once they reproduced in the wild. This effect was more pronounced in males than in same-aged females. Hatchery spawned fish that were released as unfed fry (age 0), as well as hatchery fish raised for one year in the hatchery (released as smolts, age 1), both experienced lower lifetime reproductive success (RS) than wild fish. However, the subset of hatchery males that returned as 2-year olds (jacks) did not exhibit the same fitness decrease as males that returned as 3-year olds. Thus, we report three lines of evidence pointing to the absence of sexual selection in the hatchery as a contributing mechanism for fitness declines of hatchery fish in the wild: (i) hatchery fish released as unfed fry that survived to adulthood still had low RS relative to wild fish, (ii) age-3 male hatchery fish consistently showed a lower relative RS than female hatchery fish (suggesting a role for sexual selection), and (iii) age-2 jacks, which use a sneaker mating strategy, did not show the same declines as 3-year olds, which compete differently for females (again, implicating sexual selection).

Edited from published abstract.

For peer-review see attached below:

Thériault, V., G. R. Moyer, L.S. Jackson, M.S. Blouin, and M.A. Banks. 2011. Reduced reproductive success of hatchery coho salmon in the wild: insights into most likely mechanisms. *Molecular Ecology*. 20(9): 1860–1869.

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)*

We Found: Observed N_e was significantly less than expected for each of the hatchery reared stocks (either for single-generation hatchery spawning and rearing of naturally produced coho (*vis a vis* supplementation programs) or for a multiple-generation integrated hatchery 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 the effective number of hatchery parents in applications of the Ryman–Laikre formula (at least for programs such as ours that attempt to equalize sex ratios and family sizes). *Note that comparisons reported here under task 4 are from early assessments of first generation return progeny and do not include findings from when these stock spawn along with truly 'wild' coho in a natural spawning context (as was achieved under task 3 above).*

Edited from published abstract.

For peer-review see attached below:

Moyer, G. R., M. S. Blouin, and M. A. Banks. 2007. The influence of family-correlated survival on *Nb/N* for progeny from integrated multi- and single-generation hatchery stocks of coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Fisheries and Aquatic Science. 64: 1258-1265.*

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

Not assessed explicitly: We submit, however, that this particular study is not suited to this question. The Umpqua multiple-generation hatchery stock has been managed using a 30% wild-broodstock integrated approach. This wild-brood used to 'integrate' the hatchery stock is the very same 'wild stock' as used for the single generation naturally spawned single-generation hatchery stock. Effective population size or inbreeding levels of the two stocks considered in this study are thus not likely markedly different as was indeed noted in Moyer et al. 2007. Other hatchery/wild stock situations exist, however, where N_e (effective population size) and likely inbreeding levels differ largely between hatchery and wild. Such a situation would provide a much better context to address this question.

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;*

We Found: Evidence for reduced reproductive success (RS) of wild spawning hatchery-reared fish invites serious consideration with regard to the detrimental effects on subsequent generations of wild populations. Mate choice was evaluated as a potential mechanism contributing to these observed RS differences using a previous pedigree of wild spawning hatchery-reared and wild origin coho salmon (*Oncorhynchus kisutch*). Genetic variance at immune-relevant genes was used as a metric to examine mate choice. Two years (2005 and 2006) of three wild spawning mate pair classes were examined: wild x wild (W x W), hatchery x hatchery (H x H), and wild x hatchery (W x H). We tested for: (1) a departure from random expectations with regard to mate pair allelic diversity at immune-relevant markers, (2) a correlation between immune-relevant gene diversity and mate pair RS, and (3) distinguishable differences between mate choice strategies used by hatchery-reared and wild origin coho. Eight immune-relevant gene-linked microsatellite markers were used to evaluate mate choice; four linked to immune-relevant expressed sequence tags (ESTs) and four linked to the Major Histocompatibility Complex (MHC). We found evidence for non-random mating between 2006 W x H mate pairs at *BHMS429*, an MHC-linked marker, and at *SsalR016TKU*, an immune-relevant EST marker, which is homologous to a vasodilator-stimulated phosphoprotein. Non-random mating was also evident between 2005 H x H pairs at *SsalR015TKU*, an immune-relevant EST, though no homology was found. All other pair classes did not display a significant mate choice signature. We found a significant correlation between mate pair RS and immune gene diversity among 2005 and 2006 W x W mate pairs as well as 2006 W x H

mate pairs. Notably, H x H mate pair RS was not correlated to immune gene diversity in either year. Results suggest that mate choice and genetic compatibility may influence fitness of wild spawning coho.

Abstract from Amelia's thesis

For peer-review see:

Amelia C. Whitcomb 2012. Mate choice of wild spawning coho salmon (Oncorhynchus kisutch) in the Umpqua River, Oregon. MS thesis. http://ir.library.oregonstate.edu/xmlui/handle/1957/35596?show=full

Peer-review manuscript is in final preparation for Molecular Ecology

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)?*

We Found: That hatchery coho salmon (*Oncorhynchus kisutch*) had lower reproductive success than wild fish once they reproduced in the wild. This effect was more pronounced in males than in same-aged females. Hatchery spawned fish that were released as unfed fry (age 0), as well as hatchery fish raised for one year in the hatchery (released as smolts, age 1), both experienced lower lifetime reproductive success (RS) than wild fish. However, the subset of hatchery males that returned as 2-year olds (jacks) did not exhibit the same fitness decrease as males that returned as 3-year olds. Fish released as smolts were significantly smaller upon returning as adults than unfed fry or wild returns. Mean run timing was also significantly biased towards an earlier run time for hatchery released fish when compared to the wild component. The incidence of jacking (males maturing at the age of 2) was greater among fish stocked as smolts than for fish stocked as fry. Differences in survival, RRS, and life history appeared the result of hatchery practices and indicated that a fry stocking strategy produced fish more similar to the wild component of the population than that of fish released as smolts.

Edited from two published abstract

For peer-review see:

Thériault, V., G. R. Moyer, L.S. Jackson, M.S. Blouin, and M.A. Banks. 2011. Reduced reproductive success of hatchery coho salmon in the wild: insights into most likely mechanisms. *Molecular Ecology*. 20(9): 1860–1869.

Thériault, V., G. R. Moyer, and M.A. Banks. 2010. Survival and life history characteristics among wild and hatchery coho salmon (*Oncorhynchus kisutch*) returns: how do unfed fry differ from smolt releases? *Canadian Journal of Fisheries and Aquatic Sciences*. 67:486-497.

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?*

We did not address this task explicitly: However, overall pedigree findings provide conclusive evidence that all classes of hatchery spawned and reared fish considered in this study (both fry- and smolt-releases from single and multiple generation hatchery stocks) indeed contribute to returns and demonstrated that some of them mate (either as H x H or H X W) to provide adult progeny among returns of later years.

For peer-review see:

Thériault, V., G. R. Moyer, L.S. Jackson, M.S. Blouin, and M.A. Banks. 2011. Reduced reproductive success of hatchery coho salmon in the wild: insights into most likely mechanisms. *Molecular Ecology*. 20(9): 1860–1869.

Thériault, V., G. R. Moyer, and M.A. Banks. 2010. Survival and life history characteristics among wild and hatchery coho salmon (Oncorhynchus kisutch) returns: how do unfed fry differ from smolt releases? *Canadian Journal of Fisheries and Aquatic Sciences*. 67:486-497.

Moyer, G. R., M. S. Blouin, and M. A. Banks. 2007. The influence of family-correlated survival on *Nb/N* for progeny from integrated multi- and single-generation hatchery stocks of coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Fisheries and Aquatic Science. 64: 1258-1265.*

Amelia C. Whitcomb 2012. Mate choice of wild spawning coho salmon (Oncorhynchus kisutch) in the Umpqua River, Oregon. MS thesis. http://ir.library.oregonstate.edu/xmlui/handle/1957/35596?show=full

Other peer-review manuscripts that received support from this project include:

Johnson M.A., and M.A. Banks. 2011. Sequence conservation among orthologous vomeronasal type 1 receptor-like (Ora) genes does not support the differential tuning hypothesis in Salmonidae. *Gene. 485(1):16-21.*

Marc A. Johnson. 2009. Patterns of Natural Selection and demography in Coastal Oregon Coho salmon (*Oncorhynchus kisutch)* Populations: Evidence from Neutral and Olfactory receptor Gene-linked Markers. PhD thesis. http://ir.library.oregonstate.edu/jspui/handle/1957/11595

Johnson, M.A. and M.A. Banks. 2009. Interlocus variance of Fst provides evidence for directional selection over an olfactory receptor gene in Coho salmon (Oncorhynchus kisutch) populations *Marine Genomics* 2(2):127-131 [+]Among top 25 Articles in Earth & Planetary Science > Marine Genomics October to December 2009 http://top25.sciencedirect.com/subject/earth-and-planetary-sciences/9/journal/marine-genomics/18747787/archive/24/.

Johnson, M.A., and M. A. Banks. 2008. Genetic structure, migration and patterns of allelic richness among coho salmon (*Oncorhynchus kisutch*) populations of the Oregon Coast. *Canadian Journal of Fisheries and Aquatic Science* 75(7): 1274-1285.

Regarding intellectual and people resource development from this project:

Dr. Greg Moyer (Post Doc) is a Regional Geneticist, US Fish & Wildlife Service, Warm Springs Fish Technology Center, Conservation Genetics, 5308 Spring Street, Warm Springs, Georgia.

Dr. Veronique Theriaulst (Post Doc) is Biologist, Ministry of Natural Resources Quebec Government, Nord-du-Quebec, Chibougamau.

Dr. Marc A Johnson (PhD student) is a Technical Analyst, Willamette BiOp Programs, Oregon Department of Fish and Wildlife, Corvallis Research Lab

Ms Amelia Whitcomb (MS student) is currently revising her research write-up for peer-review.

I would like to acknowledge sincere gratefulness to 1) OWEB for funding this project, 2) Michael Blouin for project conception and consistent contributions along the way, 3) ODFW partners for substantial contribution in breeding, rearing and monitoring these fish and 4) Dr. Kathleen O'Malley for co-major professor advising Amelia Whitcomb's study. I am also grateful that both Amelia and Marc Johnson's graduate research projects were co-supported by other fund sources.

We are currently archiving full pedigree, life history and genotype data as well as all nine peer-review writings detailed above:

http://cdss.library.oregonstate.edu/data-services

http://ir.library.oregonstate.edu/xmlui/

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*)

G.R. Moyer, M.S. Blouin, and M.A. Banks

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 effective population size (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 multigeneration stock obtained by spawning 70% hatchery with 30% naturally reproducing fish, whereas 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 the effective number of hatchery parents in applications of the Ryman–Laikre formula (at least for programs such as ours that attempt to equalize sex ratios and family sizes).

Résumé : Il existe étonnamment peu de données sur la variance et la moyenne finales des tailles des familles au stade adulte chez les poissons nés en pisciculture. Il est ainsi difficile, dans une pisciculture de conservation qui minimise la variance des nombres dans la progéniture, de prédire de combien inférieure sera la véritable taille effective de la population (N_e) d'une cohorte d'adultes nés en pisciculture par rapport au N_e prédit simplement par le nombre des parents qui les ont produits. Nous utilisons une analyse de parenté pour estimer la survie et le N_e chez deux stocks intégrés de saumons coho (*Oncorhynchus kisutch*) de pisciculture. Une des piscicultures utilise un stock de plusieurs générations obtenu en croisant 70 % de poissons de pisciculture avec 30 % de poissons à reproduction naturelle, alors que l'autre est formé d'un seul stock de saumons coho à reproduction naturelle. Il n'y a pas de différence de survie globale entre les stocks, mais le N_e observé est significativement inférieur à la valeur attendue pour chacun des stocks. La survie en fonction de la famille contribue grosso modo 20 % de la réduction de N_e au cours des stades du cycle en eau douce et en mer. Cette réduction est semblable à celle des estimations antérieures, ce qui fournit une valeur qui peut être utilisée pour calculer le nombre effectif de parents de pisciculture lors de l'application de la formule de Ryman–Laikre (au moins dans les programmes comme le nôtre qui cherchent à égaliser les rapports mâles–femelles et les tailles des familles).

[Traduit par la Rédaction]

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). Although 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

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

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(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, where 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, 2002*a*; 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 brood stock 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 brood stock that produced them. For example, if 200 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, $N = 400$. Nonrandom (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 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 brood stock or have integrated captive with naturally born brood stock (Mobrand et al. 2005). It is unknown whether the mean ($\bar{k}$) and variance (V) in final family size differ between the two brood-stock strategies adopted by conservation hatcheries. Differences, if large, might cause one to substantially under- or over-estimate the effective size for a cohort of single-generation stock, relative to 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 multigeneration 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-generation and multigeneration stocks, with both stocks experiencing similar reductions in N_e. This reduction was significantly less than expected, indicating that nonrandom survival occurred throughout the freshwater–marine life stage. Our data suggest that the hatchery coho (*Oncorhynchus kisutch*) 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 multigeneration hatchery origin. In fall and early winter 2001, the Oregon Department of Fish and Wildlife (ODFW) collected 100 female and 100 male coho salmon having marked adipose fins (considered multigeneration brood 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 single-generation brood 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 the multigeneration brood stock 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). Single-generation broods 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 per mating pair and rearing them to the smolt stage (progeny from MGHS and SGHS were reared separately). Although 140–150 eggs per mating pair was a goal, our observed mean in eyed eggs per mating pair was less than expected (see Results) because of high egg mortality from a few mating pairs. As a result, our variance in progeny number was also greater than expected.

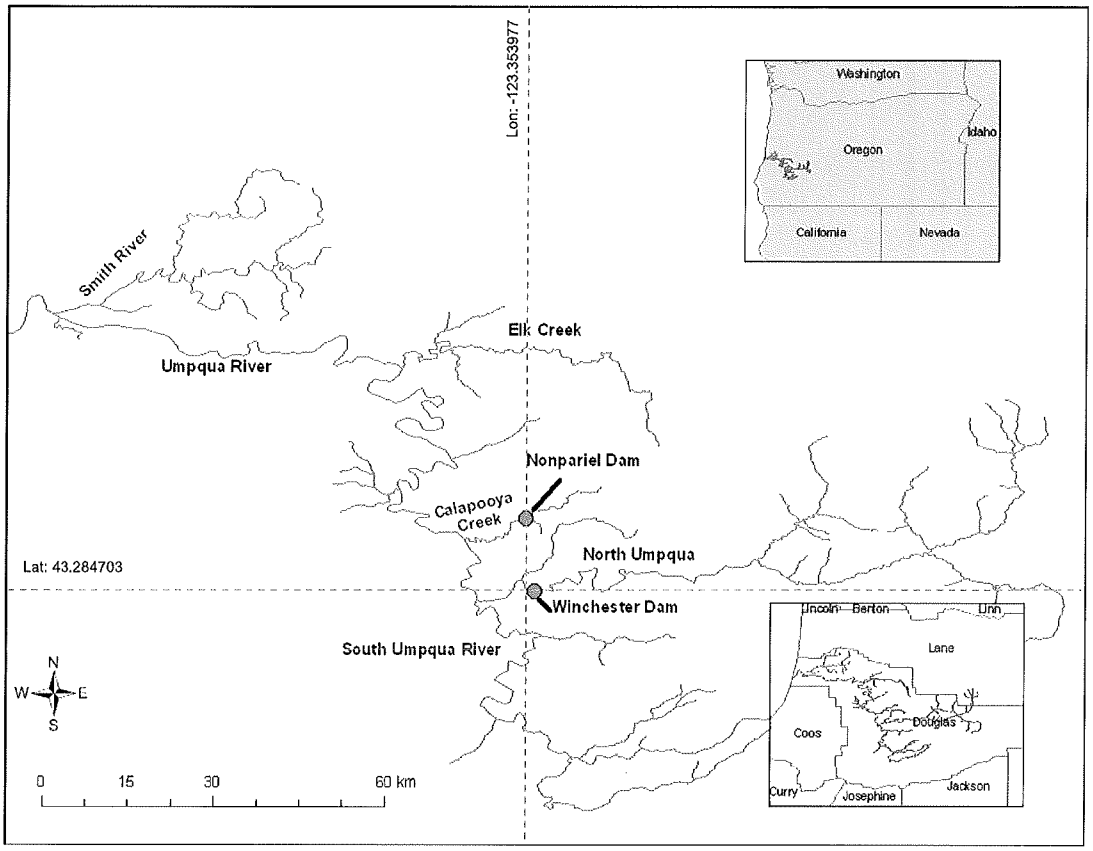
Sampling design — year 2002

Sampling design for the 2002 brood stock was similar to that of 2001. However, the SGHS were 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 conceivable that some of these fish historically could be of hatchery origin.

In fall and early winter 2002, ODFW personnel collected 100 female and 100 male coho salmon having unmarked adipose fins (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 left maxillary or adipose right maxillary to designate progeny from MGHS or SGHS, respectively. Coho have a 3-year life cycle. Mature

Fig. 1. Map of the Umpqua basin, Oregon, USA. 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 brood stock through parentage analysis.



adults typically return to spawn at age 3, but reproductively mature males, called jacks, can return at age 2. 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 2002 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 (~4–24 $ng \cdot \mu L^{-1}$) was quantified using a Victor^{3}V 1420 multilabel counter (PerkinElmer Inc., Boston. Mass.).

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 (tetranucleotides were chosen over dinucleotide repeats because of increased scoring error for dinucleotides), 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. Because of inconsistent scoring, presence of null alleles, and deviation from Hardy–Weinberg expectations (HWE), 10 of these loci where 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 polymerase chain reaction (PCR) amplifications were performed in 5 µL reactions using 0.175 $mmol \cdot L^{-1}$ each of dNTP, 0.15 $\mu mol \cdot L^{-1}$ each primer, and 0.025 U *Taq* polymerase (1 U = 16.67 nkat; Promega Corp., Madison, Wis.) (see Table 1 for buffer and $MgCl_2$ concentrations). PCR conditions were an initial denaturation at 94 °C (3 min), 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 1 $°C \cdot cycle^{-1}$ (see Table 1 for initial and final annealing temperatures). After initial cycles, reactions were run for 30 cycles at the final annealing temperature.

Before electrophoresis, 0.8 µL PCR product from three to five separate reactions were combined (Table 1) and mixed

Table 1. Description and polymerase chain reaction (PCR) conditions of microsatellite loci used for this study.

Marker	k	H	Range of alleles	Exclusion probability	Annealing temperature (°C)	$MgCl_2$ ($mmol \cdot L^{-1}$)	Buffer pH	Reference
OTS519[A]	8	0.72	286–310	0.35	58–54	1.0	9.0*	Naish and Park 2002
OTS520[A]	24	0.87	190–252	0.72	58–54	1.0	8.5†	Naish and Park 2002
ONE111[A]	6	0.84	180–190	0.32	52–48	2.5	9.0*	Olsen et al. 2000
P53[A]	19	0.91	165–197	0.73	58–54	1.0	9.0*	de Fromentel et al. 1992
OTS3[B]	10	0.89	142–162	0.53	58–54	1.0	9.0*	Banks et al. 1999
ONEμ2[B]	25	0.89	196–257	0.72	63–59	1.0	9.0*	Currens et al. 1997
OCL8[B]	20	0.88	90–121	0.65	50–46	1.5	9.0*	Condrey and Bentzen 1998
OTS215[C]	8	0.72	155–160	0.35	60–56	1.0	9.0*	M. Banks (unpublished data)
ONEμ13[C]	15	0.81	194–236	0.57	60–56	1.5	9.0*	Scribner et al. 1996
OMY1011[C]	11	0.84	178–212	0.58	50–46	1.5	9.0*	P. Bentzen (unpublished data)‡
OKI23[D]	20	0.80	120–180	0.74	60–56	1.5	8.5†	A. Spidle (unpublished data)§

Note: 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.

*1× *Taq* reaction buffer (Promega).

†20 $mmol \cdot L^{-1}$ Tris, pH 8.5, and 50 $mmol \cdot L^{-1}$ KCl (Williamson et al. 2002).

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with a 4 μL solution containing 98% formamide and 2% Genescan LIZ 500 size standard (Applied Biosystems, Foster City, Calif.). 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 pippetting robot (Matrix Technologies, Hudson, N.H.).

Parentage analysis and estimating mean and variance in progeny number

We assessed the statistical power of our 11 loci for successful parentage analysis ($\alpha = 0.20$ and 0.05) via simulations as implemented by Cervus v3.0 (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 per parent between SGHS and MGHS (each year was 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 2002*b*). 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

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

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 $k_2 = 2$). If survival was random from egg to adult stage with

Table 2. Count of hatchery coho passed above Nonpareil Dam (Calapooya Creek) for 2004–2005 cohorts.

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

Note: M, male; J, jack; F, female. 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 (Dropped; see text for details).

$\bar{k}_2 = 2$, 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

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

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 2002*b*). 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 was analyzed separately).

Results

Parentage simulations using Cervus concluded that our predicted success rate for the 11 loci used in this study (when sexes are known a priori) was 100% at $\alpha = 0.05$. We obtained similar estimates for the number of progeny per 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 per mating pair ($\bar{k}_e$) for SGHS cohort collected for smolt production was 147 ($V = 433.47$), and the mean number of eggs per 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 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) (Table 2). Four of eight fish were deemed dropped and excluded from further analyses. Three were probably due to spilling of gametes during hatchery spawning (WhichParent assigned returns to a male or female but not to a known mating pair), and one had less than eight genotyped loci scored. The 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 4 (but see below) or stray marked fish from another system.

The mean number of eggs per 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 right maxillary clipped (progeny from SGHS) smolts were released in the Calapooya Creek above Nonpareil Dam. The returning 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) (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 data set, indicating they where not age-4 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 in 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 was nonsignificant 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 vari-

Table 3. Estimated demographic parameters for juvenile and adult coho salmon from 2001 and 2002 hatchery stocks.

	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	136	397.34	2.92	0.97 (1.00–1.08)	1.02 (0.96–1.00)	2.14	3.62	1.68 (1.25–2.00)	1.64 (1.27–197)	0.76 (0.66–0.86)
SGHS 2001	147	433.47	2.94	0.97 (1.00–1.09)	1.02 (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	139	108.16	0.78	1.00 (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	138	273.92	1.99	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)

Note: 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 eqs. 2 and 3, respectively. Confidence intervals are reported in parenthesis. The designation "n/a" indicates confidence values that were unascertainable via bootsrapping due to the small variance among replicates.

ances between MGHS and SGHS for the 2004 cohort, estimates of $\bar{k}_a$ between groups were nonsignificant 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 nonsignificant (all $P > 0.05$) between MGHS and SGHS for each cohort; however, confidence intervals for R_e^* and R_a^* were nonoverlapping for all within-stock comparisons (Table 3), indicating that nonrandom survival occurred between egg and adult stages for the 2004 and 2005 cohorts. Likewise, confidence intervals were nonoverlapping for all egg per adult N_e/N comparisons (Table3).

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., nonoverlapping confidence intervals) less than expected (2004 cohort, $N_{b\ MGHS} = 152$, $N_{b\ SGHS} = 148$; 2005 cohort, $N_{b\ MGHS} = 168$, $N_{b\ SGHS} = 164$), 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 only uses naturally produced fish as brood stock. These findings seem to contradict those of previous studies (Fleming and Gross 1993; Berejikian et al. 1997, 2001), i.e., 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. However, there is quite a distinction between present and previous studies. Previous studies compared segregated hatchery brood fish (i.e., managed as if they are a distinct population relative to natural populations) with 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 gene-flow rate of 10%–20% per generation can quickly achieve the fitness level of the donor population (Ford 2002; Lynch and O'Hely 2001). Our study provides empirical evidence indicating that relative survival of progeny from a multigeneration hatchery stock exposed to an integrated breeding program is similar to that of 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 conclusions 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 brood-stock history.

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 (2002*b*), 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, nonrandom survival was not extreme enough to substantially reduce N_b, a finding similar to that of Waples (2002*b*), who also applied demographic data to predict N_b/N for coho salmon.

It is difficult to discern the exact stage at which 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. Although 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 those of Hobday and Boehlert (2001) and Linley (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 (2002*b*) showed that N_b/N ranged from 0.59 to 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 to 0.84 (mean = 0.8), are similar to those of Waples (2002*b*), suggesting that N_b for hatchery salmon might be generally predictable from knowledge of the number of parents used as brood stock, 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 (2002*b*) and current study), a 22% (±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 2002*b*), are similar to those for coho salmon, suggesting that this value (22%) may be used in conjunction with census data for calculating N_h of other salmon species.

Although 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 is mutually exclusive. It should also be stressed that this study attempted to equalize or 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 these parameters 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 (2002*b*) 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 2002*a*). 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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Survival and life history characteristics among wild and hatchery coho salmon (*Oncorhynchus kisutch*) returns: how do unfed fry differ from smolt releases?

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Abstract: Survival and life history characteristics were evaluated for a coho salmon (*Oncorhynchus kisutch*) integrated hatchery program using two stocking strategies. Fish were released as unfed fry or smolts and returned as adults, and then molecular analysis was employed to pedigree the entire population. We showed that mean adult survival of individuals released as unfed fry was less than that of individuals released as smolts (0.03% vs. 2.39%). The relative reproductive success (RRS) of the fry release strategy to wild spawning was significantly greater for one of two cohorts, whereas the smolt release strategy to wild RRS was significantly greater for both cohorts. Fish released as smolts were significantly smaller upon returning as adults than either those released as unfed fry or wild returns. Mean run timing was also significantly biased towards an earlier run time for hatchery-released fish when compared with the wild component. The incidence of jacking (males maturing at age 2) was greater among fish stocked as smolts than for fish stocked as fry. Differences in survival, RRS, and life history appeared to be the result of hatchery practices and indicated that a fry stocking strategy produced fish more similar to the wild component of the population than to that of fish released as smolts.

Résumé : Nous avons déterminé la survie et les caractéristiques du cycle biologique de saumons coho (*Oncorhynchus kisutch*) dans un programme de pisciculture intégré qui utilise deux stratégies d'empoissonnement. Les poissons ont été ensemencés comme alevins ou saumoneaux à jeun et recapturés au stade adulte; des analyses moléculaires ont alors servi à établir la filiation de toute la population. Nous avons démontré que les poissons ensemencés au stade alevin à jeun avaient un taux de survie moyen jusqu'à l'âge adulte inférieur à celui des poissons ensemencés au stade saumoneau (0,03 % vs 2,39 %). Le succès reproducteur relatif (RRS) de la stratégie alevin par rapport aux individus en milieu naturel était supérieur pour une année, mais inférieur pour l'autre année. Le RRS de la stratégie saumoneau était supérieur pour les deux années. Les poissons ensemencés au stade saumoneau avaient une taille corporelle inférieure à l'âge adulte à celle des poissons relâchés en tant qu'alevins à jeun et des poissons sauvages. La date de montaison moyenne était significativement devancée pour les poissons d'élevage. L'incidence de la maturation sexuelle précoce (« jacking ») était plus grande parmi les poissons ensemencés au stade saumoneau que parmi les poissons relâchés au stade alevin. Ces différences dans la survie, le RRS et les traits d'histoire de vie semblent découler des pratiques d'élevage en captivité et indiquent que la stratégie d'ensemencement d'alevins produit des poissons qui sont plus semblables à l'âge adulte aux poissons sauvages que les ensemencements de saumoneaux.

[Traduit par la Rédaction]

Introduction

Worldwide declines in anadromous salmonid populations have led to the widespread use of hatchery propagation in efforts to boost population size and recover threatened or endangered populations. However, there are ecological and genetic risks associated with these substantial and sustained releases of hatchery fish (Ford et al. 2006; Kostow 2009). Integrated hatchery programs that obtain a proportion of brood stock from the local population (Goodman 2005) have been applied to mitigate potential negative genetic impacts of traditional hatchery practices (e.g., reduction of genetic diversity, local maladaptation, domestication selection, and outbreeding depression; reviewed by Araki et al. 2008; Hutchings and Fraser 2008). Despite the theoretical prediction that genetic risks should be lower in these state-of-the-art conservation hatchery programs, recent empirical evidence (Araki et al. 2007, 2008, 2009) has shown that as lit-

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tle as one generation in captivity is sufficient to generate differential reproductive success (RS) between hatchery and wild components. Although captive rearing may generate differences in important life history traits likely to affect reproductive success (Knudsen et al. 2006; Fritts et al. 2007), causal genetic and (or) environmental mechanism(s) responsible for these differences remain unclear.

Salmonid populations have been supplemented at various life stages (fertilized eggs to adults) in the past century. The choice of which life stage to stock depends on various tradeoffs between the species and systems considered, the hatchery capability, the likelihood and environmental costs of artificial selection, budgetary considerations, and the relative survival and return rate of fish released at different life stages (Letcher and Terrick 2001). In Oregon, USA, coho salmon (*Oncorhynchus kisutch*) were stocked exclusively as fry from 1890 until 1940. Improvements in hatchery practices and the finding that smolts (the life stage in which juvenile salmon first migrate from freshwater to marine environments) had a higher postrelease survival led to an increased number of smolt releases by the 1950s (Solazzi et al. 1999). Smolt releases are now the dominant stocking strategy used by most federal and state agencies for salmonids of the Pacific Northwest. However, fry releases still occur regularly in Oregon (Kostow 2009) because of the popularity of Oregon Department of Fish and Wildlife's (ODFW) Salmon and Trout Enhancement Program (STEP), which was implemented in 1981 as a means to engage the public in the restoration of salmonid species.

Despite decades of implementation, few attempts have been made to evaluate the efficiency of fry stocking programs, primarily because it is difficult to tag fry and track them through adulthood using conventional methods such as fin clipping or coded-wire tagging. Previous studies that evaluated the efficiency of unfed fry releases or presmolt releases (i.e., young-of-the-year juveniles fed in the hatchery for a few months before release) concluded that they were not effective at increasing either the rearing density of juvenile coho salmon or adult returns (McGie 1980; Nickelson et al. 1986; Solazzi et al. 1999). One caveat to these studies was the use of out-of-basin and multigenerational hatchery-origin stocks that could confound the limited success of stocking young life stages with poor performance of maladapted stocks (Hutchings and Fraser 2008). More recently, Caroffino et al. (2008) used a genetic parentage reconstruction approach and local-origin brood stock to conclude that hatchery steelhead (*Oncorhynchus mykiss*) whose offspring were released as unfed fry produced more age-2 juveniles than adults reproducing in the wild. Theoretically, if domestication selection that occurs during rearing reduces mean fitness of the hatchery population, then fish released at an earlier life stage (e.g., the fry stage) should be better adapted to the natural environment than releases of later life stages because there is little (if any) time for domestication selection to occur. There is anecdotal empirical evidence to support this hypothesis in Atlantic salmon (*Salmo salar*), where fish stocked as fry show greater survival from smolt to adulthood when compared with fish released as smolts (Rideout and Stolte 1988). No study has rigorously compared the fry versus smolt release strategies, thus very limited knowledge is currently available to assess whether unfed fry releases have different survival rates and life history characteristics than smolt releases.

The major objective of this study was to evaluate the relative efficiency of coho salmon released as unfed fry versus smolts for a conservation hatchery program (one that uses random 1:1 paired matings and equalizes parent contributions). By using microsatellite markers to assign returning adults to their most likely parents, we were able to (*i*) compare survival rates of fish returning to the river from which they were released as unfed fry or smolts, (*ii*) evaluate if releasing progeny of brood stock as unfed fry or smolts increased individual reproductive success (number of progeny that survived to reproduction) relative to those breeding in the wild, and (*iii*) compare size at age, run timing, and incidence of maturing at age 2 (i.e., jacking) for returning adults between coho salmon stocked as unfed fry and those stocked as smolts, as well as with the natural population.

Materials and methods

Sampling design

The North Umpqua River, Oregon, USA, is supplemented with hatchery coho salmon every year as a way of augmenting the recreational and commercial fisheries. This integrated hatchery program incorporates local brood stock by including natural fish (30%, non-adipose-fin-clipped) each year with their collection of hatchery fish (70%, adipose-fin-clipped), producing smolts that are used to supplement the natural-spawning population. Each smolt released from the hatchery is adipose-fin-clipped to identify it as a "hatchery" fish. In 2001, 2002, and 2003, ODFW collected 100 adipose-fin-clipped coho salmon of each sex (hereafter referred to as hatchery-reared, H), as well as 94, 100, and 102 non-adipose-fin-clipped coho salmon of each sex, respectively (hereafter referred to as wild-born, W; note that although W is designated as wild-born, W fish may be of hatchery ancestry because of the occasional mating of hatchery fish in the wild). The H brood stock was collected at Winchester Dam for the three years sampled (Fig. 1). In 2001, the W brood stock was also collected at Winchester Dam, whereas for 2002 and 2003, W fish were taken from Calapooya Creek at Nonpareil Dam (Fig. 1). Calapooya Creek is a tributary of the Umpqua River and has no previous history of supplementation; however, hatchery strays have been known to influence the system. In addition to the W fish taken as brood stock, we also have records of virtually all W fish passed above Nonpareil Dam for 2002 and 2003 that were allowed to spawn naturally (783 and 520 fish, respectively). These data are not available for 2001, as relevant sampling equipment was not installed that year. For each of the three years, males and females were randomly paired within each group ([H×H] and [W×W]) and spawned at ODFW's Rock Creek hatchery facility using single-pair mating (i.e., each male and female was used only once). The progeny from these crosses are referred to as F_1 hatchery fish. Eggs from each mating pair were incubated separately until the eyed-egg stage. At this point, family sizes were equalized by randomly sampling 140–150 eyed eggs per mating pair, which were then reared together to the smolt stage (for more details, see Moyer et al. 2007). The remaining eyed eggs were transferred to hatchboxes

Fig. 1. Map of the study area showing sampling sites of brood stock (Winchester and Nonpareil dams).

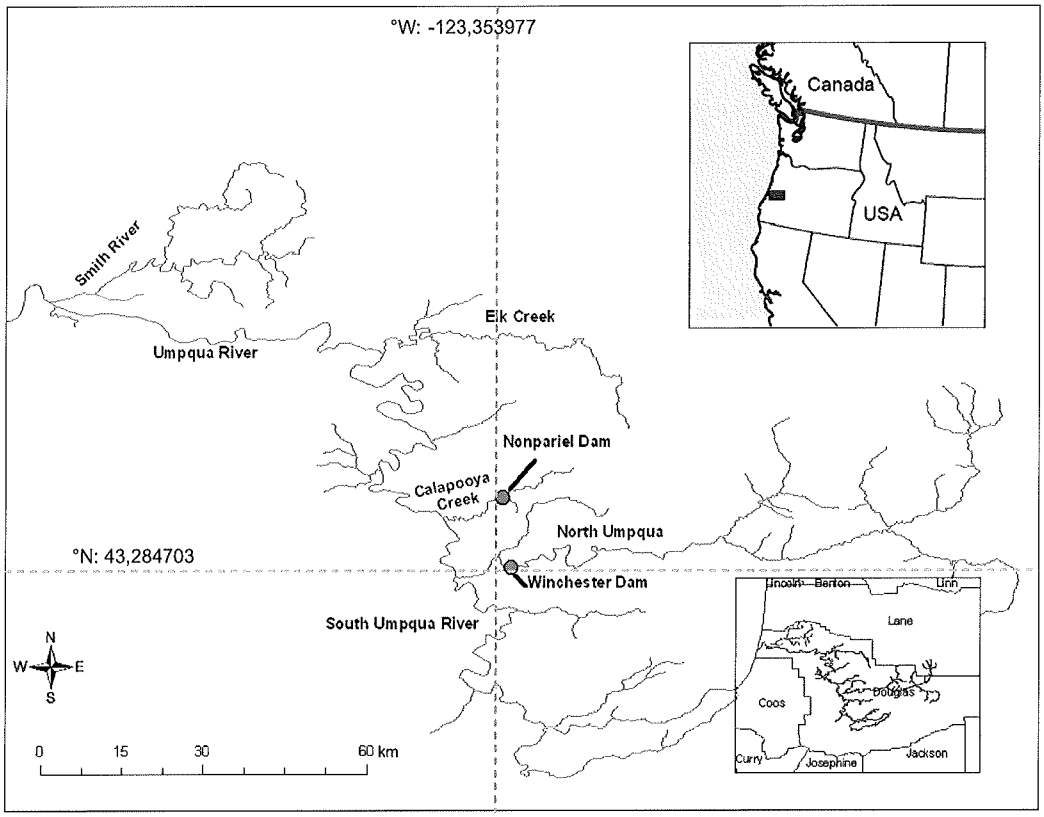


Table 1. Coho (*Oncorhynchus kisutch*) hatchery spawning and release information for the evaluation of survival in unfed fry and smolt release strategies for each brood year (BY).

		No. released		No. of returns		Survival (%)	
BY	No. of pairs spawned	Fry	Smolt	Fry	Smolt	Fry	Smolt
2001	194	370 576	24 373	79	360	0.02	1.48
2002	200	491 866	21 997	207	693	0.04	3.15
2003	202	445 628	24 372	398	622	0.09	2.55

Note: Numbers of returns as adults are given based on pedigree analysis, and survival rate is calculated as the number of fish that returned over the number released.

where three families were mixed to get a total of between 4000 and 6000 eggs and reared together in the same hatchbox for later release as unfed fry (see below).

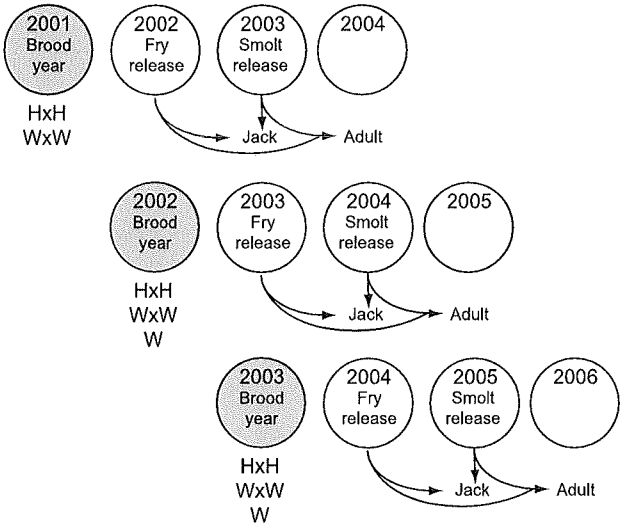
In spring 2002, 2003, and 2004, a few days after the absorption of the yolk sac, F_1 unfed fry were released in nine different sites along Calapooya Creek and two of its tributaries (Coon and Gassy creeks) above Nonpareil Dam (Fig. 1; Table 1). In spring 2003, 2004, and 2005, F_1 smolts of each brood stock were also released above Nonpareil Dam in Calapooya Creek (Fig. 1; Table 1). Smolts were clipped adipose left maxillary or adipose right maxillary to designate H×H or W×W origin, respectively. Unfed fry remained unmarked.

Most coho salmon migrate to the ocean after spending a year in freshwater. Some males spend only one summer at sea and return to freshwater in the following fall as mature males that are two years of age (termed jacks). The majority of males and all females spend two summers in the ocean before their migration to spawning habitat at three years of age. Therefore, fry and smolts released in 2002–2005 returned as jacks and adults in 2003–2006 (designed as return year (RY); Fig. 2). All returns to Calapooya Creek were captured, measured, sexed by visual identification, aged (only for 2- or 3-year-old males, based on size), and caudal-fin-clipped at a fish trap constructed at the base of Nonpareil Dam. All were released above the dam after handling.

Parentage analysis

The DNA from all brood stock and progeny returning to Calapooya Creek from 2002 to 2006 was extracted, amplified via polymerase chain reaction (PCR), and genotyped for 10 microsatellite loci (*OTS*519, *OTS*520, *ONE*111, *P*53,

Fig. 2. Study design showing the three brood years (shaded) with their associated unfed fry and smolt releases and the returning jacks and adults in return years 2003 to 2006. H×H and W×W represent the crosses performed in the hatchery in each of the three brood years, and W represents the wild fish sampled and passed above Nonpareil Dam in BY 2002 and 2003 that were allowed to reproduce naturally.



*OTS*3, *ONE*μ2, *OCL*8, *OTS*215, *ONE*μ13, *OMY*1011) following the methods of Moyer et al. 2007.

Parentage analysis, which was done separately for each brood year (BY; refers to the year in which the brood stock was sampled), was performed using a five-step approach. First, all returning offspring (ages 2 and 3) from a given BY were allocated to hatchery parental pairs using the software PAPA 2.0 (Duchesne et al. 2002). Allocations were performed based on maximum likelihood and were restricted by using the known structured mating design and a 2% error model distributed on the next adjacent allele. This error model takes into account scoring errors that usually occur by scoring the allele next to the true one. To take null alleles into account, we chose to add any allocation that yielded the most likely pair from the known hatchery mating design but was rejected by PAPA because of mismatches resulting from homozygous loci. Errors were more prevalent among *One*μ2 data, and frequency analysis showed evidence of null alleles for this locus with a frequency of 0.039 (MICRO-CHECKER; Van Oosterhout et al. 2004). Second, parentage analysis of any unassigned offspring from the previous step was performed using wild parents passed above Nonpareil Dam. Parentage analysis for this step was conducted using PASOS 1.0 (Duchesne et al. 2005). Analyses were only performed for BY 2002 and 2003 because Calapooya Creek was not sampled for wild coho salmon in BY 2001. Like PAPA, PASOS uses a maximum likelihood approach and an error model, but it differs from PAPA in that it also combines an exclusion approach and allocates parental pairs in an open system where some parents are potentially missing. We used a 2% error model and a similar procedure as described above for rejected parent pairs resulting from homozygote mismatches. Third, simulations were conducted using PASOS to estimate the number of missing wild spawners in 2002 and 2003 (see Duchesne et al. (2005) for description of simulation and Thériault et al. (2007) for an example). Fourth, to verify that the unassigned individuals from step 2 were of wild parents, we simulated wild offspring using the number of missing spawners estimated in step 3 for each year and allocated these offspring to hatchery pairs as detailed in step 1. The simulated offspring should not assign (or assign in very low proportion) to hatchery pairs if our allocation procedure is correct. Finally, the accuracy of our allocations was assessed via simulations as implemented in PAPA and PASOS as follows. Artificial offspring were produced from sampled parents used in this study and from simulated parents based on the number of missing spawners for each BY (for PASOS simulations only, because there are no missing hatchery spawners for PAPA simulations). Simulated offspring were then allocated to known sampled parents and the percentage of correctly assigned individuals was assessed.

Estimation and comparisons of survival rate

The number of offspring returning as 2- and 3-year-old adults divided by the total number released (i.e., survival rate) was compared between hatchery releases of unfed fry and smolts for each BY separately using a Yates corrected χ^2 test. We also compared survival rates among BYs to assess temporal differences. The number of returning offspring was obtained using the parental allocation described above.

Estimation and comparison of individual reproductive success

The average number of returning adult offspring produced per individual (i.e., individual reproductive success) was estimated from parentage analysis. Mean reproductive success of hatchery-spawned fish (number of returning offspring that were released as either unfed fry and smolts) was compared with mean reproductive success of fish spawned in the wild for BYs 2002 and 2003 (wild parents were not available in 2001). Estimates were calculated for both males and females combined (similar results were seen when data were analyzed by sex; data not shown). Significance in the difference between reproductive success among hatchery and wild fish and among BYs was assessed using two-tailed permutation tests as implemented in PERM 1.0 (Duchesne et al. 2006). Numbers of offspring were permuted 10 000 times, and the probability of obtaining a smaller or larger difference from the observed value was evaluated.

Comparison of life history traits

Size at age

Within each return year (RYs 2003–2005), average length (fork length) of returning fish to Calapooya Creek was compared for age-2 and age-3 fish, separately, among three groups as follows: returns stocked as fry, returns stocked as smolts, and wild returns (naturally occurring wild-born fish). For age-2 males (jacks), a one-way analysis of variance (ANOVA) was used to test for the effect of stocking history (fry, smolt, or wild) on length. If stocking was significant, we performed pairwise comparisons using a Student's *t* test. For age-3 returns, we first used a two-way ANOVA to test

for the effect of stocking history and sex, as well as the interaction between these factors, on length. If the interaction was significant, a one-way ANOVA, which tested the effect of stocking history on length, was performed for each sex separately. If stocking was significant, then we performed pairwise comparisons using a Student's *t* test.

Run time

Similar to the length analysis, the date that progeny returned to Calapooya Creek (i.e., run time) was compared within each return year (RYs 2003–2005). Separate comparisons were made for age-2 and age-3 fish among the following three groups: returns stocked as fry, returns stocked as smolts, and wild returns. Run time was scored as the number of days after 1 October for each RY. For age-2 males, we used a Kruskal–Wallis test (run time is not normally distributed) on ranks for the effect of stocking history on run time. If stocking was significant, we used a nonparametric Wilcoxon test to compare each group. For age-3 fish, we first used a two-way ANOVA to test the effect of stocking history and sex, as well as the interaction between these factors, on run time. If the interaction term was significant, we performed a Kruskal–Wallis test on ranks for each sex separately. Furthermore, if stocking was significant, we tested each pairwise group comparison using a nonparametric Wilcoxon test.

Jacking

We also explored the incidence of returning age-2 males by comparing the proportion of jacks with that of the total number of male returns from each BY. Comparisons were made between each of three groups as follows: returns from unfed fry releases, returns from smolt releases, and wild returning individuals. Pearson's χ^2 analysis was used to assess significance. If significance was found, post hoc exact tests (Fisher) were performed to assess which group(s) was significant.

Results

Offspring from H×H and W×W crosses were pooled together and considered as F_1 hatchery fish, regardless of the origin of their parents, for all the results presented below. An ANOVA including parental captive history (H×H or W×W) and stocking strategy (unfed fry, smolt, or wild) revealed a significant interaction between these two effects in BY 2001 only (data not shown). However, there are no qualitative differences in any of our analysis when we separate by parental history, i.e., the patterns are the same and our conclusions are not affected whether the data were pooled or not (data not shown). Moreover, the low number of unfed fry jack returns necessitated the pooling of the data to provide sufficient sample size to fulfill requirements for statistical analysis. Captive parental history has already been dealt with in part in Moyer et al. (2007) with a subset of the data presented here.

Parentage analysis

A total of 1340, 1652, and 1639 progeny returned from BYs 2001, 2002, and 2003, respectively (Table 2). Twenty fish were excluded from analyses because they were missing tissue or genotypes for more than five loci. PAPA and PASOS allocation rates combined (i.e., percentage of fish that were allocated to parental pairs or single parent) were 34%, 87%, and 81% for BYs 2001, 2002, and 2003, respectively (the BY 2001 allocation rate was less than the other BYs because wild parents were unattainable). Simulations indicated that our allocation method was accurate because no wild offspring were falsely allocated to hatchery pairs using PAPA during the first step of our allocation process. The correctness rate (i.e., the percentage of fish allocated to the correct parents according to simulations) for allocation to hatchery parents in all three brood years using PAPA was 100%. The correctness rate for the allocation to wild parents using PASOS was 97% and 91% for BYs 2002 and 2003, respectively.

Approximately 3% of the marked returns were unassigned to a hatchery pair according to the known mating matrix (Table 2). The exact reason for these unresolved assignments is unclear, but it could be due to the spilling of gametes during hatchery spawning, mutations in offspring, genotype error, error in data collection or processing, or wrong age determination. Similarly, another 4% of the marked returns were dropped from analyses because of a mismatch between the mark and the hatchery pair assigned (for example, a fish marked adipose left maxillary should be assigned to a H×H pair but was assigned to a W×W pair). Because we did not want to prioritize the mark over the allocation results, or vice versa, we removed these fish from our analyses. Moreover, 38 returns from BY 2003 were, in fact, progeny of 66 F_1 jacks (returns from BY 2001) spawning in the wild in 2003. These fish were removed from subsequent analyses as they are F_2 returns.

Estimation and comparisons of survival rate

The survival rate for fish released as smolts was significantly greater than that of fish released as fry (X^2_{Yates} = 4351.7, 1161.79, and 6461.01 for BYs 2001, 2002, and 2003 respectively; $\chi^2_{0.05[1]} = 3.841$; all $p < 0.0001$; Table 1). Survival rates for unfed fry were also significantly different among BYs, where the survival rate for fry from BY 2003 was the highest, followed by BYs 2002 and 2001 (X^2_{Yates} = 26.87 for BY 2001 vs. BY 2002, 158.99 for BY 2001 vs. BY 2003, and 80.13 for BY 2002 vs. BY 2003; $\chi^2_{0.05[1]}$ = 3.841, all $p < 0.0001$); Table 1). Survival rate comparisons for smolts among BYs indicated that BY 2002 had the greatest survival followed by BYs 2003 and 2001 (X^2_{Yates} = 145.13 for BY 2001 vs. BY 2002, 70.81 for BY 2001 vs. BY 2003, and 14.8 for BY 2002 vs. BY 2003; $\chi^2_{0.05[1]}$ = 3.841, all $p < 0.001$; Table 1).

Estimation and comparison of individual reproductive success

The average individual reproductive success of brood stock whose offspring were released as unfed fry was significantly ($p < 0.001$) greater than that of fish that spawned in the wild for BY 2003 (Table 3). In contrast, average individual reproductive success for brood stock in BY 2002 was significantly ($p = 0.001$) less than that of the wild population in 2002. Average individual reproductive success of brood stock whose offspring were released as smolts was significantly ($p < 0.0001$) greater than that of fish that spawned in the wild for both BYs (Table 3). Comparisons across BYs

Table 2. Count of coho (*Oncorhynchus kisutch*) that passed above Nonpareil Dam (Calapooya Creek) for each brood year (BY).

BY	Total returns	Jacks returns (RY)	Adults returns (RY)	Assigned to H pairs	Assigned to W parents	Not used	Unresolved	Dropped
2001	1340	160 (2003)	1180 (2004)	439	866*	9	11	15
2002	1652	131 (2004)	1521 (2005)	900	686	7	27	32
2003	1639	165 (2005)	1474 (2006)	1020	580†	4	18	17

Note: The actual year of return (return year, RY) is given in parenthesis. The category labeled "numbers not used" corresponds to missing genotype information. Likewise, those labeled "unresolved" are marked fish unassigned, and those of the category "dropped" are marked fish that were misassigned. W, wild; H, hatchery.

*Identity of W spawners is not known. All fish not assigned to hatchery pairs are considered of W origin.

†Thirty-eight of the fish allocated to W parents were coming from 61 F_1 jacks (returns from BY 2001) that spawned in the wild in 2003. These 38 fish were removed from subsequent analysis, as they are, in fact, F_2 returns.

Table 3. Average individual reproductive success (RS) and relative reproductive success (RRS) of hatchery and wild coho salmon (*Oncorhynchus kisutch*) for brood years (BYs) 2002 and 2003.

	RS hatchery (SE)			RS wild (SE)		RRS	
BY	*N*	Fry	Smolt	*N*	Wild	Fry/wild	Smolt/wild
2002	400	1.04 (0.08)	3.46 (0.18)	783	1.41 (0.08)	0.74**	2.45**
2003	404	1.97 (0.14)	3.08 (0.15)	520	1.27 (0.1)	1.47**	2.43**

Note: RS and RSS were evaluated for unfed fry and smolt stocking strategies. **, RSS values significantly different from one at $p \leq 0.001$.

Table 4. Mean fork length (mm) of coho (*Oncorhynchus kisutch*) that passed above Nonpareil Dam for each brood year (BY), according to their stocking origin (fry, smolts, or wild born).

	Fry		Smolt		Wild	
BY	*N*	Mean (SD)	*N*	Mean (SD)	*N*	Mean (SD)
Jacks (age 2)						
2001	5	406.0 (24.8)	61	420.6 (27.0)	94	411.2 (38.0)
2002	7	425.7 (22.4)	66	421.2 (33.2)	56	417.8 (55.8)
2003	20	443.3 (30.9)	67	428.2 (28.7)	72	445.3 (28.8)
Adults (age 3)						
2001	74	728.6 (62.3)	299	711.6 (61.1)	784	723.2 (55.8)
2002	200	709.9 (66.2)	627	700.3 (63.2)	657	716.9 (60.7)
2003	377	739.5 (57.3)	555	719.3 (56.9)	488	743.2 (60.7)

Table 5. Two-way analysis of variance (ANOVA) results for age-3 coho comparing length between stocking origin (fry, smolts, or wild born) and sex for each brood year (BY).

Source	df	Sum of squares	*F* ratio	*p*
BY 2001				
Stocking	2	32 692.43	4.92	0.007
Sex	1	3 414.54	1.03	0.311
Stocking × sex	2	3 583.30	0.54	0.583
BY 2002				
Stocking	2	93 636.27	12.30	<0.0001
Sex	1	115 369.26	30.30	<0.0001
Stocking × sex	2	21 957.14	2.88	0.056
BY 2003				
Stocking	2	180 206.56	26.96	<0.0001
Sex	1	15 957.76	4.77	0.029
Stocking × sex	2	16 299.35	2.44	0.088

for average individual reproductive success of brood stock whose offspring were released as unfed fry showed that BY 2003 was significantly greater than BY 2002 ($p < 0.0001$). The average individual reproductive success estimate for brood stock whose offspring were released as smolts was significantly ($p = 0.02$) greater in BY 2002 than in BY 2003. There was no difference ($p = 0.18$) in the estimate of average individual reproductive success for wild fish between the two years sampled.

Comparison of life history traits

Size at age

The average length for fish passed above Nonpareil Dam each year, according to their stocking origin, are shown (Table 4). There was a significant difference (all $p < 0.01$) in average length for jacks stocked as fry, smolts, or naturally occurring in the wild for BY 2003. The significant difference in length for progeny of BY 2003 can be explained by an increase in average length of jacks returning from fry releases and from naturally spawned individuals when com-

Table 6. Mean run time (number of days since 1 October of each run year) of coho that passed above Nonpareil Dam for each brood year (BY), according to their stocking origin (fry, smolts, or wild born).

	Fry		Smolt		Wild	
BY	*N*	Mean (SD)	*N*	Mean (SD)	*N*	Mean (SD)
Jacks (age 2)						
2001	5	64.2 (7.4)	61	61.7 (6.4)	94	67.1 (10.4)
2002	7	61.3 (16.4)	66	51.7 (18.1)	56	66.6 (14.8)
2003	20	57.0 (13.8)	67	53.0 (15.2)	72	59.9 (15.2)
Adults (age 3)						
2001	74	65.8 (13.6)	299	58.1 (18.6)	784	71.1 (12.0)
2002	200	54.3 (13.6)	627	52.7 (12.8)	657	63.3 (15.4)
2003	377	45.7 (8.7)	555	44.5 (7.3)	488	53.0 (18.9)

Table 7. Two-way analysis of variance (ANOVA) results for age-3 coho comparing run time between stocking origin (fry, smolts, or wild born) and sex for each brood year (BY).

Source	df	Sum of squares	*F* ratio	*p*
BY 2001				
Stocking	2	36 449.04	92.04	<0.0001
Sex	1	520.22	2.63	0.1053
Stocking × sex	2	2.96	0.008	0.9926
BY 2002				
Stocking	2	38 134.54	96.35	<0.0001
Sex	1	796.75	4.03	0.045
Stocking × sex	2	76.43	0.19	0.824
BY 2003				
Stocking	2	19 914.75	125.82	<0.0001
Sex	1	2 553.12	32.26	<0.0001
Stocking × sex	2	204.17	1.29	0.2756

pared with that of jacks returning from smolt releases (smolts vs. wild, $p = 0.0007$; smolts vs. fry, $p = 0.044$). Note that for BYs 2001 and 2002, sample sizes were very small for fry ($n = 5$ and 7, respectively), limiting our power to determine significant size differences.

There was a significant difference (all $p < 0.01$) in length for age-3 returns stocked as fry, smolts, or naturally produced in the wild for all BYs (Table 5; there was no significant interaction for stocking origin and sex of fish). For all BYs, significant differences in length at return is attributed to an increase in average length of adults returning from fry releases and from naturally spawned individuals when compared with that of adults returning from smolt releases (BY 2001: smolts vs. wild, $p = 0.003$; smolts vs. fry, $p = 0.021$; BY 2002: smolts vs. wild, $p < 0.0001$; smolts vs. fry, $p = 0.06$; BY 2003: both comparisons, $p < 0.0001$).

Run timing

Mean run time of fish passed above Nonpareil Dam each year according to their stocking origin are shown (Table 6). There were significant differences (all $p < 0.01$) in run time for jacks stocked as fry, smolts, or naturally occurring for BYs 2001, 2002, and 2003. Differences in run time were attributed to a significantly (all $p < 0.01$) earlier average run time in progeny released as smolts when compared with that of progeny from naturally spawning coho salmon. Again, small sample sizes for fry in BYs 2001 and 2002 limit our analytical power when considering pairwise comparisons with fry.

There were significant differences (all $p < 0.0001$) in run time for age-3 returns stocked as fry, smolts, or naturally occurring in the wild for all BYs (Table 7; there was no significant interaction for stocking origin and sex of fish). Significance for BY 2001 is attributed to a different run time for every group of fish. On average, adults stocked as smolts returned to Nonpareil Dam earliest, followed by adults stocked as fry and then naturally occurring adults (all $p < 0.001$; Fig. 3). For BYs 2002 and 2003, differences were attributed to a later average run time of naturally occurring adults (all $p < 0.0001$).

Jacking

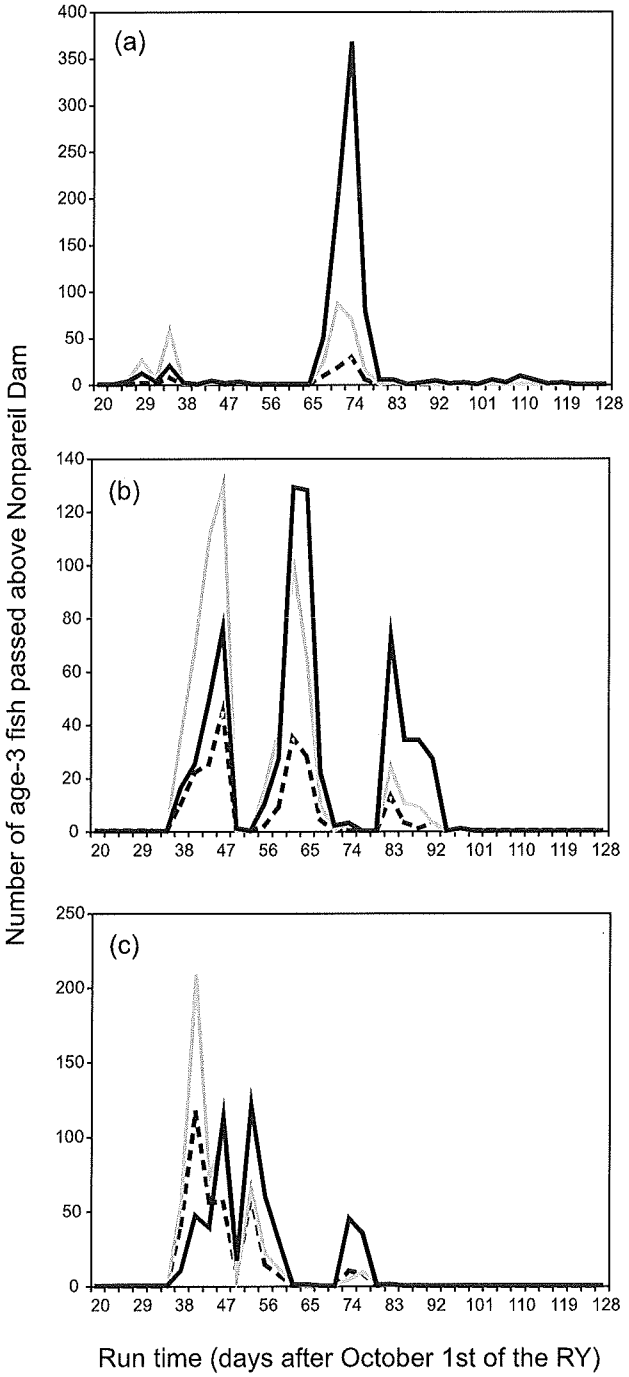
We found significant (all $p < 0.01$) differences in the proportion of jacks produced from progeny stocked as fry versus progeny stocked as smolts for each BY (Fig. 4). Post hoc testing revealed that the probability of being a jack was greater for an individual released as a smolt than for an individual released as a fry (all $p < 0.01$). When comparing the incidence of jacking between progeny stocked as fry and those naturally occurring in the wild, significant (all $p < 0.01$) differences were found in BYs 2002 and 2003 (Fig. 4). For both BYs, post hoc testing revealed that the probability of being a jack was greater for wild offspring than for hatchery offspring released as fry (all $p < 0.01$). The incidence of jacking was also different between individuals stocked as smolts and those naturally occurring in the wild for BY 2001 ($p = 0.008$). Individuals stocked as smolts had a greater probability of being a jack than did naturally occurring fish ($p = 0.006$).

Discussion

Evaluation of hatchery strategies

The main goal of this study was to evaluate the success of an integrated conservation hatchery program (i.e., random 1:1 paired matings and equalized parent contributions) that implemented two different stocking strategies — outplanting unfed fry and smolts. One of the primary measures of success for such a program is the evaluation of reproductive success between stocking strategies and between hatchery

Fig. 3. Run time of age-3 coho (*Oncorhynchus kisutch*) returning to Calapooya Creek for (*a*) brood year (BY) 2001, returning in run year (RY) 2004; (*b*) BY 2002, returning in RY 2005, and (*c*) BY 2003, returning in RY 2006 according to their stocking origin (fry, broken line; smolts, shaded line; wild-born, continuous line).



and wild components. A successful hatchery program should increase egg-to-adult survival over naturally produced fish because the hatchery is intended to minimize mortality at critical early life history stages, otherwise observed in the natural population. We found this to be the case for smolt releases and one of two unfed fry releases.

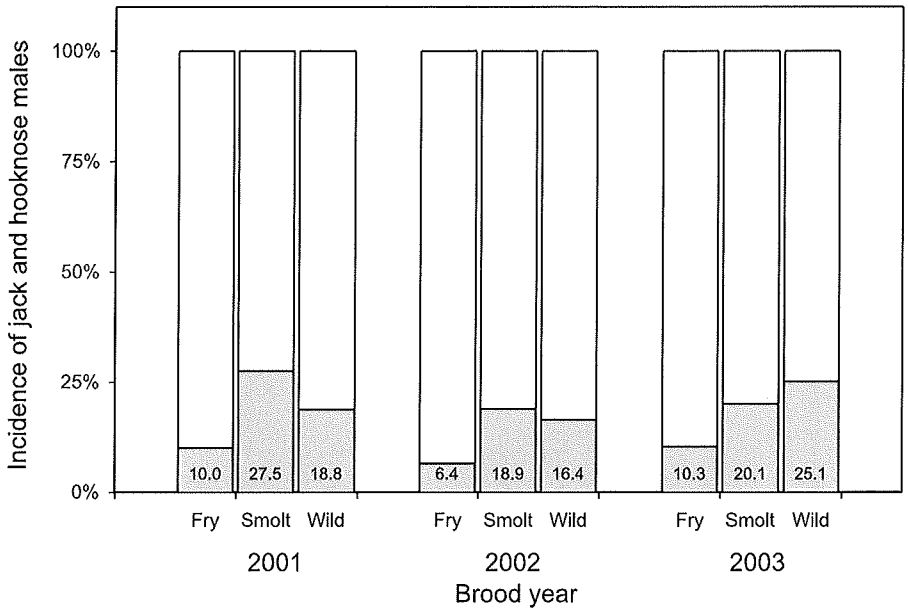
A significantly lower estimate of RRS for hatchery fish whose progeny were stocked as fry in BY 2002 could be explained by detrimental environmental conditions experienced by hatchery fry once supplemented in the river. Indeed, time of release in 2003 (for BY 2002) corresponded to a period of higher flows than for releases in 2004 (for BY 2003) (L. Jackson, ODFW, Roseburg, Oregon, USA, personal communication). Data from smolt releases in 2003 also support this hypothesis because they had the lowest survival rate of the three years under study. The fact that only hatchery fry and not wild fry would have been impacted by these higher flows could result from the prior residence of wild fry in safer territories or because wild fry may still have been in the gravel. Regardless, our data showed that the unfed fry release strategy appeared to increase the number of returns relative to wild spawning under certain conditions (BY 2003); however, the increase was not as substantial as with stocking smolts.

A caveat to our findings is that the estimate of RRS could be influenced by the relative number of offspring released as smolts versus unfed fry per family group. For example, in our study, approximately 95% of the gametes produced by an individual were released as unfed fry. If more smolts were released per paired mating, then there exists a possibility that the expected RS could be greater than our observed value, although the conclusion would be in the same direction (i.e., the smolt strategy increases RS relative to wild spawning). Unfortunately we have no way of estimating RS on a per-gamete basis (e.g., if 100% of the offspring from a paired mating were released as smolts) because survival is likely density-dependant. Therefore we urge caution when applying our RRS results to other such hatchery programs, especially those releasing a large portion of smolts relative to fry, unless density-dependent factors influencing survival are known.

Our study also found important differences in survival depending on the hatchery release strategy. On average, returning fish released as smolts had a significantly greater survival rate (25- to 75-fold) compared with fish released as unfed fry. This finding is consistent with our expectations because hatcheries keep juvenile coho salmon in captivity for longer periods to increase survival during the critical freshwater life stage that unfed fry would experience outside the hatchery (Unwin 1997). It is important to note that we compared survival of unfed fry to adulthood with survival of smolts to adulthood. Acknowledging that this is an unbalanced comparison, we are unable to discern if survival from smolt to adulthood is different between fish stocked as unfed fry and those stocked as smolts. Therefore, we were unable to offer direct support for the 10-fold increase in survival to adulthood described in Rideout and Stolte (1988), where smolts from fry releases were shown to be better adapted to facing oceanic migration than fish released as smolts.

Increased survival and RRS are not the only parameters that should define success of a conservation hatchery program. Maintaining genetic diversity from generation to generation is also important. For example, if the majority of smolts that survived to adulthood were from a few paired matings, a considerable loss of genetic diversity would be

Fig. 4. Incidence of jacks (age 2) compared with hooknose (age 3) among males that returned to Calapooya Creek for each brood year that were stocked as fry, smolts, or wild born. The numbers within the bars represent the percentage of jacks for each group.



associated with the program. Although this study does not deal with the conservation of genetic diversity (see Moyer et al. 2007), it provides insight into the efficiency of the unfed fry release strategy, which has not been rigorously evaluated, despite decades of STEP implementation in the Pacific Northwest. From a conservation genetics viewpoint, we note that alternate rearing strategies may have different outcomes depending on a myriad of genetic and environmental parameters and interactions. We illuminate this consideration by comparing smolt and fry releases with wild fish for three different life history characteristics.

Evaluation of life history characteristics

In this study, wild-born fish and fish stocked as unfed fry had the same average length at return, whereas smolts were significantly smaller. Smaller size at return for hatchery smolts has been reported in other hatchery programs (McLean et al. 2004; Knudsen et al. 2006). There are several competing hypotheses (none of which is mutually exclusive) that explain these differences. First, hatchery smolts could experience depressed growth during their transition from artificial to natural food. In turn, this depressed growth may not be recovered later in life. Second, hatchery smolts could have a slower outmigration rate than their wild counterparts that would translate into earlier and increased feeding opportunities and subsequent greater growth of wild fish in the ocean phase of their life cycle. Third, hatchery smolts could also experience an earlier run time than wild fish, causing hatchery smolts to spend less time at sea. Finally, there could be a genetic component if length was a heritable trait such that small brood stock (when compared with the wild) on average produced smaller offspring.

Though it is difficult to disentangle competing hypotheses that might explain our length data, we can eliminate the latter hypothesis because if there were a genetic effect due to the heritability of length, then we would expect that the average length of the hatchery brood would be significantly less than that of the wild population. Instead, we saw that fish used as brood stock for our study were significantly larger than the wild fish (data not shown), indicating that there is a strong environmental influence on length of returning hatchery fish.

We also found significant differences in run timing among returning progeny from smolts when compared with unfed fry releases and the wild population. Although run time overlapped for all groups, fish stocked as smolts returned earlier to their stream of origin, followed closely by fish stocked as fry, and then by wild fish. Earlier run timing of hatchery fish has been observed in other hatchery programs (Unwin and Glova 1997; McLean et al. 2003; Ford et al. 2006). Run timing is a heritable trait in salmonids with the capacity to rapidly evolve and adapt to local environmental conditions (Hansen and Jonsson 1991; Quinn et al. 2000; Carlson and Seamons 2008). Relaxation of natural selection on run timing in the hatchery environment may potentially lead to the survival of certain genotypes and phenotypes that would otherwise be selected against in nature. For example, early spawning in nature can be selected against because of redd disturbance by later spawners (van den Berghe and Gross 1989) or because fry that emerge too early may face limiting food resources or unsuitable stream flow conditions (Nickelson et al. 1986). These selection processes do not take place in the hatchery environment. Additionally, artificial selection resulting from hatchery managers that unintentionally spawn the earliest fish caught as brood stock to secure a brood stock quota could result in an over-representation of early run-time phenotypes.

Differences in run time found in our study appeared to be the result of direct artificial selection. Despite efforts to collect a representative sample from the entire spawning run, brood stock used in this study were captured at a significantly earlier date. Brood stock sampled at Winchester Dam

and Nonpareil Dam had a run time on average 46 and 6 days earlier, respectively, than the natural run in Calapooya (data not shown). Differences between hatchery and wild fish are likely the result of passing these differences from parent to offspring. Therefore, we would expect that fry releases would show the same pattern as smolts, as they have the same genetic background. Although returns from fry releases were consistently later than returns from smolt releases, generally, the trend was not significantly different. Discrepancies could be artifacts of low sample size (fry returns) or because environmental influences on run time were greater for fry releases.

Differences in the incidence of jacking between stocking strategies may be attributed to complex interactions among environmental and genetic effects. Alternative mating tactics in salmonids often associated with early maturation have been shown to be heritable (Silverstein and Hershberger 1992; Heath et al. 1994; Wild et al. 1994) but are also influenced by environmental conditions acting on growth and other energetic traits (Rowe and Thorpe 1990; Forseth et al. 1999; Olsson et al. 2006). It is well established that age at maturity is negatively correlated with growth rate in fishes (Stearns and Koella 1986; Hutchings 2004), and fast growth has been shown to be associated with a greater probability of jacking (Vøllestad et al. 2004). Based on these findings, we would expect fish reared to the smolt stage to exhibit the highest incidence of jacking because of their faster growth in the hatchery environment (for example, see Unwin and Glova 1997; Larsen et al. 2004; Knudsen et al. 2006). Our data supported these conclusions. The incidence of jacking was greater in fish stocked as smolts than in those stocked as fry. We also noted a higher tendency for fish stocked as smolts to return as jacks in BYs 2001 and 2002 than for wild fish, but this tendency is reversed in BY 2003. In 2003, 44% of the wild males passed above Nonpareil Dam were jacks. This was a much greater percentage than reported in any other year among available data (average of 17% over 3 years). However, only 16% of the males sampled for hatchery brood in 2003 were jacks. If we consider jacking to be heritable, then the greater incidence of jacking among wild returns from BY 2003 could be the result of an increased proportion of natural jack spawning among BY 2003 relative to the proportion spawned in the hatchery. Similarly, lower incidences of jacking from hatchery fry releases compared with the wild (when we would expect them to be similar because these two groups are hypothetically experiencing similar environmental conditions) may be the result of fewer jacks spawned among hatchery crosses relative to the natural population (BY 2002, 7% of the hatchery males were jacks, whereas 17% of the wild spawning males were jacks; see above for BY 2003; no data on wild fish were available for BY 2001).

Our findings illustrate that juvenile release strategies in supplementation programs have important consequences for coho salmon survival rates, numbers of adult returns, and other key life history characteristics. Irrespective of the genetic or environmental origin of these differences, any changes in these critical features are likely to affect the fitness of the population (Stearns 1992; Goodman 2005). Accumulating evidence demonstrates reduced fitness of hatchery fish in the wild (Araki et al. 2008; Araki et al. 2009), and researchers are currently exploring causal mechanisms. Overall, our study indicated that a fry stocking strategy produced fish more similar to the wild component of the population than that of fish released as smolts. However, the extent to which hatchery fish are influenced by the differences found in life history traits, once they reproduce in the wild, remains unresolved. Assessing the relative reproductive success in the wild of unfed fry releases versus smolt releases and wild-born fish is critical for a complete understanding of the efficiency of the two release strategies.

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MOLECULAR ECOLOGY

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Reduced reproductive success of hatchery coho salmon in the wild: insights into most likely mechanisms

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Abstract

Supplementation of wild salmonids with captive-bred fish is a common practice for both commercial and conservation purposes. However, evidence for lower fitness of captive-reared fish relative to wild fish has accumulated in recent years, diminishing the apparent effectiveness of supplementation as a management tool. To date, the mechanism(s) responsible for these fitness declines remain unknown. In this study, we showed with molecular parentage analysis that hatchery coho salmon (*Oncorhynchus kisutch*) had lower reproductive success than wild fish once they reproduced in the wild. This effect was more pronounced in males than in same-aged females. Hatchery spawned fish that were released as unfed fry (age 0), as well as hatchery fish raised for one year in the hatchery (released as smolts, age 1), both experienced lower lifetime reproductive success (RS) than wild fish. However, the subset of hatchery males that returned as 2-year olds (jacks) did not exhibit the same fitness decrease as males that returned as 3-year olds. Thus, we report three lines of evidence pointing to the absence of sexual selection in the hatchery as a contributing mechanism for fitness declines of hatchery fish in the wild: (i) hatchery fish released as unfed fry that survived to adulthood still had low RS relative to wild fish, (ii) age-3 male hatchery fish consistently showed a lower relative RS than female hatchery fish (suggesting a role for sexual selection), and (iii) age-2 jacks, which use a sneaker mating strategy, did not show the same declines as 3-year olds, which compete differently for females (again, implicating sexual selection).

Keywords: captive breeding, parentage analysis, reproductive success, salmonids, sexual selection, supplementation

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Introduction

Hatchery programs have been used for decades to compensate for worldwide declines in salmonid populations. Despite years of supplementation, there is scarce evidence that such programs have helped the recovery of, or increased the long-term productivity of wild salmonid populations (Waples *et al*. 2007; Fraser 2008). Instead, recent studies have provided convincing evidence that hatchery fish have lower reproductive success (RS) than wild fish when both breed in the wild. This difference is detectable after as little as one or a few generations spent in the hatchery environment (review in Araki *et al*. 2008; Recovery Implementation Science Team 2009). However, most hatchery/wild fitness comparisons to date have been limited to studies using steelhead (*Oncorhynchus mykiss*). Thus, it is still unclear whether the same phenomenon occurs in other salmonid species.

Fitness differences between hatchery and wild salmonids can be genetically based (Araki *et al*. 2007, 2009) or could result from carry-over effects of the environment

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experienced in the hatchery (Fleming *et al.* 1997). Genetic differences are hypothesized to result from relaxed natural selection and/or domestication selection in the captive environment (Ford 2002; Araki *et al.* 2008). There are two main stages in the life cycle where genetic effects of hatchery rearing are expected. These are adult reproduction and juvenile survival. Because adults are spawned artificially in the hatchery, there can be relaxed natural selection or inadvertent artificial selection on adult reproductive traits (McLean *et al.* 2005). For example, hatchery breeders do not allow mates to choose, compete, find suitable spawning sites, defend nests, and so on. Alternately, viability selection (survival) could act on juveniles in the hatchery, or later in life on phenotypic variation generated in the hatchery (Reisenbichler *et al.* 2004). To date, the actual causal genetic and/or environmental mechanisms responsible for the fitness differences remain unclear.

Most studies comparing the relative reproductive success of hatchery and wild fish involve salmonid species that spend one-third to one-half of their lifetime in the hatchery (e.g. steelhead, coho salmon *O. kisutch*, Atlantic salmon *Salmo salar*, brown trout *S. trutta*) prior to release as smolts (i.e. juvenile fish undergoing osmoregulatory changes necessary to migrate from fresh water to the marine environment). Time spent in a captive environment typically ensures higher juvenile survival rates when compared to that of the wild. On the other hand, the relatively long time spent in the hatchery presents substantial opportunities for domestication selection and for environmental influences that can carry over into adulthood. In this study, we compared the relative fitness of two types of coho salmon hatchery fish: those that were created in the hatchery, but released into the wild as unfed fry, vs. their siblings that were raised for a year in the hatchery and released as smolts.

Reproductive success is measured as the number of adult offspring produced per adult individual. Coho only spawn once; therefore, fitness is measured over one full generation from returning adult to returning adult (thus including components of adult reproduction and offspring survival) and this is a true measure of lifetime fitness. Unfed fry came from the same mating pairs as the smolts, but were released upon emergence without being fed in the hatchery. They experienced the same environmental conditions and high mortality (i.e. opportunity for selection) as wild fish, except for the circumstances of their fertilization and their incubation as eggs and newly hatched fry. We hypothesize that if selection on juvenile traits in the hatchery is the principal mechanism causing the decline of hatchery fish fitness, then reproductive success of unfed fry releases should be the same as that of wild fish. On the other hand, if selection (or lack thereof) acts on some other stage in the life cycle in the hatchery (e.g. adult reproductive behaviours), then the success of unfed fry (that return to spawn) should also be lower than that of wild spawners. Therefore, the goals of this study were to: (i) determine whether the phenomenon of lower fitness of hatchery fish in the wild that was principally documented in steelhead (Araki *et al.* 2007, 2008, 2009) also occurs in coho salmon (a congener with a number of significant life-cycle differences), and (ii) test the prediction that any fitness difference between hatchery and wild coho results from selection or environmental effects during the hatchery juvenile phase of the life cycle.

Methods

Sampling design

Hatchery programs were conducted on the Umpqua River basin sporadically from 1948 to 1980, using both local and non-local brood stocks. The North Umpqua River hatchery coho program was started in 1980, and began to use only North Umpqua fish as brood stock in 1983. This integrated hatchery program used local North Umpqua brood stock which included 30% natural fish (non-adipose fin-clipped) and 70% hatchery-born fish recaptured (adipose-fin clipped), to produce smolts that are used to supplement the natural-spawning population. Each smolt released from the hatchery was adipose-clipped and/or coded-wire tagged (CWT) to ensure the designation 'hatchery fish'. This program mainly produced smolts, but was also part of the Salmon and Trout Enhancement Program (STEP) that released non adipose-clipped unfed fry in tributaries throughout the Umpqua basin. Stream releases of both fry and smolt ended in 2006. In 2001, 2002 and 2003 ODFW (Oregon Department of Fish and Wildlife) collected 100 CWT/adipose-clipped adult coho salmon of each sex at Winchester Dam on the North Umpqua. These fish were designated hatchery-reared brood stock, H. They had at least one generation of hatchery ancestry, and possibly more. Over the same time period 94, 100, and 102 non-CWT/non-adipose-clipped adult coho salmon of each sex were collected and designated as wild-born brood stock, W. In 2001, the W brood stock was collected at Winchester Dam, while for 2002 and 2003, W fish were taken from the Nonpareil Dam at Calapooya Creek, a tributary of the Mainstem Umpqua (Fig. 1). For each of the 3 years, males and females were randomly paired within each group (H × H and W × W) and spawned at ODFW's Rock Creek hatchery facility using single-pair mating (i.e. each male and female was used only once). Hatcheries methods in

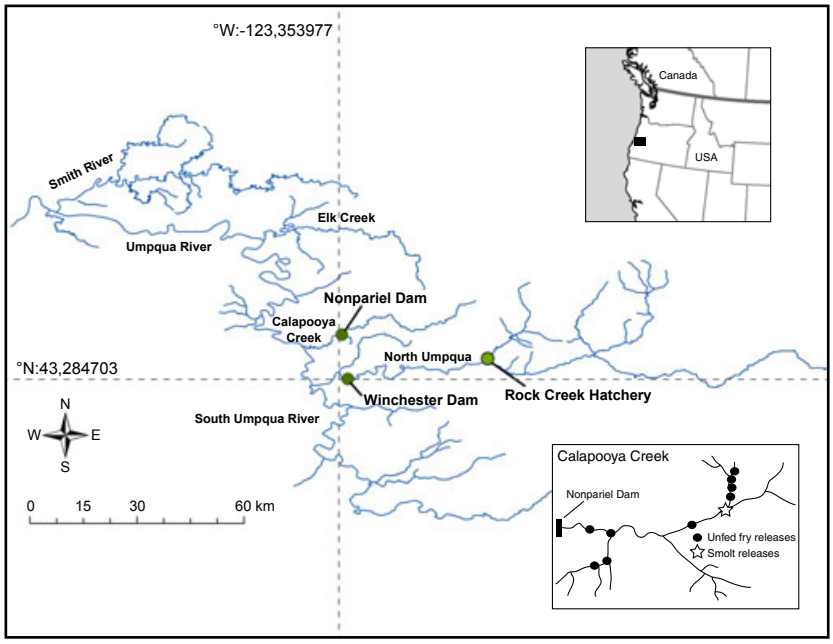

Fig. 1 Map of the study area showing sampling sites of brood stock (Winchester and Nonpareil Dam) and the location of Rock Creek hatchery. All offspring resulting from hatchery crosses were released in Calapooya Creek. The inset shows specific locations of release for unfed fry and smolts in Calapooya Creek.

Oregon vary from a random matrix design (many males with many females) to a random single-pair design, as we used in the present study. Thus, our hatchery method is representative of what can be found in a 'real' supplementation program. The progeny from these single-pair crosses are referred to as F1 hatchery fish. Note that while W is designated as wild-born, W fish may have hatchery ancestry owing to past mating of hatchery fish in the wild. Moreover, W fish collected as brood stock from Nonpareil Dam could also be composed of unfed fry returns, that is, fish born in the hatchery but released upon emergence as part of the STEP program. Calapooya Creek was supplemented in 2000 and 2001 with unfed fry, which returned as adults in 2002 and 2003. Based on analysis of returns from 2004 to 2006 in Calapooya Creek (Thériault *et al.* 2010 and the present study), we estimated the proportion of the run composed of unfed fry to be on average 15% (range 6–25%). Thus, if some W fish are in fact unfed fry returns from previous stocking, they likely form a small proportion of the brood stock. We thus assume that the majority of the W fish used in our study were born and reared in the wild, and that W fish had at least one less generation of hatchery rearing as juvenile than the H fish. Note that hatchery ancestry in W fish will just reduce our ability to detect differences between H and W.

The progeny of each pair was split at the eyed-egg stage. A portion of the offspring was transferred to hatchboxes and mixed upon emergence to be randomly released as unmarked unfed fry at nine sites along the Calapooya Creek in spring of 2002, 2003 and 2004 (Fig. 1). The remaining eggs of each pair were mixed when ponded as fry in rearing tanks and were released as smolts in spring of 2003, 2004 and 2005 at one specific location on the Calapooya Creek (Fig. 1). These two release strategies resulted in two groups with different times spent in captivity. We released an average of 438 332 fry and 22 160 smolts per year. As expected, fish released as smolt experienced much higher survival from release to adulthood (2.4% relative to 0.05% for fish released as unfed fed, Thériault *et al.* 2010). See Moyer *et al.* (2007) and Thériault *et al.* (2010) for additional methodological details.

Most coho salmon migrate to the ocean after spending a year in freshwater. Some of the males spend only one summer at sea and return to freshwater the following fall as small but sexually mature males that are two years of age (termed jacks). The majority of males and all females spend two summers in the ocean before their fall migration to freshwater spawning habitat at three years of age. Therefore, unfed fry and smolts released in Calapooya Creek from 2002 to 2005 returned as F1 2-year-old males (hereafter 'jacks') and 3-year-old males (hereafter 'males') and 3-year-old females, alongside their naturally born counterparts, from 2003 to 2006 (designated as brood year, BY, Fig. 2). Note that spawning migration starts in fall and continue until

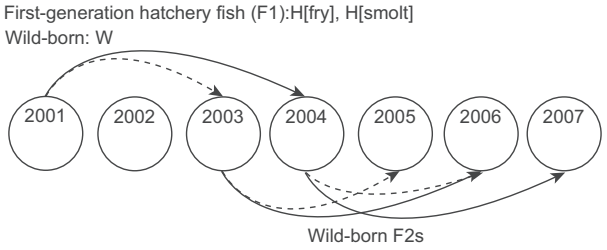

Fig. 2 Sampling design of the study. Illustrated is the sampling design for 2001, but the same scheme applies to brood stock collections in 2002 and 2003. Circles represent the brood year (BY), which correspond to the year (fall) at which the first fish enters the river during the spawning migration. For example, the first generation of hatchery fish (F1) was created from paired matings in the hatchery in the fall of 2001 and released as unfed fry (H[fry]) in spring 2002 and as smolts (H[smolt]) in spring 2003. Fish released as unfed fry and as 1-year-old smolts return on the same schedule, alongside their wild-born counterpart (W). These hatchery and wild fish returned as age-2 males (jacks; dotted line) and age-3 males and females (solid line) in BY 2003 and 2004 and mated in the wild, mixing together. F2s resulting from these matings returned as jacks and age-3 fish from BY 2005 to 2007.

February. Thus, the 'brood year' in which an adult returns is actually the fall calendar year before the year in which their offspring were born (Fig. 2). At the fish trap at the base of Nonpareil Dam, all returning coho were captured, measured, checked for fin-clips, sexed by visual identification, aged (for males only, age-2 or age-3 based on size) and had tissue samples taken via a caudal clip for genetic identification. All the fish were released above the dam after handling and allowed to spawn naturally. The offspring resulting from mating by F1s in the wild were termed F2, and returned as jacks and age-3 males and females at Nonpareil Dam from BY 2005 to 2009 (Fig. 2). All F2 returns were also caudal-clipped for genetic identification, which allowed us to match them back to their F1 parents (see parentage analysis below).

Parentage analysis

DNA was extracted from fin tissue, amplified via PCR and scored from all brood stock (BY 2001–2003), F1 and F2 progeny returning to Calapooya Creek (BY 2003–2009) at 10 microsatellites loci (*OTS*519, *OTS*520, *ONE*111, *P*53, *OTS*3, *ONE*μ2, *OCL*8, *OTS*215, *ONE*μ13, *OMY*1011) following the methods of Moyer *et al.* (2007).

The F1 returns were allocated to either a hatchery parental pair (H × H or W × W) or a wild pair using the allocation method described in Thériault *et al.* (2010). Briefly, we used the software PAPA 2.0 that uses a maximum-likelihood allocation approach, to allocate F1 returns to hatchery pairs (Duchesne *et al.* 2002). We then used PASOS 1.0 that combines a maximum-likelihood approach followed by exclusion, to allocate F1 returns to wild pairs or single parents (Duchesne *et al.* 2005). Note that these likelihood methods do not rely on significance thresholds. Therefore, our estimates of difference in reproductive success among groups should not suffer from the bias identified by Ford & Williamson (2010) (i.e. groups having a lower effective size, such as hatchery fish, wind up with an under-estimated relative RS). The following notation will be used hereafter: H[fry] and H[smolt] designate F1 fish born in the hatchery and released as unfed fry or smolts, respectively, while W designates fish born in the wild. The F2 returns were allocated to their F1 parents using PASOS 1.0. Allocation was performed for each cohort separately; F2 jacks and age-3 returns from BY 2005 to 2009 were reallocated to their F1 parents, which returned in BY 2003, 2004, 2005 and 2006 (see Fig. 2). Simulations were conducted to estimate the number of missing spawners for each BY of the F1 (see Duchesne *et al.* 2005 for description of simulation and Thériault *et al.* 2007 for an example). The accuracy of our allocations was then accessed via further simulations. Simulated offspring were produced from sampled parents used in this study and from artificially created parents based on the number of missing spawners estimated above. Simulated offspring were then allocated to known sampled parents, and the percentage of correctly assigned individuals was assessed. All the simulations were performed using the simulation procedure implemented in PASOS.

Estimation and comparison of individual reproductive success

Using parentage analysis, we estimated lifetime individual RS of F1 fish that bred in the wild (number of returning F2 mature offspring produced per individual). Mean RS was estimated separately for females, males and jacks as well as for brood stock type (W × W and H × H) and stocking strategy (unfed fry and smolt). Three years of F1 mating in the wild were available for jacks (BY 2003, 2004 and 2005), and for males and females (BY 2004, 2005 and 2006). For each gender type of fish (males, females and jacks) we estimated relative reproductive success (RRS) in each year by dividing average RS of hatchery fish by average RS of W fish of the same type. The broodstock type (H × H and W × W) did not seem to affect later reproductive success (see below) so we pooled those two types for the comparison of H vs. W. To test for statistical significance between RS of H and W fish within each year and fish type, we used two-tailed permutation

tests. Numbers of offspring assigned to each parent were permuted 10 000 times and the probability of obtaining a smaller or larger difference than the observed value was evaluated using an algorithm as applied in PERM 1.0 (Duchesne *et al.* 2006). We evaluated the power of our analysis by calculating the minimum difference in RS we could have detected with 80% and 95% probability. The minimum difference was obtained from distribution of RS differences in the permutation tests.

We then used a three-factor ANOVA to test the effects of gender type (females, males, jacks), brood stock type (W × W, H × H) and stocking strategy (unfed fry, smolt), as well as their two and three-way interactions, on the yearly RRS values. Here the data points are the individual RRS values calculated as above. We used the log values of RRS to conform to the homoscedasticity and normality assumptions of the ANOVA.

Results

Parentage analysis

Details of parentage allocation results of F1 fish to the parental generation can be found in Thériault *et al.* (2010). The number of F2 fish (progeny of F1 spawning events) returning each year over the course of this study is shown in Table 1. The proportion of returns (jacks and age-3 males and females combined) that was assigned to a parental pair or a single parent was 76%, 90%, 94% and 97% for BY 2003, 2004, 2005 and 2006 respectively. A few reasons (not mutually exclusive) can explain the unassigned fish: these include genotyping errors, errors in data recording and processing, or failure to collect parents (as a result of individuals straying from another system or spawning below the dam). The correctness rate (i.e. the percentage of fish allocated to the correct parents according to simulations) was 91%, 96%, 96% and 97% for BY 2003, 2004, 2005 and 2006 respectively.

Relative reproductive success

Point estimates of RRS and a comparison between RS of H[fry] relative to wild fish via permutation tests are shown for each gender-type (jacks, males, females,) in Table 2. For females, RRS was less than 1.00 in all 3 years comparisons (range from 0.76 to 0.91) but none of the comparisons was significant (Table 2, Fig. 3a). For males, RRS was also less than 1.00 in all three years (range from 0.48 to 0.74) and was significant in 2005 and 2006, and for the 3 years combined (Table 2,

Table 2 Reproductive success in the wild of fish stocked as unfed fry relative to wild fish (RRS) for each brood year (BY)

BY	*N* (fry/wild)	RRS*	*P*-value	Power† (80%/95%)
Females				
2004	26/358	0.91	0.80	0.63 / 0.45
2005	97/352	0.76	0.12	0.81 / 0.70
2006	201/218	0.87	0.21	0.87 / 0.81
Overall female		0.84	0.26	
Males (3-year-old)				
2004	41/384	0.74	0.29	0.80 / 0.61
2005	101/277	0.48	0.001	0.87 / 0.75
2006	172/188	0.68	0.005	0.85 / 0.78
Overall male		0.62	<0.001	
Jacks (2-year-old)				
2003	5/93	2.13	0.30	2.17 / 3.67
2004	7/54	2.09	0.26	2.53 / 11.17
2005	18/66	1.49	0.24	1.55 / 2.10
Overall jack		1.75	0.24	

N is the number of F1 fish that spawned in the wild used in the analysis. *P*-values are based on two-tailed permutation tests (see Methods for details).
*RRS is calculated as the RS of hatchery fish stocked as fry over RS of wild fish. Overall RRS was estimated using weighted geometric means and the according *P*-values were calculated on the basis of Fisher's combined probability.
†Statistical power represents the minimum effect size (displayed as RRS) detectable with 80% and 95% power.

Table 1 Numbers of F1 fish spawning in the wild in brood year (BY) 2003 to 2006 used in parentage analysis and corresponding number of F2 fish returning in BY 2005–2009

F1 hatchery/ wild (BY)	F2 total returns	F2 age-2 jacks returns (BY)	F2 age-3 adults returns (BY)	Not used	Unresolved
66/93 (2003)	616	78 (2005)	538 (2006)	4	150
428/796 (2004)	569	86 (2006)	483 (2007)	8	52
937/625 (2005)	1427	104 (2007)	1323 (2008)	9	87
935/406 (2006)	2057	120 (2008)	1937 (2009)	1	53

The numbers not used correspond to F2 missing genotype information. 'Unresolved' are F2 fish left unassigned by parentage analysis. In BY 2003, F1 are only composed of age-2 males (first F1 age-3 fish returned in BY 2004). In BY 2006, F1 are only composed of age-3 fish.

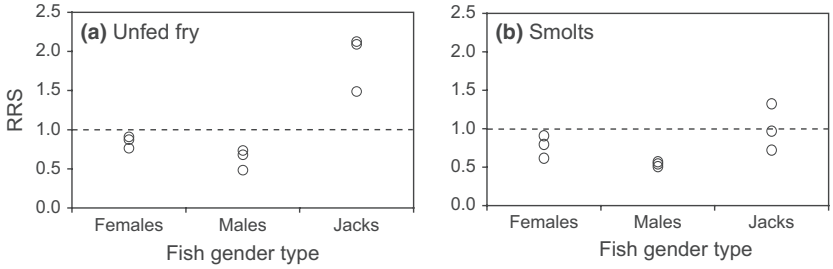

Fig. 3 Relative reproductive success (RRS, reproductive success of F1 fish of hatchery origin relative to wild fish) for each gender type (females, males, jacks) for (a) fish released as unfed fry and (b) fish released as smolts. Fish of W × W and H × H origin were pooled as brood stock type had no effect (see 'Results' section). Each point is the yearly RRS estimate. The dotted line represents the value where reproductive success of hatchery fish is equal to reproductive success of wild fish (RRS = 1).

Fig. 3a). For jacks, sample size limited the power of our analysis but RRS was greater than 1.00 (range from 1.49 to 2.13) for all comparisons, although not significantly so in any BY (Table 2, Fig. 3a).

Point estimates of RRS and a comparison between RS of H[smolt] relative to wild fish via permutation tests are shown in Table 3. For females, RRS was less than 1.00 for all comparisons (range from 0.62 to 0.91) and was significant in 2005 and 2006, and for the 3 years combined (Table 3, Fig. 3b). For males, RRS was also significantly less than 1.00 in all three years (range from 0.51 to 0.57, Table 3, Fig. 3b). For jacks, RRS ranged from 0.72 to 1.33 and was not significantly different than 1.00 in any comparison (Table 3, Fig. 3b).

If we pool both types of hatchery fish (H[fry] and H[smolt]), their RRS over all three years was significantly less than 1 for females and for males (RRS = 0.77 and 0.56 respectively, both *p*-value two-tailed <0.001). For jacks, RRS was not significantly different than 1 (RRS = 1.11, *P*-value two-tailed = 0.68).

The overall ANOVA testing the effects of gender type, brood stock type and stocking strategy on RRS was significant (F ratio = 3.48, $P = 0.0051$), with the result clearly driven by the main effect of gender type ($P < 0.001$) (Table 4; Fig. 3). A post hoc Tukey HSD test revealed that RRS for jacks differs from males and females, but that the latter two were indistinguishable at the 0.05 significance level. None of the other main effects or interactions were significant, although the effect of stocking type (H[fry] vs. H[smolt]) was close ($P = 0.065$). Here females and males that were released as unfed fry did slightly better than those released as smolts, and the difference was even larger for jacks

Table 3 Reproductive success in the wild of F1 fish stocked as smolts relative to wild fish (RRS) for each brood year (BY)

BY	*N* (smolt/wild)	RRS*	*P*-value	Power† (80%/95%)
Females				
2004	135/358	0.91	0.54	0.82/0.73
2005	361/352	0.62	<0.001	0.88/0.81
2006	295/218	0.80	0.03	0.88/0.81
Overall female		0.75	<0.001	
Males (3-year-old)				
2004	156/384	0.57	0.001	0.83/0.75
2005	287/277	0.54	<0.001	0.87/0.81
2006	267/188	0.51	<0.001	0.88/0.81
Overall male		0.53	<0.001	
Jacks (2-year-old)				
2003	61/93	1.33	0.35	1.48/2.00
2004	63/54	0.97	1	0.47 / 0.21
2005	73/66	0.72	0.35	0.64/0.49
Overall jack		0.94	0.65	

N is the number of F1 fish that spawned in the wild used in the analysis. *P*-values are based on two-tailed permutation tests (see Methods for details).
*RRS is calculated as the RS of hatchery fish stocked as smolts over RS of wild fish. Overall RRS was estimated using weighted geometric means and the according *P*-values were calculated on the basis of Fisher's combined probability.
†Statistical power represents the minimum effect size (displayed as RRS) detectable with 80% and 95% power.

Table 4 Three-way analysis of variance (ANOVA) testing the effect of fish gender type (females, males, jacks), brood stock (W × W, H × H) and stocking strategy (unfed fry, smolt) on RRS (RS of hatchery fish over wild fish). The log values of RRS were used

Term	d.f.	Sum of squares	*F* ratio	*P*
Fish gender	2	4.123	13.915	<0.001
Brood stock	1	0.0007	0.005	0.946
Stocking	1	0.553	3.732	0.065
Fish gender × brood stock	2	0.226	1.528	0.228
Fish gender × stocking	2	0.540	1.824	0.183
Brood stock × stocking	1	0.226	1.53	0.228
Fish gender × brood stock × stocking	2	0.023	0.078	0.925

(Fig. 4). Interestingly, H fish of the two brood stock types (H × H vs. W × W) were indistinguishable ($P = 0.946$).

Discussion

Lower fitness of hatchery fish in the wild

The first important result of this study is the finding that age-3 hatchery coho exhibit a lower fitness in the wild than wild coho. This result confirms the phenomenon documented for steelhead (Araki *et al.* 2007), which is the only other published study that estimated lifetime reproductive success in the natural environment for local-origin hatchery brood stock. Thus, for both steelhead and coho, only a single generation of integrated hatchery rearing (i.e. where local wild-born brood stock was used for supplementation) is sufficient to decrease the RS of those hatchery fish in the wild. Surprisingly, we observed no difference between hatchery fish of H × H parents and those of W × W parents. Thus, the additional generation of hatchery rearing had minimal effect, which is in stark contrast to results from steelhead in which the fitness of second-generation hatchery fish was much lower than that of first-generation fish (Araki *et al.* 2007).

The second major finding of this study is that although the RS of age-3 hatchery fish released as unfed fry was slightly higher than that of hatchery fish released as smolts (Fig. 4), it was still substantially less than that of the wild fish. This trend was seen in both age-3 males and in females (Fig. 3; Tables 2 and 3). We hypothesized that if selection or environmental effects of hatchery rearing occurred during the captive juvenile phase, then hatchery fish released as unfed fry would have similar RS to that of wild fish, with both having higher RS than hatchery fish released as smolts. These results, therefore, suggest that the causal mechanism for lowered fitness is not associated only with the juvenile stage of the life cycle, but also involves some effect during the adult mating phase or during incubation of eggs or newly hatched fry (see also Berejikian *et al.* 2009).

Where in the life cycle?

Incubation and homing. Given these data that, in part, point away from the juvenile phase, it is worth reconsidering other potential stages in the life cycle that make hatchery and wild fish different. First, the incubation environment was different (i.e. hatch box/tray *vs.* natural gravel bed). Kihslinger & Nevitt (2006) found a larger cerebellum (part of the brain involved in spatial recognition and social learning) for emerging fry that were incubated in tanks with structural complexity (stones) relative to fry incubated in tanks without stones. Thus, it is possible that some environmental effects experienced as early as incubation can initiate a developmental trajectory that facilitates survival in the local environment. How this might translate into reproductive success differences later in life is still unclear.

Second, our study did not take into account individuals straying to rivers other than the Calapooya. If the straying rate was higher for the wild-born descendants of F1 hatchery fish (the F2s) when compared to descendants of wild-born fish, then, this could bias our results and explain the observed lower RS. However, it is unclear why hatchery F2s would have a higher straying rate because they were reared under the same environmental conditions as their wild counterparts where olfactory learning and imprinting take place (Dittman & Quinn 1996). Moreover, these fish are not from a highly domesticated strain, where many generations in captivity could potentially alter homing abilities, but rather from an integrated hatchery program designed to minimize domestication selection. The lower RS of F1 hatchery fish could be associated with the result of fine scale homing to spawning locations where they were released within the Calapooya River if those locations are of poorer quality than locations where wild fish spawn. While information on spawning locations is lacking, that hatchery and wild fish frequently mated with each other (data not shown) suggests they are actually abundant on the same spawning grounds.

Breeding stage. The other portion of the life-cycle that is shared between unfed fry and smolt releases is the

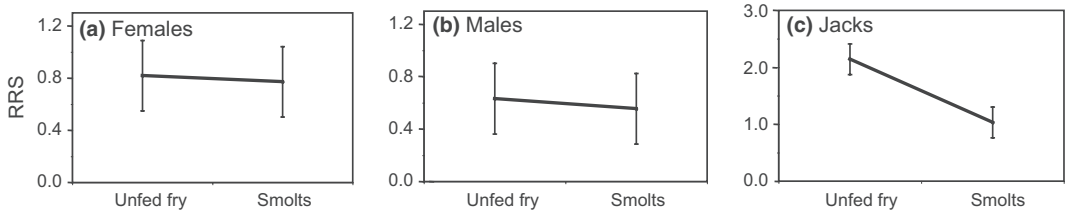

Fig. 4 Relative reproductive success (RRS, reproductive success of F1 fish of hatchery origin relative to wild fish) for fish released as unfed fry and fish released as smolts for (a) females, (b) males and (c) jacks. Each point represents the least squares means over 3 years. Error bar represents one standard error.

breeding stage. Hatchery breeding, where there is no opportunity for mate choice, intra-sexual competition, redd construction, or any other type of natural breeding behaviour, differs substantially from breeding in the wild (Wedekind 2002). During the course of this study, fish released as unfed fry and as smolts both returned to the river earlier than wild fish (Thériault *et al.* 2010). The difference in return time was attributable to the unintentional collection of earlier spawners that were used as brood. An earlier run time (a life history trait shown to be heritable in salmon; Carlson & Seamons 2008) could be associated with a lower RS providing a possible explanation for the observed lower RS of hatchery fish. However, linear and quadratic relationships between run time and RS (data not shown) were not significant or consistent over years suggesting that the magnitude of the difference in RS could not be explained by the unintentional selection of hatchery brood.

Although we cannot entirely exclude environmental effects that could happen as early as the incubation period (see above), we suggest that the absence of sexual selection, owing to artificial breeding in the hatchery, is a plausible explanation for some of the lower RS observed in fish released both as unfed fry and as smolts. In particular, three pieces of evidence in our dataset implicate selection (or lack thereof) on adult reproductive traits as a contributing cause of fitness declines in hatchery fish: (i) unfed fry release have a lower RS than wild fish (discussed above), (ii) males consistently show a more severe decrease in RRS than females, and (iii) jacks show striking divergence in RRS from age-3 males.

We observed that 3-year-old hatchery males averaged 56% of the RS of wild fish, which was 21% less than that observed in females (77%). Studies on reproductive behaviour have found hatchery fish, particularly males, to be competitively inferior to wild fish, showing less aggression and more submissive behaviour (Fleming & Gross 1993; Fleming *et al.* 1996; Berejikian *et al.* 2001). This competitive disadvantage translated into lower RS, with males more severely affected than females (Fleming & Gross 1993; Fleming *et al.* 1996). Competition among males for spawning females is usually more intense than intra-sexual female competition for spawning territories and nest defence (Fleming & Gross 1992; Quinn & Foote 1994). Thus, intense selection for breeding opportunities among males may magnify the differences in behaviour (and consequently breeding success) between hatchery and wild fish. By circumventing sexual competition that takes place on the natural spawning grounds, artificial breeding could allow less competitively fit fish to reproduce (e.g. less aggressive and more submissive fish that would otherwise be evicted by dominant males). Agonistic behaviour has been shown to have a significant additive genetic component in salmonids, and can thus be inherited (Rosenau & McPhail 1987; Swain & Riddell 1990; Vollestad & Quinn 2003). Therefore, the offspring resulting from artificial mating in the hatchery could show an inherited behaviour that subsequently proves to be less fit in a natural spawning context.

Hatchery jacks did not show the same fitness decline as age-3 hatchery males, suggesting that the absence of natural selection or inadvertent artificial selection on adult reproductive traits was a causal mechanism for reduced RS of hatchery fish in the wild. Jacks favour a different reproductive tactic – they stay in the periphery of the dominance hierarchy established by older males and sneak into the nest at the time of egg deposition (Gross 1985). Despite the low sample size for jacks, the pattern is very different than what we found for age-3 males. The mechanism that is causing the discrepancy in fitness between wild and hatchery age-3 males does not seem to affect jacks in the same way or as severely. Interestingly, a similar result has been observed in hatchery Atlantic salmon. Hatchery adult males have been found to have a poor fertilization success relative to wild males due to their reduced aggressiveness (Fleming *et al.* 1996) while hatchery precocious parr (males that mature younger without seawater migration) were found to be more successful, in part because they showed more aggression and dominated their wild counterparts (Garant *et al.* 2003). These results suggest that the relative level of aggression can change with life-stage and age (e.g. Berejikian *et al.* 1996) or that the genetic basis of reproductive behaviour differs between the two life-histories. Another explanation for our observed discrepancy between jacks and age-3 males could be related to the proximate mechanisms leading to the adoption of alternative mating tactics. Jacking is associated with higher growth rates as juveniles (Vollestad *et al.* 2004). Higher growth rate is often linked to higher level of aggression through the increased level of growth hormone (Jönsson *et al.* 1998; Fleming *et al.* 2002). Therefore, if we assume that the lower RS of hatchery age-3 males is due to their reduced competitiveness and aggressiveness, it is possible that this does not show up in jacks because they are among the faster growers, and hence more aggressive fish of their cohort.

Summary

Here we have shown that age-3, first-generation hatchery coho have lower RS than wild fish, with age-3 males doing worse, on average, than females. Furthermore, the same effect was observed in hatchery fish that were released as fry and in those released as smolts.

Interestingly, hatchery jacks did at least as well as wild fish in all comparisons. Finally, we observed no effect of broodstock type (H × H *vs.* W × W) on the performance of hatchery fish, which suggests that the additional year(s) of captive rearing in the hatchery had minimal effect. The actual causal mechanism(s) responsible for the fitness decline of hatchery fish are currently unknown. Although our study only provides indirect evidence, we suggest that differences in spawning behaviour resulting from the absence of sexual selection through artificial breeding may contribute to the problem. It is not clear whether this potential mechanism would apply to all salmonids and all systems or could be extended to non-salmonids. Furthermore, selection on juvenile traits could still occur in concert with relaxation of selection on adult behaviours, especially in hatchery programs where fish are grown quickly to a size at age suitable for migration, a treatment that does not necessarily correspond to their natural life-cycle (for example steelhead, where fish are commonly released at age-1 despite the age-2 migration pattern typical in nature).

Our findings have important management and conservation implications. We suggest that releasing unfed fry may not be the panacea for circumventing negative hatchery effects. On the one hand, released unfed fry do survive to return as adults to the wild populations, and they are phenotypically more like wild fish as adults than those hatchery juveniles released as smolts (Caroffino *et al.* 2008; Thériault *et al.* 2010). But on the other hand, our results indicate that fish released as unfed fry also exhibit reduced RS once they return as adults to reproduce in the wild. If that reduced RS has a genetic origin and is carried over generations (e.g. Araki *et al.* 2009), then even unfed fry releases have the potential to negatively impact wild populations.

Finally, our results support the finding outlined by others that even contemporary hatchery practices (e.g. using wild brood stock, pairwise matings) can produce fish that have lowered reproductive success in the wild. This evidence suggests that hatcheries may need to consider how to replicate the intricacies of natural breeding behaviours if they are to produce fish for supplementation programs that truly help recover endangered populations.

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Sequence conservation among orthologous vomeronasal type 1 receptor-like (*ora*) genes does not support the differential tuning hypothesis in Salmonidae

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ABSTRACT

Salmon utilize olfactory cues to guide natal stream homing during spawning migrations. Both inorganic and biogenic chemicals have been proposed as odorants that might be used by salmon during homing. In this study, we used genomic DNA sequence data from nine salmonid species to compare nucleotide identities for orthologous main olfactory receptor (*mOR*) genes with nucleotide identities for orthologous vomeronasal type 1-like (*ora*) receptor genes. We found that orthologs for both classes of olfactory receptor genes (*mOR*s and *Ora*s) appear to be highly conserved among species. Our findings do not support the differential tuning hypothesis in Salmonidae, which predicts higher sequence conservation for *mOR*s than *ora*. We did, however, find convincing evidence for site-specific positive selection acting on paralogous main olfactory receptor genes.

1. Introduction

Anadromous salmonids demonstrate a remarkable ability to return to their natal streams to spawn after spending a year or more in the ocean, often traversing thousands of kilometers during the course of migration. Natal stream homing is believed to mediate the accumulation of heritable, locally adapted traits within breeding populations, by subjecting successive generations to relatively similar freshwater selective pressures (Dittman and Quinn, 1996).

Although geomagnetic cues have been proposed as a means by which salmon might navigate the open ocean (Lohmann et al., 2008), the seminal works of Arthur Hasler revealed the nexus between olfaction and home stream recognition by salmon in the nearshore environment (Hasler and Wisby, 1951; Scholz et al., 1976). Numerous authors have since proposed a variety of water borne chemicals that might serve as naturally occurring odorants used by salmon during homing. These range from inorganic solutes such as calcium (Bodznick, 1978) to species-specific pheromones (Nordeng, 1977) and dissolved amino acids (Shoji et al., 2003).

Like other vertebrates, salmon detect odorants through the activity of olfactory receptors (ORs); seven transmembrane G-protein coupled receptors that are expressed by sensory neurons of the olfactory epithelium (Buck and Axel, 1991). Four distinct classes of vertebrate ORs have been identified to date: main olfactory receptors (MORs), trace amine-associated receptors (TAARs), vomeronasal type 1 receptors (V1Rs) and vomeronasal type 2 receptors (V2Rs; all reviewed by Mombaerts, 2004). Evidence from receptor-ligand studies have suggested that terrestrial vertebrate MORs are broadly tuned to bind a variety of odorants, whereas vomeronasal receptors (V1Rs and V2Rs) appear to be finely tuned to species-specific odorants such as pheromones (Boschat et al., 2002; Emes et al., 2004; Grus and Zhang, 2008). These receptor-ligand relationships constitute the basis of the "differential tuning hypothesis", which further predicts that selective constraints should result in high sequence conservation among orthologous *mOR*s, while positive Darwinian selection on genes encoding vomeronasal receptors should favor taxon-specific genetic variation that could facilitate species recognition.

Although teleosts do not possess a vomeronasal organ, *V1R*- and *V2R*-like genes (sometimes referred to as *ora* and *olfC* receptor genes) are present in teleost genomes (Alioto and Ngai, 2006; Saraiva and Korsching, 2007). Moreover, a variety of biogenic chemicals, including amino acids, prostaglandins, and steroid hormones, have been proposed to function as pheromones in teleosts (Sorensen and Stacey, 2004; Yambe et al., 2006). Accordingly, expectations of the differential tuning hypothesis may apply to teleost *OR* genes. Plausibly, finely tuned Ora and OlfC receptors could contribute to home stream recognition by detecting species- or even population-specific biogenic odorants.

Abbreviations: ANOVA, Analysis of variance; cDNA, DNA complementary to RNA; ddH_2O, Double-distilled water; dN/dS, Ratio of non-synonymous to synonymous nucleotide substitutions; DNA, Deoxyribonucleic acid; dNTP, Deoxynucleotide triphosphate; LRT, Likelihood ratio test; MgCl, Magnesium chloride; *mOR*, Main olfactory receptor gene; MOR, Main olfactory receptor; *olfC*, Olfactory C family receptor gene; OlfC, Olfactory C family receptor; *OR*, Olfactory receptor gene; OR, Olfactory receptor; *Ora*, Class A-related olfactory receptor gene; PCR, Polymerase chain reaction; *salmOR*, Salmonid olfactory receptor gene; TAAR, Trace amine-associated receptor; *V1R*, Vomeronasal receptor type 1 gene; V1R, Vomeronasal receptor type 1; *V2R*, Vomeronasal receptor type 2 gene; V2R, Vomeronasal receptor type 2.

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In this study, we test the differential tuning hypothesis in Salmonidae by comparing nucleotide sequence conservation among orthologs of seven *mOR* and two *ora* genes from nine salmonid species, whereby

H_0. Sequence identity of orthologous *mOR*s ≤ sequence identity of orthologous *ora*s; and

H_1. Sequence identity of orthologous *mOR*s > sequence identity of orthologous *ora*s

We then examine salmonid *OR* gene nucleotide sequences for signals of site-specific positive selection to gain insight to the evolutionary processes shaping OR gene diversity in this widely distributed teleost family.

2. Materials and methods

2.1. Isolation of OR genes

We utilized a combination of degenerate PCR and genome walking techniques to isolate salmonid *OR* gene sequences. We first designed degenerate PCR primers with the program CODEHOP (Rose et al., 2003), using multiple amino acid sequence alignments for receptors encoded by diverse teleost *mOR* genes. Alignments were performed with ClustalW (Thompson et al., 1994) implemented in BioEdit (Hall, 1999) and included sequences representative of zebrafish (*Danio rerio*) OR gene families C, D, E, F, G and H (Alioto and Ngai, 2005), as well as similar sequences from other teleost species, identified through tBLASTx searches (Altschul et al., 1997). Degenerate PCR primers for *ora* were developed according to the methods of Johansson and Banks (2011).

We isolated genomic DNA from a single coho salmon (*Oncorhynchus kisutch*) tissue sample with a DNeasy® kit (Qiagen Inc., Valencia, CA) and performed individual PCRs using our degenerate primers in 50 μL reactions containing 30.5 μL ddH_2O, 5 μL 5× reaction buffer, 3 μL 25 mM MgCl, 2.5 μL of each 10 μM forward and reverse primers, 1 μL 10 mM dNTPs, 0.5 μL Taq polymerase and 5 μL DNA template at 100 ng/μL. Thermocycling followed a modified "touchdown" PCR profile (Korbie and Mattick, 2008), initiated through 4′ at 94°, 1′ at 68°, 1′30″ at 72°, followed by 26 additional cycles wherein the annealing temperature was incrementally reduced by 2° until reaching 56° in the final 6 cycles, followed by a 2′ extension at 72°.

We separated PCR products by agarose gel electrophoresis and purified them with a QIAquick® gel extraction kit (Qiagen, Inc.). We then direct sequenced PCR products with the same degenerate primers used during PCR. When sequence reads were ambiguous or incomplete, we cloned products using a TOPO TA Cloning® Kit, (Invitrogen, Inc.) and sequenced inserts using T3/T7 or M13 forward and reverse primers according to the manufacturer's protocol. All sequencing was carried out on an ABI 3730XL DNA Analyzer (Applied Biosystems, Inc.) and reads were aligned and edited with BioEdit software (Hall, 1999).

For sequences that presented high similarity to previously described *OR* genes, as determined through tBLASTx searches, we used Vector NTI software (Invitrogen, Inc.) to design high stringency (T_m > 67 °C) nested PCR primers from the putative *OR* gene sequences. We then used these primers in conjunction with a Universal GenomeWalker™ Kit (BD Biosciences, Inc.) to obtain sequence from the 5′ and 3′ flanking regions of each putative *OR* gene. As with degenerate PCR products, amplicons generated through genome walking were separated by gel electrophoresis, extracted, purified and sequenced, using the nested (secondary) walking primers to initiate sequencing reactions.

Once we had obtained complete putative *OR* gene sequences from coho salmon, we designed medium stringency ($T_m \approx 55$ °C) PCR primers in the sequences flanking each gene. We used these primers to amplify DNA from a single individual for each of the following salmonid species: Atlantic salmon (*Salmo salar*), brown trout (*S. trutta*), cutthroat trout (*O. clarkii*), steelhead rainbow trout (*O. mykiss*), sockeye salmon (*O. nerka*), Chinook salmon (*O. tshawytscha*), pink salmon (*O. gorbuscha*), chum salmon (*O. keta*), and another coho salmon (*O. kisutch*). PCRs were performed through 3′ at 94°; followed by 5 touchdown cycles of 30″ at 94°; 30″at 58°–54° (annealing), 45″ at 72°; followed by 30 cycles of 30″ at 94°; 30″at 54°, 45″ at 72°; and a 2′ extension at 72°. We then isolated, purified, cloned and sequenced all PCR products of expected size.

2.2. Nomenclature

For the purposes of this study, we devised a system of nomenclature for putative salmonid *OR* genes, based on similarities with previously described teleost *OR* genes. Briefly, we used ClustalW in BioEdit to align the amino acid translations for *O. kisutch* and *O. mykiss* gene sequences with zebrafish sequences from the eight MOR families described by Alioto and Ngai (2005). We used this alignment to infer relationships between salmonid MORs and established teleost MOR families by constructing a maximum likelihood tree with the program PROML in the PHYLIP analysis package (Felsenstein, 2005). We assessed node support of this tree by bootstrapping the data with the program SEQBOOT and constructing a consensus tree with the program CONSENSE (Felsenstein, 2005). Trees were visualized with TREEVIEW (Page, 1996).

In our system of salmonid *OR* gene nomenclature, we converted the alphabetic gene family designations of Alioto and Ngai (2005) to numeric designations, such that salmonid genes similar to zebrafish *OR* family "A" genes received a *salmOR* family designation of "100". In suite, zebrafish *OR* family B-like genes were designated *salmOR200* gene family members, and zebrafish *OR* family C-like genes were designated *salmOR300* gene family members, etc. Subfamilies within gene families were identified by single digit increments of the centennial gene family designator (eg. *salmOR300*, *salmOR301*, *salmOR302*) and multiple genes within subfamilies were designated by a terminal single digit preceded by a dash (eg. *salmOR300-1*). Percent amino acid sequence identities greater than 60% and 40% were used to verify subfamily and family gene clades, respectively (*sensu*Alioto and Ngai, 2005). This objective nomenclature system facilitated organization and analyses of our data, which included distinctly novel gene sequences, as well as sequences similar or identical to salmonid *OR* genes previously described with various nomenclatures (Dukes et al., 2006; Morinishi et al., 2007; Wickens et al., 2001).

We refer to teleost *V1R*- and *V2R*-like genes as *ora* and *olfC* genes, respectively, as suggested by Saraiva and Korsching (2007) and Alioto and Ngai (2005), and applied by Johnstone et al. (2008, 2009) for salmonids.

2.3. Sequence analyses

Given the short (800–1200 bp) and typically single exon nature of *mOR* and *ora* genes, we chose to use percent nucleotide sequence identity as an initial measure of conservation among orthologous genes. We used ClustalW to align nucleotide sequences for all orthologous salmonid *OR* genes, then used the program BioEdit to calculate percent nucleotide sequence identities for all sequence pairs in the alignment. We then used a one-way ANOVA to perform a planned comparison between percent nucleotide sequence identities of orthologous salmonid *ora* genes and orthologous *mOR* genes, with $\alpha = 0.05$. If salmonid *mOR* and *ora* genes have evolved in a manner similar to their mammalian *mOR* and *V1R* analogs, we predict that sequence identity for *mOR*s will be greater than that of *ora* genes.

We used the program CODEML from the PAML 4 analysis package (Yang, 2007) to calculate global non-synonymous to synonymous nucleotide substitution rates (dN/dS or ω) for all groups of orthologous genes, under the basic M0 model. But because positive selection can

drive sequence divergence for specific codons amid global sequence conservation, we used site models to test for evidence of codon-specific positive selection among orthologous salmonid *OR* genes. We estimated log likelihood scores for ancestral sequence reconstruction under CODEML models 7 (β model) and 8 (β and ω model), which vary constraints on site-wise (dN/dS or ω), where $\omega>1$, $\omega=1$, and $\omega<1$ indicate positive selection, neutrality, and purifying selection, respectively (Yang et al., 2000). We then compared model 7 and 8 log likelihood scores through likelihood ratio tests, where $LRT=2(\ell_0-\ell_1)$ was evaluated over a χ^2 distribution with $\alpha=0.05$. This approach allowed us to investigate for site-specific signals of genetic divergence among orthologs, which might otherwise be masked by global sequence conservation.

In a final analysis, we investigated for site-specific signals of positive selection among paralogous salmonid *OR* genes with the PAML "branch-site test" (Zhang et al., 2005). Branch-site tests were used to identify lineages of salmonid *OR* genes likely to have been influenced by positive selection during the course of gene family radiation. For these tests, we performed LRTs with log likelihood scores for the branch-site model A and a null model, using conservative critical values of 3.84 at 5% and 5.99 at 1%, to guard against possible violations of model assumptions (Zhang et al., 2005). We then employed a Bayes Empirical Bayes analysis to identify amino acid sites likely to have been subjected to selection (Yang et al., 2005) and used the TMHMM Server v. 2.0 (Krogh et al., 2001) to predict the genic locations of these sites relative to OR transmembrane, intra- and extracellular coding domains.

3. Results

Numerous studies have demonstrated that salmon are able to detect and respond to conspecific odors (Quinn et al., 1983; Groot et al., 1986; Courtenay et al., 2001). If these odors are utilized by salmon during natal stream homing or mate choice, it might be expected that positive Darwinian selection would diversify orthologous *OR*s involved in species recognition. In mammals, such diversification has been observed for *V1R* and *V2R* orthologs, but not for *mOR* orthologs, forming the basis of the differential tuning hypothesis (Grus and Zhang, 2008). Our study represents the first effort to address this hypothesis in Salmonidae, by comparing nucleotide sequence identities of orthologous *mOR*s with those of orthologous *ora* genes.

3.1. Isolation of OR genes

In total, we isolated seven putative *mOR*s and a novel *ora* gene from the coho salmon genome. We then characterized these eight genes in a majority of eight additional salmonid species, though PCR failed to amplify one or more target genes in some species (Table 1). PCR failures may have been the result of multiple primer binding site mutations (various forward and reverse primer combinations were tested), but could also have been due to gene absence in some lineages.

The *mOR* gene sequences that we obtained cluster with zebrafish *mOR* gene families C, D, E and F (Fig. 1). Amino acid sequence identities further suggest that four *mOR* families are present in our data, with each family represented by a single subfamily (Table 2). We refer to these putative *mOR* genes as members of salmonid *OR* gene subfamilies *salmOR300*, *salmOR400*, *salmOR500* and *salmOR600*.

Several of the *OR* gene sequences that we obtained resemble previously described salmonid *OR* genes. For example, the first *mOR* that we isolated from the *O. kisutch* genome is nearly identical to a gene described from five other Pacific salmon by Morinishi et al. (2007), with high synteny in 5′ and 3′ flanking sequences. We found this gene also to be present in *S. salar*, *O. clarkii* and *O. tschawycha*. We included the sequences of Morinishi et al. (2007) in our analyses (GenBank

Table 1
PCR primers and GenBank accession numbers for olfactory receptor genes isolated from *Oncorhynchus* and *Salmo* species. Dashes indicate no amplification during PCR.

Putative OR gene Forward primer Reverse primer	*O. clarkii*	*O. gorbuscha*	*O. keta*	*O. kisutch*	*O. nerka*	*O. tshawytscha*	*O. mykiss*	*S. salar*	*S. trutta*
salmOR300-1 GGTTTCACGATGAAGCAGATAACA GCATTGGCTTACATCAAAGACATT	FJ611230	AB255033[a]	AB255035[a]	FJ611229	AB232550[a]	FJ611228	AB255034[a]	FJ611227	-
salmOR300-2 AGGACCTGAAGCAAGGATATGC GTACACCATAAGGAATTGGATGAATT	FJ613850	FJ613851	FJ613852	FJ613853	FJ613855	FJ613856	FJ613854	FJ613857	FJ613858
salmOR300-3 TCCGCAGGTTGTCTAAGAGACCATCATC GGCATTGAGGTCAGGATTTGGTA	FJ613859	-	-	FJ613860	FJ613862	FJ613863	FJ613861	FJ613864	FJ613865
salmOR300-4 CCAGTGTTGGTTCTAAGAGAACACAT CATGGAAATCAGCAATGCAACA	FJ613866	FJ613867	FJ613868	FJ613869	FJ613871	FJ613872	FJ613870	FJ613873	FJ613874
salmOR400-1 GAGAGGTATACAATTAACAGGAAGGCTG GGGAATGTCACCTTACTTGGAAACTT	FJ716225	FJ716226	FJ716227	FJ716228	FJ716230	FJ716231	FJ716229	FJ716232	FJ716233
salmOR500-1 GGAGCAGTATCACTCTCTCAAGCAAG CTAACTGTCTCGCAGGTTGGTAGAA	FJ613875	FJ613876	FJ613877	FJ613878	-	FJ613880	FJ613879	-	FJ613881
salmOR600-1 GGAAGCTGCCCCACAGTCT GGGCTATTGATGGCTGAGAGAA	FJ613882	FJ613883	-	FJ613884	-	FJ613886	FJ613885	FJ613887	FJ613888
ora1-1 CCAATAGAATATGACCATTGATGGCT TCACAAGACCATCCATGAGGTATTAA	-	-	-	-	-	-	-	EU143808[b]	FJ716224
ora1-2 CCTGTGCTGCTGTGTGCCAA CAGCAAAACCCT CCATGAGGTAT	FJ613841	FJ613842	FJ613843	FJ613844	FJ613846	FJ613847	FJ613845	FJ613848	FJ613849

[a]Sequences characterized by Morinishi et al. (2007). [b]Sequence characterized by Johnstone et al. (2008).

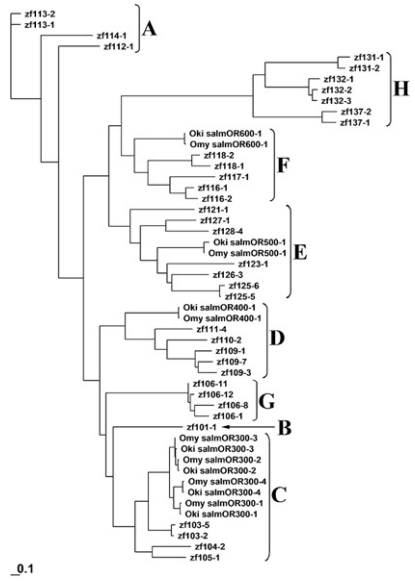

Fig. 1. Maximum likelihood tree from amino acid sequence alignment, depicting relationships among salmonid olfactory receptors from *Oncorhynchus mykiss* (Omy) and *O. kisutch* (Oki) with *Danio rerio* (zf) olfactory receptors from eight gene families (letters A-H). Bootstrap values >70% appear at left of corresponding nodes. Branch lengths represent the number of substitutions per amino acid site.

AB232550, AB255033–AB255036) and treated these genes as orthologs of *salmOR300-1*. In addition to *salmOR300-1*, we identified three novel genes from this *mOR* subfamily: *salmOR300-2*, *salmOR300-3* and *salmOR300-4* (Table 2).

The novel *salmOR400-1* gene closely resembles the Atlantic salmon *ASOR1* gene described by Wickens et al. (2001), but differs through the unusual presence of a single intron in all orthologs and lacks synteny with *ASOR1* in the 5′ untranslated region. Although *salmOR400-1* may be a pseudogene, as suggested by the presence of an intron, we treated this sequence as a bi-exonic gene in our analyses. To this end, we defined *salmOR400-1* intron-exon boundaries through alignment with the *ASOR1* gene sequence, which was characterized from cDNA (Wickens et al., 2001). The novel *salmOR600-1* gene appears to be a member of the same subfamily as the Atlantic salmon *SORB* gene described by Dukes et al. (2006), with 62% predicted amino acid sequence identity between these two genes in *S. salar*.

We identified a novel *ora1* gene (which we name *ora1-2*, see following discussion) from each of the nine salmonid species included in our study. The predicted coding region of this gene in *S. salar* presents 92.4% predicted amino acid sequence identity with the *ora1* gene previously described from *S. salar* by Johnstone et al. (2008), yet carries 99% mean predicted amino acid sequence identity with apparent orthologs from other salmonids.

We confirmed the presence of two copies of *ora1* in *S. salar* by amplifying both copies of this gene from a single *S. salar* individual. We accomplished this by designing primers in unique flanking sequences of the two putative genes (Table 1), identified through an alignment of the BAC sequence analyzed by Johnstone et al. (2008; GenBank EU147784.1) with the genomic sequence obtained through our degenerate PCR-genome walking approach. The *S. salar* sequence that we obtained with BAC clone-designed primers presented 100% nucleotide sequence identity with *ora1* from Johnstone et al. (2008). We hereafter refer to the novel gene as *ora1-2*, and refer to the gene described by Johnstone et al. (2008) as *ora1-1*. We successfully isolated both *ora* genes from *S. trutta* (as in *S. salar*), but failed to isolate *ora1-1* from any *Oncorhynchus* species.

3.2. Sequence conservation and positive selection

We observed mean percent nucleotide sequence identities ranging from 89.4% (*salmOR400-1*) to 98.5% (*salmOR300-3*) for *mOR* orthologs. Significantly higher percent identities were observed among orthologs of *ora1-2* (99.0%) than among orthologs of any *mOR* gene ($F = 13.183$, $p < 0.001$ on 2 and 233 d.f.). Similarly, *ora1-1* genes from *S. trutta* and *S. salar* presented a higher percent nucleotide sequence identity (99.1%) than the mean percent nucleotide sequence identity for any group of *mOR* orthologs. Accordingly, we do not reject the null hypothesis of *mOR* sequence identities being less than or equal to sequence identities among *ora* orthologs. We emphasize that this finding is not consistent with the differential tuning hypothesis, which predicts greater conservation of *mOR* genes.

Global dN/dS ratios did not exceed unity for any group of orthologs, suggesting that the protein structures of the ORs here examined are globally conserved among species. Moreover, we found no convincing evidence for site-specific positive selection driving sequence divergence among either *mOR* or *ora1* gene orthologs (Table 3).

Similarly, a branch-site test provided no evidence for site-specific positive selection driving divergence between the paralogous *ora1-1* and *ora1-2* genes ($LRT = 1.76$, $p > 0.05$ on 2 d.f.). In contrast, however, a branch-site test provided convincing evidence for site-specific positive selection having acted over residues of *salmOR300* subfamily paralogs ($LRT = 14.22$, $p < 0.01$ on 2 d.f.). A Bayes Empirical Bayes analysis (Yang et al., 2005) indicated codon sites 54 ($p = 0.939$), 113 ($p = 0.952$) and 156 ($p = 0.993$) as those most likely to have experienced selection. These codon sites correspond to an amino acid residue of the first intracellular domain, and residues of the third and fourth transmembrane domains, respectively. A phylogeny of *salmOR300* subfamily genes, indicating residue changes at these sites is presented in Fig. 2.

4. Discussion

Comparative genomic studies have provided evidence to reveal the dynamic nature of *OR* gene evolution among vertebrate species. Through duplication, diversification and pseudogenization, *OR* gene

Table 2
Mean percent amino acid sequence identities among seven putative main olfactory receptors (MORs) isolated from nine species of salmon and trout. Mean values among orthologs in bold.

	SalmOR300-1*	SalmOR300-2	SalmOR300-3	SalmOR300-4	SalmOR400-1	SalmOR500-1	SalmOR600-1
SalmOR600-1	27	27	27	27	24	31	**97**
SalmOR500-1	25	22	23	24	22	**93**	
SalmOR400-1	33	31	31	31	**94**		
SalmOR300-4	82	80	80	**98**			
SalmOR300-3	82	94	**98**				
SalmOR300-2	82	**96**					
SalmOR300-1[a]	**97**						

* Includes sequences from Morinishi et al. (2007).

Table 3
For orthologous salmonid *OR* genes, mean global dN/dS ratios (standard errors) and log likelihood scores for reconstruction of ancestral sequences under CODEML models 7 and 8. The number of sequences analyzed (n) and amino acid sites predicted to be under positive selection are also presented. Bottom Rows: Log likelihood scores for null and Model A branch-site models, and associated LRT statistic. Likelihood ratio tests ($LRT = 2(\ell_0 - \ell_1)$) were evaluated over a χ^2 distribution. See text for additional details.

Site models	n	Global dN/dS (SE)	Model 7	Model 8	LRT	p (df = 2)	AA sites
salmOR300-1	9	0.38 (0.00)	−1719.39	−1719.35	0.083	0.96	
salmOR300-2	9	0.34 (0.00)	−1710.81	−1709.75	2.119	0.35	
salmOR300-3	7	0.15 (0.00)	−1640.03	−1639.82	0.414	0.81	
salmOR300-4	9	0.44 (0.00)	−1599.36	−1599.17	0.372	0.83	
salmOR600-1	7	0.23 (0.00)	−1641.76	−1640.25	3.028	0.22	
salmOR400-1	8	0.40 (0.00)	−1281.98	−1281.38	1.196	0.55	
salmOR500-1	7	0.48 (0.00)	−1939.86	−1939.80	0.123	0.94	
ora1-2	9	0.32 (0.00)	−1443.43	−1441.16	4.544	0.10	
Branch-sites models	n	**Global dN/dS (SE)**	**Null model**	**Model A**	**LRT**	p^*	**AA sites**
salmOR300 subfamily	34		−3621.56	−3614.45	14.22	<<0.05	54, 113, 156
ora1 subfamily	11		−1678.23	−1677.35	1.76	>0.05	

* Evaluated over critical values of 3.84 at 5% and 5.99 at 1%.

families have expanded and contracted as vertebrates have colonized new environments (Niimura and Nei, 2005) and experienced selective pressures favoring species recognition (Grus and Zhang, 2004). The putative *OR* genes that we have examined likely represent only a fraction of the presumably large salmonid *OR* gene superfamily, yet our data lend novel insight to the nature of *OR* gene evolution in this teleost family.

We found *ora* genes to be more highly conserved among salmonid species than *mOR* genes, as evidenced by a comparison of percent nucleotide sequence identities among orthologs from these two classes of *OR* genes. Our data provided no significant evidence for site-specific positive selection influencing diversity among either *ora* or *mOR* orthologs. Accordingly, these results do not support the differential tuning hypothesis in Salmonidae, but are instead consistent with previous reports of high *ora* gene sequence conservation among teleost species (Saraiva and Korsching, 2007; Johansson and Banks, 2011). The high similarity among *ora1-2* orthologs suggests that the receptor encoded by this gene likely does not provide species-specific olfactory

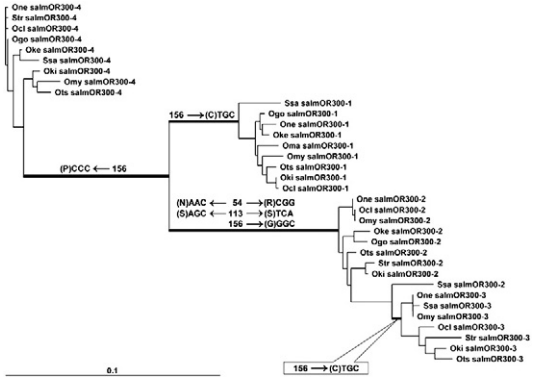

Fig. 2. Maximum likelihood tree inferred from nucleotide sequence alignment of 4 olfactory receptor genes from the *salmOR300* subfamily*, isolated from *Oncorhynchus kisutch* (Oki), *O. clarkii* (Ocl), *O. mykiss* (Omy), *O. tshawytscha* (Ots), *O. gorbuscha* (Ogo), *O. keta* (Oke), *O. nerka* (One), *O. masu* (Oma) *Salmo salar* (Ssa), and *S. trutta* (Str). Branch lengths represent the number of substitutions per nucleotide site. Amino acid sites under positive selection are presented on foreground branches delineating paralog clades (in bold). Arrows point toward clades with indicated residues (in parentheses) and codons for each selected site. * Sequences of Morinishi et al. (2007) here as orthologs of *salmOR300-1*.

information during natal stream homing. In mammals, conservation of V1R orthologs is believed to explain physiological responses to pheromones produced and detected among diverse ruminant species (Ohara et al., 2009). It is unknown whether salmonids respond to the biogenic odors of other, potentially sympatric congeners. Carefully designed behavioral studies could provide relevant answers.

Interestingly, the *ora1-1* gene, isolated from both *Salmo* species of our study, failed to amplify in any *Oncorhynchus* species, despite repeated efforts. This result may be an artifact of multiple PCR primer site mutations, or it could reflect a lineage-specific absence of this *ora* gene, as has been observed for some teleost *ora* (Pfister et al., 2007) and tetrapod *V1R* genes (Grus and Zhang, 2004; Grus et al., 2005). If the latter explanation were accurate, it would suggest that salmonid *ora* gene diversification proceeds through birth-death evolution (Nei and Rooney, 2005), as opposed to sequence divergence among functional orthologs. Indeed, *ora1-1* may have evolved to detect biogenic odorants specifically associated with the *Salmo* genus.

A branch-site analysis of all *salmOR300* subfamily genes suggested that, in a common ancestor of *Salmo* and *Oncorhynchus*, positive Darwinian selection influenced certain codons of paralogous *mOR* genes, favoring fixation of non-synonymous nucleotide substitutions relative to synonymous substitutions. A Bayes Empirical Bayes analysis did not provide highly significant posterior probabilities for the identification of codon sites under selection, a common issue when multiple sites experience moderate levels of positive selection (Yang et al., 2005). However, two of the three lowest p-values were associated with residues of transmembrane domains, which could potentially affect ligand binding sites (Mombaerts, 2004; Katada et al., 2005). This interpretation would be consistent with the findings of several previous studies, which have reported evidence of positive selection acting on transmembrane domain residues for paralogous *OR* genes (Alioto and Ngai, 2005; Kondo et al., 2002; Ngai et al., 1993).

We found that high levels of sequence conservation among *salmOR300-1* orthologs, as previously described by Morinishi et al. (2007), are not unique to this salmonid *OR* gene. Instead sequences for other genes from this *mOR* subfamily are conserved at similar or even higher levels among salmonid species (Table 2). In fact, all of the *OR* genes that we isolated presented high levels of ortholog conservation in Salmonidae.

Taken together, our results demonstrate that both *mOR* and *ora* orthologs are highly conserved in diverse salmonid species. The model for *OR* gene evolution proposed through the differential tuning hypothesis is therefore not supported by our data, and species recognition among salmonids is not likely to be facilitated by sequence variation of *ora* gene orthologs. However, it should be noted that our findings do not preclude the possible (and likely) existence of *functional* differences among salmonid *OR* gene repertoires, which could arise through variable gene copy numbers or gene expression. Moreover, interspecific diversity of salmonid *olfC* and *taar* genes has not been examined in this study or elsewhere. Additional research of salmonid *OR* gene diversity and expression, both within and among species, is needed to advance our understanding of the molecular bases of olfaction and homing by salmon.

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Interlocus variance of F_{ST} provides evidence for directional selection over an olfactory receptor gene in Coho salmon (*Oncorhynchus kisutch*) populations

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ABSTRACT

Coho salmon (*Oncorhynchus kisutch*) utilize olfactory cues to recognize and home to natal streams during spawning migrations. Chemically distinct river systems may promote directional selection for appropriately tuned olfactory receptor repertoires among Coho populations. Here, we use F_{ST} outlier methods to test for a signal of selection over olfactory receptor gene-linked markers, characterized in Coho populations from four geographically proximate, but ecologically distinct rivers. We report evidence for directional selection over one such marker, *OkiOR3001*, and document substantially higher levels of genetic structure among Coho populations from Oregon coastal lakes than previously observed with putatively neutral microsatellites.

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1. Introduction

In recent years, Oregon coastal Coho salmon (*Oncorhynchus kisutch*) populations have experienced dramatic fluctuations in census size. Several years of low escapements and the uncertainty of future ocean conditions prompted managers to list this evolutionarily significant unit (ESU) as threatened under the Federal Endangered Species Act (NMFS, 2008). During this time, molecular genetic studies have aimed to provide insight into the extent and scale of structure and connectivity among populations. For example, microsatellite analyses have produced estimates of effective size, migration rates and genetic structure for Coho populations from this region (Ford et al., 2004; Johnson and Banks, 2008), and this information has been used to identify recovery goals and modify resource use practices (Wainwright et al., 2008). The presumed neutrality of microsatellite markers has, however, precluded their application toward any inference of adaptive genetic diversity within and among populations.

Nevertheless, data from genetic markers are routinely used to describe the effects of both demographic processes and selection over natural populations (*e.g.* Bonin et al., 2006; Wilding et al., 2001; Hoekstra et al., 2004). To facilitate such analyses, several methods have been developed to discriminate amongst loci that either conform to, or depart from, neutral models of population divergence (reviewed by Luikart et al., 2003). For example, genome scans (Bowcock et al., 1991, Kayser et al., 2003) may be used to identify loci which present unexpectedly high (or low) F_{ST} values. With caution, selection may then be inferred for "outlier loci", as was first suggested by Lewontin and Krakaeur (1973). More recently, it has been recognized that although genome scans do not require *a priori* information of marker linkage associations, efficiency can be greatly improved by including candidate genes of likely functional significance among the markers to be examined (Stinchcombe and Hoekstra, 2008).

As adult Pacific salmon undergo spawning migrations, they recognize and home to natal streams with the aid of olfactory cues (Quinn and Dittman, 1990). Salmon detect water-borne odorants through the activation of olfactory receptors, which are expressed by sensory neurons of the olfactory epithelium, and coded for by a large and diverse superfamily of genes (Alioto and Ngai, 2005). Accurate homing presumably confers greater fitness through adaptations to local freshwater environments. Coho salmon home to ecologically diverse spawning grounds along the Oregon coast, ranging from low gradient lake dominated habitats, to coastal rivers and oligotrophic, montane streams. Accordingly, the chemical signatures of Oregon streams also differ (Wigington et al., 1998), which may drive selection for optimally tuned suites of olfactory receptors in different Coho populations.

Here, we use a panel of olfactory receptor gene-linked markers and putatively neutral microsatellites to characterize the genetic structure among naturally spawning Coho salmon populations from four geographically proximate, yet ecologically distinct coastal Oregon river systems. We use genotypic data from these populations to test for a signal of selection at olfactory receptor gene-linked loci, through an analysis of interlocus variance of F_{ST}. This approach aims to identify loci under directional selection and examine the distribution of potentially adaptive genetic diversity among coastal Oregon Coho populations.

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2. Methods

2.1. Tissue sampling

During the 2004–05 spawning season, Oregon Department of Fish and Wildlife biologists collected tissue samples from unmarked Coho spawner carcasses, while conducting stream surveys of the Umpqua, Siltcoos, Tenmile and Tahkenitch rivers (Fig. 1). During collections, biologists recorded the date and location associated with each sample taken, and tissues were stored in 95% ethanol until processed in the laboratory. The Umpqua is a large coastal river that drains an area of approximately 4560 mi^2 (11,810 km^2), including western slopes of the Cascade Mountains. In contrast, the Tenmile, Tahkenitch and Siltcoos smaller basins, characterized by expanses of lake habitat and numerous small streams.

2.2. Olfactory receptor gene-linked marker development

We designed olfactory receptor (OR) gene-linked markers by first isolating OR gene sequences from a single Coho salmon, as described in Johnson and Banks (2009). After isolating the coding sequences for OR genes, we used a Universal GenomeWalker™ Kit (BD Biosciences, Inc.) to perform additional rounds of primer walking, to further characterize sequences flanking each OR gene. PCR cycling conditions followed those recommended in the GenomeWalker™ protocol, and products were separated via agarose gel electrophoresis. We then excised bands and purified PCR products with a QIAquick® Gel Extraction kit (Qiagen Inc., Valencia, CA). The high stringency primers used for primer walking were also used to carry out direct sequencing reactions. We generated our sequence data with an ABI 3730XL DNA Analyzer (Applied Biosystems, Inc., Carlsbad, CA) and used BioEdit software (Hall, 1999) to align and edit sequence reads. Through this procedure, we identified micro- and minisatellites in the sequences flanking Coho OR genes. We then used Vector NTI software (Invitrogen, Inc.) to design PCR primers for these potentially variable features. We incorporated fluorescent labels onto forward primers, to facilitate high-throughput genotyping.

2.3. Genotyping

Using a Chelex and proteinase-K protocol (Estoup et al., 1993), we extracted genomic DNA from Coho tissue samples for the Umpqua ($n = 213$), Siltcoos ($n = 39$), Tahkenitch ($n = 47$) and Tenmile ($n = 41$) populations. We then used primers for OR gene-linked markers, as well as 12 previously described microsatellite loci (Table 1) to genotype all samples from the four populations. For simplicity of terms, we hereafter refer to the 12 previously described microsatellites as the "neutral markers". PCRs were carried out separately in 5 μL volumes, using a "touchdown" protocol (Korbie and Mattick, 2008). PCR began with a 3′ denature at 94 °C, followed by five denature–extention–anneal cycles in which the annealing temperature was decreased by 1 °C per cycle: 30″ annealing at 94 °C, 30″ annealing at 57 °C thru 53 °C, 30″ extension at 72 °C, then 30 cycles of 30″ at 94 °C, 30″ at 53 °C, and 30″ at 72 °C. We collected fluorescence data with an ABI 3730XL DNA Analyzer and scored genotypes with GeneMapper Software (Applied Biosystems, Inc., Carlsbad, CA).

2.4. Statistical analyses

We used the program GENETIX (Belkhir et al., 2004) to estimate F_{ST} between all population pairs, calculated as θ (Weir and Cockerham, 1984). We obtained θ estimates twice, first using data from the OR gene-linked markers, then with data from the neutral markers. We used GENETIX to perform a permutation test (1000 permutations) to test for linkage disequilibrium among all locus pairs in all populations, using a Bonferroni corrected critical value to assess significance.

We employed two approaches to test for a signal of selection among loci, both of which represent modifications of the F_{ST} outlier test proposed by Lewontin and Krakauer (1973). Utilizing information from genotypic data supplied by the user, the program FDIST2 (Beaumont

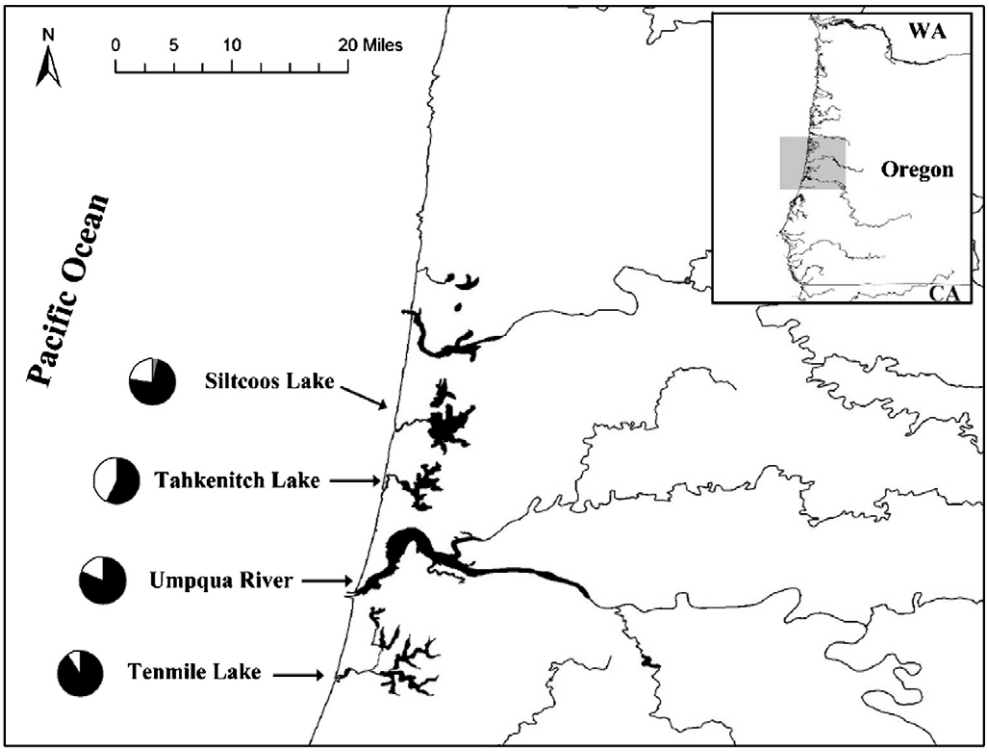

Fig. 1. Map of the study area, indicating Siltcoos, Tahkenitch, and Tenmile lakes, and the Umpqua River, Oregon, U.S.A. Pie charts depict allele frequencies of *Oki3001* for Coho salmon populations: black fill for allele 434, open for allele 436 frequency. Allele 432 (gray) was observed only in the Siltcoos population.

Table 1
Putatively neutral microsatellite loci used to characterize Coho salmon populations from the Tenmile Lake, Tahkenitch Lake, Siltcoos Lake and Umpqua River.

Locus	Source
Oke4	Buchholz et al. (2001)
Oki13	Smith et al. (1998)
Omy1011	Rexroad et al. (2002)
One111	Olsen et al. (2000)
Ots2	Banks et al. (1999)
Ots3	Banks et al. (1999)
Ots206	Greig et al. (2003)
Ots215	Greig et al. (2003)
Ots505	Naish and Park (2002)
Ots519	Naish and Park (2002)
p53	de Fromentel et al. (1992)
Ssa85	O'Reilly et al. (1996)

and Balding, 2004) performs a series of coalescent simulations to construct a 95% confidence interval for expected θ values, over a range of heterozygosities. Loci that fall outside the high probability region may be classified as outliers and, with due caution, selection may be inferred. We performed 20,000 realizations with FDIST2 to simulate loci for the construction of the 95% confidence interval (Fig. 2).

A second test was performed with the program DETSEL (Vitalis et al., 2003), which also uses coalescent simulations to model a high probability region for the joint distribution of F_{ST}. This method is executed for pairs of populations, and assumes a neutral model of population genetic divergence. We used DETSEL to perform neutrality tests for all loci, in all possible pairwise combinations. We initiated our analyses with the default nuisance parameters, 10,000 simulated loci and a 95% high probability region, q. We conducted tests using divergences times of $t=30$, 40 and 50, then varied nuisance parameter values to assess robustness of the model.

3. Results

3.1. Coho salmon olfactory receptor genes and associated markers

We isolated seven main olfactory receptor genes from the Coho salmon genome (Johnson and Banks, 2009) and identified a minisatellite and/or microsatellite physically linked to each of these. However, stutter artifacts generated unacceptable genotyping error rates for two of the gene-linked markers (data not shown), which we did not include in our analyses. Primer sequences and repeat motifs for the remaining five OR gene-linked markers are presented in Table 1.

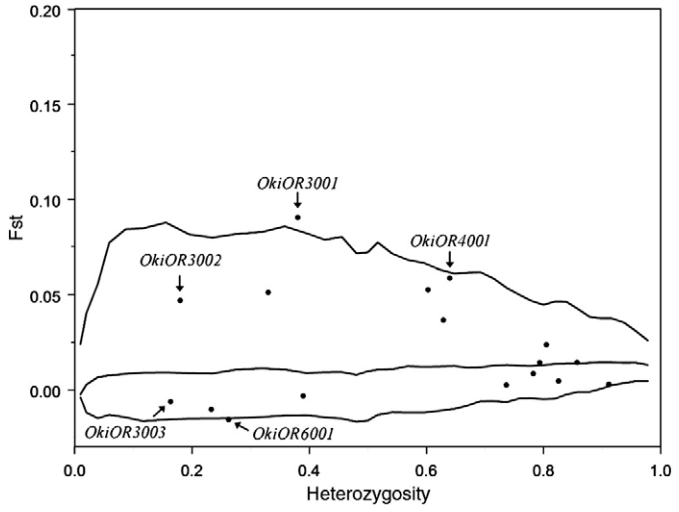

Fig. 2. Relationship between F_{ST} and heterozygosity for 12 microsatellite loci and five OR gene-linked loci (labeled) characterized from four coastal Oregon Coho salmon populations. Upper and lower lines delineate the 95% confidence interval for expected F_{ST} × heterozygosity under a neutral model of divergence, as estimated through FDIST2 simulations (Beaumont and Balding 2004). Center line represents expected median F_{ST} across the full range of possible heterozygosities.

3.2. Patterns of divergence among loci

The neutral microsatellites presented between 2 and 24 alleles, with heterozygosities ranging between 23% and 91%. OR gene-linked markers presented between 2 and 16 alleles, with heterozygosities that ranged between 16% and 81%. We observed only minimal evidence for linkage disequilibrium. In Siltcoos Lake, *Oke4* and *Ots519* appeared to be in LD ($p<0.0006$) and in the Umpqua *p53* and *One111* appeared to be in LD ($p<0.0006$). However, the majority of locus pairs presented no evidence for LD in any population, and appear to be independently segregating loci.

In all but one instance, pairwise θ values were higher for OR gene-linked markers than for neutral microsatellites (Table 2), although neither marker class provided statistically significant θ values between the Siltcoos population and those of other lake systems. We observed particularly strong divergence between Tenmile and Tahkenitch lakes for OR gene-linked markers ($\theta=0.115$), reaching a level of divergence that is nearly an order of magnitude greater than observed for neutral microsatellites ($\theta=0.015$) (Table 3).

The program FDIST2 identified two OR gene-linked markers as F_{ST} outliers. Whereas *OkiOR3001* presented a particularly high F_{ST}, *OkiOR6001* was unexpectedly low. None of the neutral microsatellites appeared to be outliers.

We obtained similar, but not entirely consistent results from the program DETSEL. Unfortunately, only two pairwise population comparisons were possible, as most simulations generated a negative joint distribution of F_{ST} that could not be plotted or used to evaluate individual loci. Nevertheless, for the Siltcoos × Umpqua analysis, all OR gene-linked markers and *One111* appeared to be outliers ($t=30$). By increasing the time since divergence, t, in the model, first *Ots2* ($t=40$), then *Ots2* and *Oki13* ($t=50$) added to the list of outliers. In simulations of divergence between Tahkenitch × Umpqua ($t=30$), outlier loci included *OkiOR3001*, *OkiOR3002*, *Oki13* and *Ots2*. With an increase in t, *Ssa85* and *OkiOR6001* also appeared as outliers (for both $t=40$ and $t=50$). These results were generally consistent across a wide range of nuisance parameter values.

4. Discussion

Studies examining sequence variation have provided mounting evidence for positive (directional) selection acting over paralogous vertebrate olfactory receptor genes, while purifying selection appears to maintain high sequence conservation among orthologues (Kondo et al., 2002; Gimelbrant et al., 2004; Grus and Zhang, 2008; Johnson and Banks, 2009). Findings from these studies have identified processes governing OR evolution following gene duplications, as well as speciation events. However, only a few studies have examined OR gene diversity among populations of any vertebrate species (Moreno-Estrada et al., 2008; Gilad et al., 2000).

The approach we have taken has allowed us to assay genetic diversity associated with multiple OR genes in four Coho salmon populations. Given the apparent ecological differences between the Umpqua River and the coastal Oregon lakes, we expected to see the greatest divergence for OR gene-linked markers to occur between populations from these two habitat types. Interestingly, this was not the case, as θ was highest between the Tenmile and Tahkenitch Lake populations. This result illustrates the difficulty associated with predicting variables driving evolution, and suggests that one or more processes are inflating OR gene associated θ values among lake populations.

Table 2
Olfactory receptor gene-linked markers used to characterize Coho salmon populations from the Tenmile Lake, Tahkenitch Lake, Siltcoos Lake and Umpqua River drainages.

OR gene-linked marker	Associated OR gene	Primers for marker (5′–3′)	Repeat motif	No. alleles	Distance from gene start/stop codon
OkiOR3001	*salmOR300-1*	F-GGACATGAACGCTCACTCATTC R-CTGACCATGCTGCTGCTCCAG	(TC-) microsatellite	3	2385 bp upstream from start
OkiOR3002	*salmOR300-2*	F-GTGGAAGTGCCGACTAATGACG R-GAACTGTGGTCAGAACGTGACACTC	41 bp minisatellite	3	954 bp downstream from stop
OkiOR3003	*salmOR300-3*	F-AGACAGGGTCCTGAAAAGGGG R-CAGCACTGTAACCGTAACGCAA	20 bp minisatellite	3	2438 bp upstream from start
OkiOR6001	*salmOR600-1*	F-GAATGAGCAGGTGCGTCCAAA R-CGCTGGAACAAACCAAGAACT	(A-) mono repeat	2	1544 bp upstream from start
OkiOR4001	*salmOR400-1*	F-GTTCACACAAAGACTGCCTCCAAAAC R-CCATCTCAAACTAAACAGCCTTCCTG	31 bp minisatellite	16	179 bp upstream from start

Table 3
Pairwise θ values for Oregon coastal Coho salmon populations, as calculated with putatively neutral microsatellites (upper right demi-matrix), and five OR gene-linked markers (lower left demi-matrix).

	Siltcoos	Tahkenitch	Tenmile	Umpqua
Siltcoos	–	0.002	0.005	**0.023**
Tahkenitch	0.015	–	**0.015**	**0.018**
Tenmile	0.022	**0.115**	–	**0.020**
Umpqua	**0.023**	**0.039**	**0.052**	–

Statistically significant values ($p<0.05$) are in bold.

The significantly low θ value observed for *OkiOR6001* could be interpreted as a signal of balancing selection over this locus. However, FDIST2 lacks power to discriminate between balancing selection and neutrality (Beaumont and Balding, 2004), and *OkiOR6001* did not appear as an outlier in some DETSEL results. Because corroboration among tests provides the strongest evidence for selection (Bonin et al., 2006), we cannot confidently infer non-neutral evolution for this locus.

In contrast, each of the tests we performed provided significant evidence for directional selection over *OkiOR3001,* and allele frequencies for this locus are visibly different among populations (Fig. 1). The gene associated with this marker, *salmOR300-1*, has been described from all Pacific salmon, as well as Atlantic salmon and several trout species (Johnson and Banks, 2009; Morinishi et al., 2007), yet little genetic diversity has been reported for orthologues of this gene. It is therefore somewhat surprising that *OkiOR3001* presents the strongest evidence for selection among Coho populations. However, selection may be acting over the regulation of this gene, and not the coding sequence *per se*.

In Atlantic salmon, striking variation has been reported for OR gene expression among full-sibling families (Dukes et al., 2004). These findings suggest that intraspecific genetic diversity may translate into functional, regulatory differences over OR genes. A number of regulatory mechanisms have been proposed for vertebrate olfactory receptor genes, many of which involve *cis* elements (Reed, 2000; Serizawa et al., 2003; Hoppe et al., 2006). As the majority of our markers are situated 5′ to nearby OR genes, it is plausible that they are linked to functionally variable promoter elements.

A logical next step would be to examine sequence data for *salmOR300-1* from multiple individuals from each population. Our preliminary efforts toward this end indicate that linkage between *salmOR300-1* and its associated marker, *OkiOR3001*, may not be particularly strong. In several instances, marker homozygotes appear to be heterozygous for the *salmOR300-1* gene (unpublished data). Additional sampling will be needed to establish linkage strength between OR genes and their associated markers.

On the other hand, if our gene-linked markers are associated with variable regulatory elements, quantitative PCR could furnish data to test for the effects of marker genotypes. Specifically, we would like to know if different marker alleles explain variation of expression for linked OR genes. Of course, selection is not likely to operate over the same genes in all populations, so it will be important to address this question with judiciously selected OR genes, populations and samples.

In summary, we have detected unexpectedly high divergence at an OR gene-linked marker among Oregon Coho salmon populations. While our findings suggest that directional selection has acted over this locus, further studies will be needed to determine the functional implications of allelic diversity for this marker. Such work promises to shed light on the evolutionary processes shaping olfactory receptor gene diversity among natural salmon populations.

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Genetic structure, migration, and patterns of allelic richness among coho salmon (*Oncorhynchus kisutch*) populations of the Oregon coast

Marc A. Johnson and Michael A. Banks

Abstract: Genotypic data from eight microsatellite loci are used to infer population structure, effective population size, migration rates, and patterns of allelic richness among wild and hatchery populations of Oregon coastal coho salmon (*Oncorhynchus kisutch*). Corroborating the results of a previous study, we found relatively weak genetic structure among coho from different river basins, although some geographically and ecologically defined clades are supported. Contemporary migration rates among basins appear to be high and asymmetrical. Hatchery populations tended to resemble the wild populations from which they were founded, but presented significantly lower levels of allelic richness. Allelic richness was also low in Oregon coastal lake populations and peaked in the central region of the evolutionarily significant unit among wild river populations. We suggest that the observed patterns may reflect both current source–sink dynamics and post-Pleistocene colonization events.

Résumé : Les données génotypiques provenant de huit locus microsatellites nous ont servi à déduire la structure de la population, la taille effective de la population, les taux de migration et les patrons de richesse allélique chez des populations sauvages et de pisciculture de saumons coho (*Oncorhynchus kisutsch*) de la région côtière de l'Oregon. Nous trouvons une structure génétique relativement faible parmi les saumons coho des divers bassins versants, ce qui corrobore les résultats d'une étude antérieure; néanmoins, il y a évidence de quelques clades définis géographiquement ou écologiquement. Les taux de migration actuels d'un bassin à l'autre semblent être élevés et asymétriques. Les populations de pisciculture tendent à ressembler aux populations sauvages dont elles sont issues, mais elles affichent des taux significativement plus bas de richesse allélique. La richesse allélique est également basse dans les populations lacustres de la côte de l'Oregon et elle atteint son maximum dans la région centrale de l'unité évolutive significative parmi les populations sauvages d'eau courante. Nous pensons que les patrons observés sont le reflet à la fois de la dynamique actuelle de type source–drain et des événements de la colonisation après le pléistocène.

[Traduit par la Rédaction]

Introduction

The life histories of Pacific salmon (*Oncorhynchus* spp.) present unique challenges for management and conservation. For example, the tendency for adult salmon to return to their natal streams as they prepare to spawn is believed to isolate populations over a spatial scale and mediate genetic divergence of locally adapted stocks (Taylor 1991). Moreover, in some species, fixed maturation ages, combined with semelparity, limits matings among individuals from different brood years to events involving less abundant precocial individuals (jacks). For example, most coho salmon (*Oncorhynchus kisutch*) mature and spawn at age 3+ years, although a fraction return to spawn at age 2+ years. Consequently, temporally isolated subpopulations can occur within a single watershed.

Given the potential for population structure at multiple scales, the rapid decline of many Pacific salmon stocks in the late 1980s prompted managers and conservation biologists to consider demographic independence and genetic distinctiveness as key criteria for the establishment of management units (Allendorf and Phelps 1981; Waples 1991; Utter et al. 1993). Allozyme studies (e.g., Utter et al. 1973; Beacham et al. 1985; Weitkamp et al. 1995) provided the first source of genetic data for the delineation of the 52 Pacific salmon evolutionarily significant units (ESUs; Waples 1991) now recognized by the US Endangered Species Act. However, the statistical power provided by allozyme data to discriminate among populations varies greatly for Pacific salmon species (Utter 1991), limiting the general applicability of these markers.

Advances in molecular genetic technology have since allowed researchers to uncover previously undetected levels of genetic diversity within and among populations of Pacific

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salmon. For example, highly polymorphic microsatellite markers have elucidated significant genetic structure among coho salmon within and among river basins of California (Bucklin et al. 2007), Oregon (Ford et al. 2004), British Columbia (Small et al. 1998; Beacham et al. 2001), and Alaska (Olsen et al. 2003). Over a larger scale, Smith et al. (2001) used mtDNA sequence data and microsatellite markers to describe patterns of coho genetic diversity throughout the species' North American range. A latitudinal cline of mtDNA haplotype diversity led Smith et al. (2001) to hypothesize that early Pleistocene glaciations had reduced the North American distribution of coho salmon to southern refugia in California and (or) Oregon. Latitudinal clines of genetic diversity in other nearshore fishes have similarly been described (e.g., Adams et al. 2006; Gysels et al. 2004). Only three coho populations from California and Oregon were considered by Smith et al. (2001), thus limiting the resolution of analyses in this region.

Recently, Ford et al. (2004) used seven microsatellite loci to more thoroughly characterize the genetic structure of coho salmon populations from the Oregon coast. In addition to structure analyses, the authors tested for a signal of genetic introgression from an aquacultural operation, which utilized non-native broodstock on the central Oregon coast. Their findings generally supported previously hypothesized population complexes and clearly acknowledged the potentially confounding effects of anthropogenic activities over coho salmon genetic diversity.

In this study, we use data from eight microsatellite loci to characterize genetic structure within and among putative coho salmon populations from 23 river basins of the Oregon coastal coho (OCC) ESU, as well as a single basin (Rogue River) from the Northern California – Southern Oregon ESU (Fig. 1). We utilize temporally replicated samples of wild populations from five river basins to estimate the effective number of breeders, immigrant fractions, and the percentage of genetic variation apportioned among river basins, brood-years, and within basin sampling sites. Directional migration rates among wild coho populations are estimated through a Bayesian assignment method (Wilson and Rannala 2003), and in a final analysis, we examine latitudinal patterns of allelic richness among wild and hatchery coho populations of the Oregon coast.

Materials and methods

Sampling and genotyping

During the spawning seasons of 2002, 2003, and 2004, tissue samples were collected from expired, presumably wild origin coho salmon spawners from 24 coastal Oregon river basins. Wild origin fish were distinguished from hatchery coho through the presence of an intact adipose fin, which is removed from nearly all coastal Oregon hatchery coho prior to release (84%, 96%, and 97% marked for brood years 2002, 2003, and 2004, respectively). In 2004, samples were also collected from adipose fin-clipped coho returning to five Oregon hatcheries. Tissue samples were preserved in 95% ethanol, and the collection location and date were recorded together with the length and sex of each fish sampled. From the 2002 collection, samples from all rivers were included in our analyses. Samples from four basins in the 2003 collection (Nehalem, Yachats, Smith, and Coos) and five basins in the 2004 collection (Nehalem, Yachats, Smith, Coos, and Coquille) were also included in our analyses. Genomic DNA was extracted through DNEasy (Qiagen Inc., Valencia, California) or Chelex – proteinase K (Estoup et al. 1993) protocols, and separate polymerase chain reactions were carried out in 5 μL volumes on a MJ Research thermocycler to amplify eight microsatellite loci, utilizing fluorescently labeled primers (*One13*, Scribner et al. 1996; *Ots2*, Banks et al. 1999; *p53*, de Fromentel et al. 1992; *Oki16*, Smith et al. 1998; *Ots215*, Greig et al. 2003; *Ots520*, Naish and Park 2002; *Ots3*, Banks et al. 1999; *Ocl8*, Condrey and Bentzen 1998). PCR products were separated via polyacrylamide gel electrophoresis on an ABI 3730XL genotyper and (or) MJ Research Basestation and binned according to size with either GeneMapper or Cartographer software. A minimum of 96 samples were analyzed at all loci on both genotyping platforms to allow for bin adjustments and consistent size scoring.

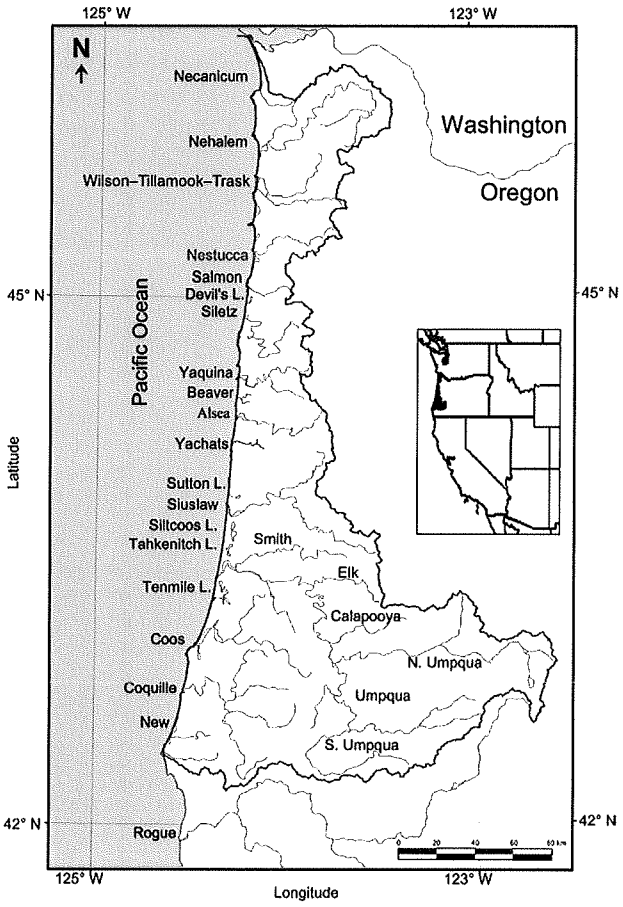


Fig. 1. Map of rivers from which tissue samples were collected. The Oregon coastal coho evolutionarily significant unit is outlined in bold. Inset shows location on the US west coast.

Statistical analyses

We used the program FSTAT (Goudet 1995) to calculate the per-locus and overall heterozygosity for each population, as well as performed permutation tests (1000 iterations) to

detect departures from Hardy–Weinberg equilibrium (HWE). The program GENETIX (Belkhir et al. 2004) was used to carry out permutation tests (1000 iterations) to detect linkage disequilibrium among loci. Sequential Bonferroni corrections (Rice 1989) were made to adjust the initial critical value of 0.05 to account for multiple comparisons made during these tests.

Pairwise values for Weir and Cockerham's inbreeding coefficient, θ, were calculated for all wild 2002 samples with the program GENETIX (Belkhir et al. 2004), and we used a permutation test with 1000 iterations to assess the statistical significance of these estimates. Similarly, we calculated all pairwise θ values for all major tributaries of the Umpqua River to assess structure within this basin. We used the program GDA (Lewis and Zaykin 2001) to perform an analysis of molecular variance (AMOVA) on data from four temporally replicated populations (Nehalem, Yachats, Smith, and Coos), simultaneously estimating the percentage of total genetic covariance explained by allele frequency differences among river basins, years, and within-basin sampling sites for each year.

We used the maximum likelihood phylogeny inference program, CONTML, in the PHYLIP analysis package (Felsenstein 2005) to construct trees depicting the structure of coho salmon genetic diversity among all 24 basins sampled in 2002. Five hatchery populations and temporal replicates (2003, 2004) of wild populations from five river basins were also included in this analysis. We used the program SEQBOOT, also in the PHYLIP package, to bootstrap the data and estimate statistical support for the topology of the best maximum likelihood tree. We displayed trees with the program TREEVIEW (Page 1996).

For the five populations that were sampled in multiple years, the number of breeders, N_B, was estimated through the methods of Waples (1990):

$$\widehat{N}_B = \frac{b}{2(\widehat{F} - 1/S)}$$

where $\widehat{F}$ is an estimator of the standardized temporal variance in allele frequencies, $1/S$ is a sampling error correction factor, and b is the number of generations between samples, adjusted to account for the relative contribution of jacks to the population. Values of b were calculated according to the methods of Tajima (1992). N_B was then used to estimate m, the per-population immigrant fraction, according to the temporal F_{ST} method (Ford et al. 2004).

Using only data from 2002, we applied a Bayesian method to estimate directional migration rates among wild coho salmon populations. This method, described by Wilson and Rannala (2003) and implemented in the program BAYESASS, relaxes several assumptions carried in the temporal F_{ST} method (e.g., constant and symmetrical geneflow between population pairs, HWE) and is designed to provide an estimate of recent migration rates. By inferring the ancestry of each sample through Markov chain Monte Carlo methods, BAYESASS provides directional estimates of migration, which may be used to infer source–sink dynamics. However, computational limits of the program necessitated some data pooling. A minimum number of pooling steps were made, utilizing evidence for natural clades identified through the maximum likelihood inference of coho population structure. Briefly, (*i*) the lower Umpqua and neighboring coastal lakes were pooled into a single clade; (*ii*) all other Umpqua tributaries were pooled into a single clade; and (*iii*) the Wilson and Tillamook rivers were pooled together as a single clade. Also, the number of alleles present at the *Oki16* locus exceeded the computational limits of the program. Thus, data from this marker were not included in the analysis. Lastly, the current version of BAYESASS limits the maximum number of populations considered to 19. We therefore performed the analysis twice, first excluding the northernmost population (Necanicum) and then excluding the southernmost population (Rogue). We used the default prior values (allele frequency, migration rate, and inbreeding coefficient all set at 0.15) and performed three million steps (999 999 burn-in) with a sampling frequency of 2000.

Allelic richness, a measure of genetic diversity that accounts for variable sample sizes through rarefaction, was calculated for all loci in all populations with the program FSTAT (Goudet 1995). An analysis of variance, carried out with the program S-PLUS 7.0 (Insightful Corp., Seattle, Washington), was used to test for associations between each population's mean allelic richness (across loci) and the following explanatory variables: "latitude" of entry from the ocean into the spawning river (river mouth), sampling "year", "ecotype" (river or lake), and "origin" (hatchery or wild). All variables were treated as fixed effects, and all variables were categorical, except for latitude, which was continuous. Owing to the unbalanced design of our study (e.g., absence of lake hatcheries and temporal replication for only wild fish from rivers), we used type III sums of squares to assess the significance of variables included in our models, but could not estimate the significance of third- and fourth-order interaction terms. We included all first-order interactions, with the exception of ecotype × year (not estimable), in an initial full model, then manually removed insignificant terms to obtain our final reduced model. We then used linear regression analysis to test for a cline in allelic richness among wild coho populations, using the continuous explanatory variable latitude.

Results

The eight microsatellites we used to characterize Oregon coastal coho salmon populations presented high yet variable levels of polymorphism, with between 10 and 71 alleles (mean = 30.12, standard deviation (SD) = 18.52). Per-locus heterozygosities ranged from 85.2% to 60.2%, with a mean of 72.8% overall (Table 1). Even after sequential Bonferroni corrections, approximately 64% of the wild populations did not conform to HWE expectations across all loci, whereas only one of five hatchery populations differed significantly from expected HWE genotypic frequencies. After making sequential Bonferroni adjustments to an initial critical value of 0.05, we detected significant evidence for linkage disequilibrium for only 0.7% of locus pairs, considering all possible locus pair combinations across all populations.

Population structure

Pairwise θ values at the basin level ranged from 0.002 to

Table 1. Data collected for all populations: mean sample sizes across loci, deviation from Hardy–Weinberg proportions (F_{IS}), observed and expected heterozygosities (H_O and H_E, respectively), latitude of river (at mouth), origin, and ecotype.

Basin	Mean n	F_{IS}	H_O	H_E	Latitude (°N)	Origin	Ecotype
Alsea	83.5	0.094	0.731	0.801	44.25	Wild	River
Beaver Creek	31.6	0.069*	0.733	0.774	44.31	Wild	River
Coos	178.5	0.099	0.729	0.807	43.21	Wild	River
Coquille	41.8	0.197	0.648	0.794	43.07	Wild	River
Devil's	37.3	0.110	0.669	0.741	44.57	Wild	Lake
Necanicum	30.0	0.115	0.696	0.771	46.01	Wild	River
Nehalem	162.1	0.089	0.708	0.775	45.40	Wild	River
Nestucca	55.4	0.089	0.714	0.776	45.09	Wild	River
New	42.8	0.055*	0.728	0.761	42.56	Wild	River
Rogue	44.4	0.092	0.672	0.730	42.25	Wild	River
Salmon	42.8	0.072	0.730	0.777	45.02	Wild	River
Siletz	46.4	0.120	0.685	0.769	44.54	Wild	River
Siltcoos	26.9	0.103	0.692	0.754	43.52	Wild	Lake
Siuslaw	130.6	0.161	0.683	0.811	44.00	Wild	River
Smith	90.5	0.109	0.722	0.805	43.40	Wild	River
Sutton	34.0	0.097	0.688	0.749	44.04	Wild	Lake
Tahkenitch	31.1	0.069*	0.725	0.765	43.48	Wild	Lake
Tenmile	57.6	0.073	0.739	0.789	43.33	Wild	Lake
Tillamook	13.3	0.082*	0.698	0.728	45.33	Wild	River
Trask	18.9	0.021*	0.750	0.744	45.33	Wild	River
Umpqua	275.25	0.074	0.757	0.816	43.40	Wild	River
Wilson	31.1	0.072*	0.739	0.782	45.33	Wild	River
Yachats	20.3	0.050*	0.770	0.789	44.18	Wild	River
Yaquina	111.6	0.166	0.668	0.796	44.37	Wild	River
Temporal replicates							
Coos 2003	54.6	0.093	0.731	0.798	43.21	Wild	River
Coos 2004	80.3	0.047*	0.775	0.808	43.21	Wild	River
Coquille 2004	44.9	0.055	0.723	0.757	43.07	Wild	River
Nehalem 2003	121.9	0.021*	0.790	0.803	45.40	Wild	River
Nehalem 2004	41.4	0.184	0.648	0.780	45.40	Wild	River
Smith 2003	58.0	0.079	0.734	0.789	43.40	Wild	River
Smith 2004	67.5	0.077	0.740	0.794	43.40	Wild	River
Yachats 2003	35.0	0.060*	0.768	0.803	44.18	Wild	River
Yachats 2004	33.9	0.073*	0.732	0.777	44.18	Wild	River
Hatcheries							
Coos 2004	91.0	0.005*	0.797	0.789	43.21	Hatchery	River
Coquille 2004	47.1	0.007*	0.794	0.780	43.07	Hatchery	River
Cow Creek (South Umpqua) 2004	42.8	0.027*	0.806	0.818	43.40	Hatchery	River
Nehalem 2004	28.3	0.100	0.703	0.764	45.40	Hatchery	River
Salmon 2004	50.0	0.002*	0.750	0.741	45.02	Hatchery	River
Total	2434.0						

Note: Samples were collected in 2002, except where indicated.
*No significant difference from Hardy–Weinberg equilibrium (HWE) after sequential Bonferroni correction.

0.068, with an overall θ value of 0.021 (95% confidence interval (CI) = 0.016, 0.027; $p < 0.0001$). Highest values tended to occur between the northerly Trask population and those of rivers and coastal lakes from the southern extreme of the ESU (see Appendix A, Table A1). Within the Umpqua River, pairwise θ values ranged between 0.004 to 0.030, with an overall value of 0.015 (95% CI = 0.010, 0.022; $p < 0.0001$). Highest pairwise θ values within this basin occurred between the Smith River and south fork Umpqua populations, followed by values associated with the lower Umpqua and all other Umpqua tributaries. For the Nehalem, Yachats, Smith, and Coos rivers, simultaneous hierarchal analyses indicated that 97.33% of the observed genetic variance could be attributed to differences among individuals. Most of the remaining 2.67% of genetic variance could be explained by allele frequency differences among basins (52.8%), followed by differences among sites within basins (29.5%), and lastly, differences observed among temporal replicates (17.7%).

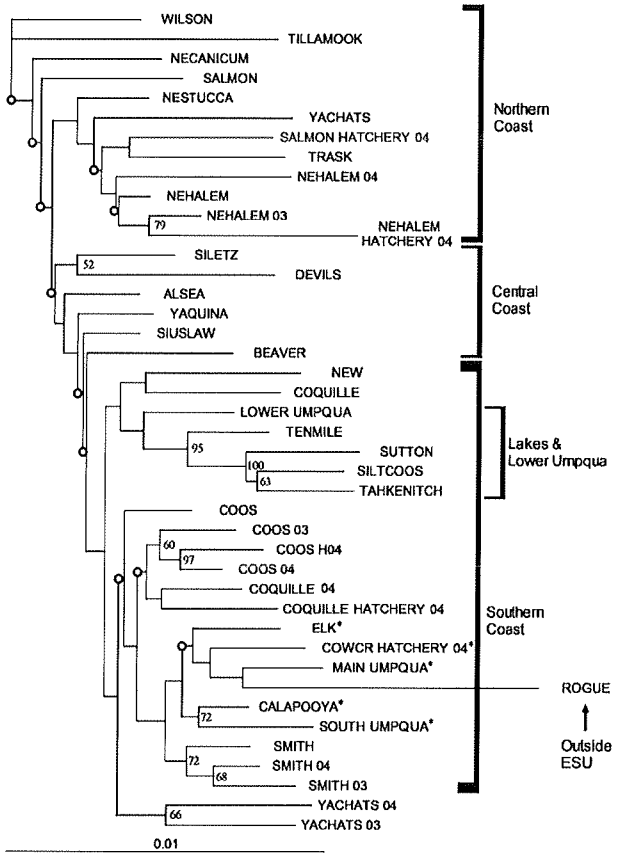
In agreement with our analyses of genetic variance, the

maximum likelihood tree inferred from our data suggests that only weak structure exists among coho salmon samples from different river basins of the OCC ESU, as many internal branch lengths are short or do not significantly differ from zero (Fig. 2). However, statistically supported clades generally reflect geographic relationships among basins. For example, the coastal lakes flanking the mouth of the Umpqua River form a clade together with the lower reaches of this river. Nearby Sutton Lake also falls into this group, whereas the more distant Devil's Lake does not, but instead appears to be most similar to its proximal Siletz River. The Coos, Umpqua, Smith, and Coquille rivers appear to form a southern group, whereas the Nehalem, Tillamook, Wilson, Necanicum, Nestucca, Trask, and Salmon rivers form a loosely defined northern group.

Samples collected from the same river in different years tended to form clades, although interannual Coquille samples alternately grouped with neighboring rivers to the north and south (Fig. 2). The Yachats River population presents another exception, as it does not fit neatly on the tree in a geographic context, but instead aligns with distant southern and northern populations between years.

In all but one case, hatchery coho salmon populations appear most similar to wild fish returning to the river where they are located (Fig. 2). The exception to this pattern is the Salmon River hatchery stock, which appears to be most similar to the Trask River wild population. Unlike other hatchery stocks considered here, the Salmon River hatchery population was not established from native fish from the river on which it operates. Instead it was founded by Siletz River hatchery stock (Salmon River Hatchery operations plan, Oregon Department of Fish and Wildlife, ODFW), which was heavily supplemented with Trask River coho during the years 1968–1977 (Beidler 1987). In concert, the pairwise θ value between the Salmon and Trask wild populations is the lowest observed between any two populations (Appendix A, Table A1), evidence that suggests that hatchery-mediated genetic introgression of Trask River stock may have occurred into the wild Salmon River coho population.

Fig. 2. Maximum likelihood tree depicting structure among Oregon coastal coho salmon (*Oncorhynchus kisutch*) populations. Open circles indicate branches of length not significantly different from zero ($\alpha = 0.05$). Values for nodes that received bootstrap support greater than 50% are presented. All samples were collected in 2002, unless otherwise indicated (03, from 2003; 04, from 2004). An asterisk (*) indicates a tributary or hatchery of the Umpqua River drainage.



Migration

Estimates of the effective number of breeders, based on the methods of Waples (1990), ranged from 66 to 384 individuals for the five populations examined (Table 2). Immigrant fraction estimates for these populations ranged from 0.02 to 0.14. The immigrant fraction point estimate in the Smith River, as calculated through the Bayesian assignment procedure implemented in BAYESASS (Wilson and Rannala 2003), exceeded those calculated through the temporal F_{ST} method of Ford et al. (2004) (Table 2), largely as a result of migration from the Umpqua and Lakes – Lower Umpqua migrant sources. In general, the Bayesian migration estimates tended to be higher, although estimates for the Nehalem River did not follow this pattern (Table 2). The Nehalem and Coos populations appeared to be the primary migrant sources for most basins, followed by the Umpqua and Lakes – Lower Umpqua (Appendix A, Table A2). Notably, our estimates for the Rogue River population indicate that strays from this basin account for less than 0.01% of the individuals present in any basin of the OCC ESU and that less than 0.01% of the Rogue population originated from any basin of the OCC ESU. This degree of isolation is not observed for any population within the OCC ESU. The BAYESASS program provided very consistent results between runs, with overlapping 95% CIs for all repeated estimates. Directional migration rate estimates among 20 population groups are presented in Appendix A (Table A2).

Allelic richness

Considerable variation in mean allelic richness was observed among Oregon coastal coho salmon populations. An analysis of variance indicated that first-order interaction terms for the explanatory variables year, origin, latitude, and ecotype were not significantly associated with variability in allelic richness ($p > 0.05$). Accordingly, the interaction terms were removed from the model, and a subsequent analysis of variance (ANOVA) identified both origin and ecotype to be significantly associated with variation in mean population allelic richness ($F_{[1,32]} = 4.430$, $p = 0.043$ and $F_{[1,32]} = 8.438$, $p = 0.007$, respectively; Table 3). Neither

Table 2. Effective number of breeders (N_B) and corresponding immigrant fractions (m) for five Oregon coastal coho salmon (*Oncorhynchus kisutch*) populations as calculated by the temporal F_{ST} method (Ford et al. 2004), under 10% and 25% jack contributions.

	10% jacks		25% jacks		
Basin	N_B	m	N_B	m	Bayesian m*
Nehalem	170 (117, 245)	0.05 (0.02, 0.10)	76 (53, 110)	0.12 (0.06, 0.22)	0.06 (0.02, 0.10)
Yachats	150 (81, 348)	0.06 (0.02, 0.14)	66 (35, 153)	0.14 (0.05, 0.32)	0.32 (0.29, 0.33)
Smith	378 (210, 824)	0.02 (0.01, 0.06)	171 (95, 373)	0.05 (0.02, 0.12)	0.15 (0.06, 0.28)
Coos	384 (238, 674)	0.02 (0.01, 0.05)	172 (107, 301)	0.05 (0.02, 0.11)	0.19 (0.15, 0.24)
Coquille	168 (98, 324)	0.06 (0.02, 0.12)	71 (41, 137)	0.13 (0.05, 0.28)	0.31 (0.28, 0.33)

Note: For all estimates, 95% confidence intervals are reported in parentheses.
*Immigrant fraction as calculated through the Bayesian method of Wilson and Rannala (2003).

Table 3. Analysis of variance (ANOVA) table testing for association between the explanatory variables origin, ecotype, year, and latitude with mean allelic richness of Oregon coastal coho salmon (*Oncorhynchus kisutch*) populations.

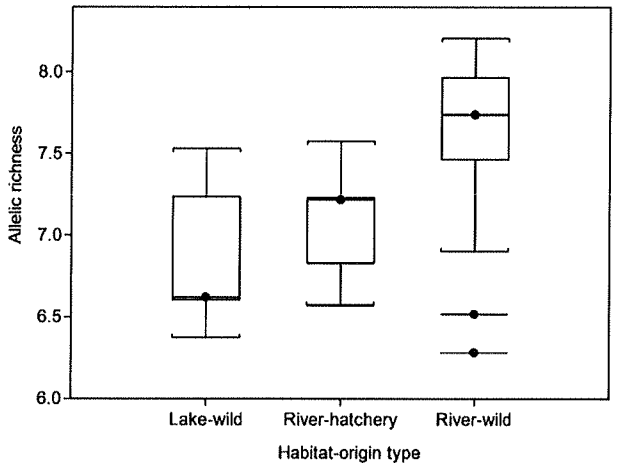
	df	Sum of squares	Mean squares	F	Pr(F)
Origin	1	0.9782	0.9782	4.4301	0.043
Ecotype	1	1.8633	1.8633	8.4384	0.007
Year	2	0.2716	0.1358	0.6150	0.547
Latitude	1	0.0045	0.0045	0.0203	0.888
Error	32	7.0661	0.2208		

Note: See text for variable descriptions.

year nor latitude appeared to be significant ($F_{[2,32]} = 0.615$, $p = 0.547$ and $F_{[1,32]} = 0.020$, $p = 0.888$, respectively Table 3). Given these results, we then collapsed the data into three categories: WildRiver, WildLake, and HatcheryRiver, recognizing that each of these population "types" could be making substantially different contributions to the overall mean of allelic richness. A Scheffé's multiple comparisons analysis indicated that mean allelic richness of wild lake rearing populations is significantly lower than wild river rearing populations (Fig. 3). One potential outlier, the Trask River population, presented markedly low mean allelic richness for a wild river population (Fig. 4). When this sample is removed, the Scheffé's multiple comparisons analysis suggests that wild river populations present significantly higher mean allelic richness than both wild lake and hatchery river origin coho.

Given the small sample sizes of both lake and hatchery populations and the disparate patterns of allelic richness observed between these groups and wild river coho salmon, linear regression analysis of mean allelic richness was conducted only for the latter. A latitudinal cline in neutral genetic diversity, peaking in the central region of the OCC ESU, can be observed by plotting mean allelic richness of wild river coho populations against the latitude at which they enter freshwater spawning grounds (Fig. 4). Initially, a model with a single linear term, latitude, was evaluated, but this variable alone was not significantly associated with variation in allelic richness ($F_{[1,26]} = 0.3509$, $p = 0.5587$). Given the curvature observed in the data (Fig. 4), a linear regression model containing a quadratic latitudinal term was tested and found to carry high significance, explaining 33.1% of

Fig. 3. Box plots depicting the range of mean allelic richness observed among coho (*Oncorhynchus kisutch*) populations from three habitat origin types: lake-wild, river-hatchery, river-wild. Boxes enclose the 25th through 75th quartile range, and whiskers are drawn to the nearest value not beyond the standard span from quartiles (i.e., 1.5 × interquartile range).



the variation observed in population mean allelic richness ($F_{[2,25]} = 6.184$, $p = 0.007$):

$$\mu\{\text{Richness}|\text{Latitude}\} = -501.93 + 23.02 \times \text{Latitude} - 0.26 \times \text{Latitude}^2$$

Moreover, when the potentially outlying Trask River sample was removed from this analysis, the model fit improved considerably, explaining 43% of the variation ($F_{[2,24]} = 9.048$, $p = 0.001$).

Discussion

Population structure and migration

The relatively weak genetic structure observed among coho salmon from Oregon coastal rivers suggests that migration among these populations has acted to reduce the effects of genetic drift and divergence. Tagging studies have suggested that during spawning migrations, coho may stray into non-natal streams more frequently than some other Pacific salmonids (Shapovalov and Taft 1954; Quinn 2005). Moreover, past human activities have served to mediate migration among Oregon coho populations through the practice

Fig. 4. Relationship between mean allelic richness of wild, river-rearing Oregon coastal coho salmon (*Oncorhynchus kisutch*) populations and the latitude at which they enter freshwater spawning grounds. The upper curve includes the Trask River sample point; the lower curve does not.

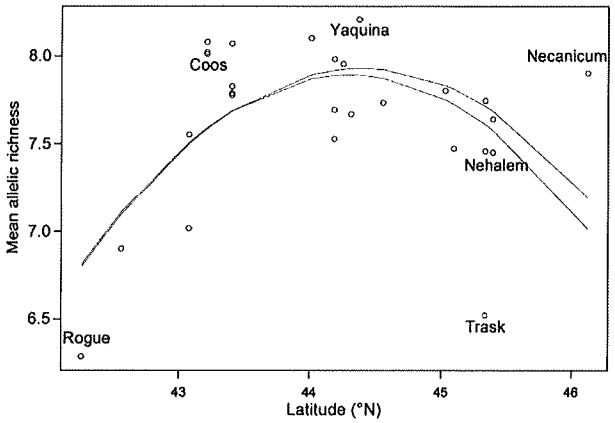
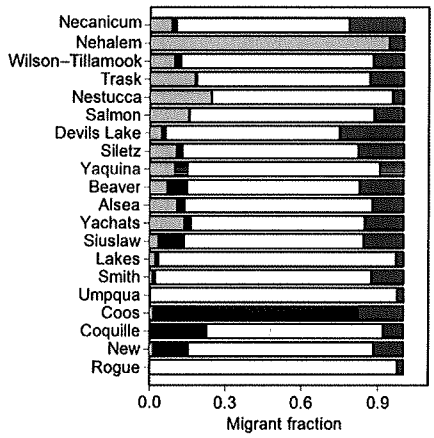


Fig. 5. Oregon coho (*Oncorhynchus kisutch*) populations ordered from north (top) to south (bottom) as composed by individuals homing to their natal river (open) and migrants from the Nehalem (light gray), Coos (black), and all other sources combined (dark gray). Migrant contributions estimated with the program BAYESASS (Wilson and Rannala 2003).



of hatchery stock transfers. Disentangling the relative influence of these two sources of migration is complicated by the shared ancestry of many hatchery and wild coho populations, as well as the unpredictable nature of genetic introgression from introduced hatchery stocks.

The immigrant fraction values we have estimated through the temporal F_{ST} method approximate those reported by Ford et al. (2004), although a direct comparison can only be made for the Smith River, which presents nearly identical values. Immigrant fractions were highest in the Yachats and Coquille populations, that in 2002 presented the lowest spawner abundance counts for the five populations examined (data not shown). Curiously, the temporal F_{ST} method provides a rather high m value for the large Nehalem population. It should be kept in mind, however, that several assumptions of the temporal F_{ST} method, namely HWE and symmetrical geneflow among populations, might not be met. By applying the Bayesian method of Wilson and Rannala (2003), we provide directional migration rate estimates that are not contingent on these assumptions.

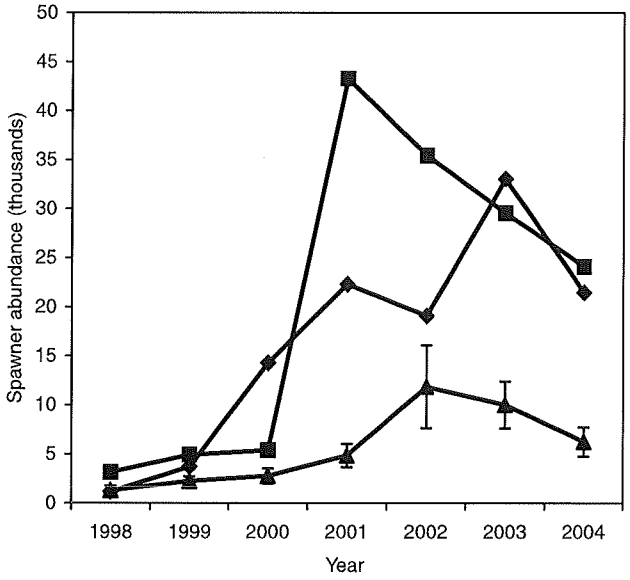
Bayesian migration rate estimates tended to be higher than those of the temporal F_{ST} method and suggest that two of the larger populations function as migrant sources for numerous smaller populations (Fig. 5 and Appendix A, Table A2). Moreover, source–sink dynamics appear to be influenced by distance, as smaller basins appear to receive more migrants from the Coos in the south, whereas the Nehalem functions as the principal migrant source in the north (Fig. 5). Consequently, populations of the central coast, such as those from the Yaquina River, Alsea River, and Beaver Creek, receive relatively balanced immigrant contributions from the major source populations.

Two noteworthy sources of error may be compromising the accuracy of our Bayesian migration rate estimates. First, simulations performed by Wilson and Rannala (2003) indicate that their method lacks power when either few loci (less than 20) are employed or when population structure is weak. Given the nature of our data set, caution must therefore be taken when interpreting these results. The influence of unmarked hatchery coho salmon may constitute a second source of error in our migration rate estimates. Most notably, in 1999, the Nehalem hatchery released 53 080 unmarked hatchery coho smolts. Given estimated smolt-to-adult survival rates of 4% to 8% (Logerwell et al. 2003), this hatchery cohort likely produced between 2123 and 4246 unmarked coho adults returning in 2002, a portion of which may have strayed into neighboring basins, thereby elevating the estimate of wild migrants from the Nehalem River. This event may also have generated major allele frequency changes within the Nehalem River population, affecting the Nehalem temporal F_{ST} estimate of m. In contrast, such confounding circumstances are not associated with the Coos source population, as only 139 unmarked Coos River hatchery smolts were released in 1999. Despite these factors, we believe that our results provide a useful index of migration rates and represent the first attempt at quantitatively describing source–sink dynamics among Oregon coho populations.

Dramatic census size increases, recorded for most coastal Oregon coho salmon populations during the sampling period of 1998–2002 (Fig. 6), have probable relevance to our findings. It is likely that the relatively high contemporary migration rates that we are reporting are the result of a spilling over effect, as the larger Coos and Nehalem populations responded more quickly to favorable conditions and provided large numbers of migrants to neighboring populations in 2002. Again, hatchery influence over coho population dynamics on the Nehalem River cannot be ignored and may have contributed to this population's rapid census size increase. Nevertheless, wild coho smolt production on the north fork of this river was estimated to be twice that of any other coastal Oregon site monitored by ODFW in 1999.

Regardless of the source, admixture resulting from migration would generate a transient signal of nonrandom mating. Such a scenario would serve to explain the major departures from HWE that we have detected in the majority of wild populations. Although recent population expansions may be

Fig. 6. Spawner abundance of coho salmon (*Oncorhynchus kisutch*) in the Nehalem (◆) and Coos (■) rivers, plotted together with the mean spawner abundance of 13 other Oregon coastal coho evolutionarily significant unit (OCC ESU) populations (▲, Necanicum, Tillamook, Nestucca, Salmon, Siletz, Yaquina, Alsea, Yachats, Siuslaw, Coquille, Tenmile Lake, Tahkenitch Lake, Siltcoos Lake) for the years 1998 through 2004. Error bars indicate standard error of mean estimate. Adapted from Oregon Department of Fisheries and Wildlife data (oregonstate.edu/Dept/ODFW/spawn/cohoabund.htm).



increasing this effect, it seems that high levels of geneflow among populations may be characteristic of Oregon coho salmon, as our $F_{ST}(\theta)$ estimates corroborate previously reported values for Oregon coho (Ford et al. 2004), yet are much lower than values reported for coho at more extreme locations of the species' North American distribution (Olsen et al. 2003; Bucklin et al. 2007).

The temporal and spatial scales over which natural populations form discrete units are seldom predictable and often dynamic. Whereas significant departures from HWE were observed when all individuals from a given basin were considered as a single population, most hatchery populations conformed to HWE expectations. Again, this observation suggests that considerable admixture or genetic substructure, not present in hatchery populations, exists within natural coho populations.

To evaluate the possibility of within-basin substructure, we conducted a hierarchical analysis of genetic covariance and tested for conformance with HWE expectations at the level of within-basin sampling sites for the Nehalem, Yachats, Smith, and Coos populations. Hierarchical analyses of genetic covariance provided only limited evidence for within-basin substructure, when contrasted with covariance levels associated with among-basin differences. However, among-site covariance did exceed among-year covariance; thus, it can be inferred that precocial males (jacks) mediate a higher level of geneflow among brood-year populations than occurs among spawning sites within the same basin. Concomitantly, nearly a third of all putative subpopulations did not conform to genotypic frequency expectations under HWE, even after sequential Bonferroni corrections. Thus, within-basin substructure appears to be both weak and more complex than a temporally stable system of spatially defined demographic units. Unfortunately, the use of carcass samples precluded our ability to detect for multiple, temporally structured populations within spawning years at sampling sites.

In all but one case, hatchery coho salmon populations appeared to be most similar to wild fish of the same river. This result is perhaps not surprising, as the hatchery populations examined were founded by local wild stock, with the exception of the Salmon River hatchery population. Moreover, the Coquille, Coos, and Cow Creek hatchery stocks receive regular supplementation from native wild fish. Although small sample sizes preclude statistical analyses, each of these three hatcheries presented higher allelic richness than both the Nehalem and Salmon hatchery populations, which do not incorporate wild stock into their breeding programs. Overall, the general pattern suggests that hatchery populations possess significantly lower levels of allelic richness than wild river populations.

Allelic richness

Founder effects, bottlenecks, and inbreeding may all contribute to the relatively low allelic richness observed in Oregon hatchery coho salmon populations. However, these processes may also act over natural populations. Coho salmon from Oregon rivers have been shown to be more diverse than more northerly populations with both allozymes (Wehrhahn and Powell 1987) and mtDNA sequence data (Smith et al. 2001). These findings have been interpreted as a signal of bottlenecks and founder effects from a limited number of post-Pleistocene colonizations by coho from Oregon and California refugia. Interestingly, the coastal lakes flanking the mouth of the Umpqua River are also believed to be of late Pleistocene origin (Cooper 1958; Johnson et al. 1985), far younger than most rivers along the Oregon coast. These basins therefore became available for colonization by coho during the same period that more northerly, glaciated rivers of Washington, British Columbia, and Alaska became inhabitable. Founder effects may then also serve to explain the relatively low levels of allelic richness observed in coho populations from Oregon coastal lakes, as compared with neighboring populations.

The highest levels of allelic richness were found in river populations from the central region of the OCC ESU. Interestingly, it appears that coho salmon populations of this region receive the most balanced contributions from the two primary, geographically distant migrant sources of the ESU: the Nehalem and Coos river populations (Fig. 5).

Only one population from another ESU was included in our analyses, that of the Rogue River in southern Oregon, and it presented the lowest level of allelic richness observed among our samples. While sampling across additional ESUs could certainly broaden the scope of our findings, the intensive within-ESU sampling strategy we have employed has provided sufficient resolution to detect both continuous and categorical patterns of allelic richness that might otherwise have been overlooked or misinterpreted.

In conclusion, our findings suggest that both natural and

anthropogenic forces have served to shape the contemporary patterns of coho salmon genetic diversity along the Oregon coast. Relatively high migration rates from a limited number of large, geographically distant source populations appear to modulate spatial genetic structure while maintaining elevated levels of allelic richness within the core of the OCC ESU. In concert with local adaptations, the source–sink dynamics we have described may very well represent a long-standing ecological mechanism responsible for generating and maintaining genetic diversity in Oregon coastal coho salmon populations.

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Appendix A

Tables A1 and A2 appear on the following pages.

Table A1. Pairwise θ values for Oregon coastal coho salmon (*Oncorhynchus kisutch*) populations as defined by basins (data above diag-

	Umpqua	Yaquina	Yachats	Wilson	Trask	Tillamook	Tenmile	Tahkenitch	Sutton	Smith	Siuslaw	Siltcoos
Alsea	0.010	0.014	0.015	0.020	0.021	0.025	0.031	0.031	0.032	0.015	0.006	0.032
Beaver	0.015	0.011	0.016	0.022	0.014	0.025	0.043	0.028	0.039	0.026	0.007	0.032
Coos	0.013	0.013	0.025	0.037	0.044	0.032	0.018	0.020	0.027	0.010	0.011	0.021
Coquille	0.026	0.025	0.040	0.047	0.066	0.038	0.023	0.031	0.035	0.022	0.021	0.028
Devil's	0.035	0.022	0.045	0.021	0.029	0.023	0.064	0.047	0.046	0.045	0.023	0.035
Necanicum	0.013	0.008	0.008	0.016	0.019	0.009*	0.030	0.031	0.041	0.019	0.011	0.032
Nehalem	0.020	0.015	0.014	0.011	0.006*	0.024	0.051	0.039	0.042	0.028	0.012	0.037
Nestucca	0.016	0.014	0.006*	0.016	0.004*	0.030	0.036	0.034	0.034	0.024	0.010	0.032
New	0.026	0.024	0.041	0.053	0.061	0.062	0.028	0.028	0.027	0.028	0.024	0.024
Salmon	0.022	0.015	0.024	0.010	0.002*	0.017	0.054	0.044	0.049	0.035	0.015	0.040
Siletz	0.015	0.016	0.019	0.018	0.013	0.022	0.047	0.040	0.040	0.028	0.013	0.028
Siltcoos	0.028	0.021	0.044	0.032	0.053	0.055	0.017	0.007*	0.005*	0.030	0.018	—
Siuslaw	0.007	0.006	0.011	0.015	0.020	0.020	0.026	0.015	0.021	0.011	—	
Smith	0.007	0.022	0.019	0.039	0.043	0.032	0.021	0.028	0.033	—		
Sutton	0.028	0.029	0.047	0.043	0.064	0.059	0.019	0.009	—			
Tahkenitch	0.025	0.024	0.037	0.041	0.056	0.056	0.019	—				
Tenmile	0.026	0.034	0.029	0.058	0.068	0.062	—					
Tillamook	0.024	0.019	0.041	0.020	0.031	—						
Trask	0.025	0.020	0.019	0.015	—							
Wilson	0.027	0.016	0.027	—								
Yachats	0.015	0.020	—									
Yaquina	0.016	—										
Umpqua	—											

*Not significantly different from zero at $p < 0.05$ with 1000 permutations.

Table A2. Directional migration rates among coho (*Oncorhynchus kisutch*) populations of the Oregon coast, as estimated by the program

Source	Necanicum	Nehalem	Wilson–Tillamook	Trask	Nestucca	Salmon	Devil's	Siletz	Yaquina	Beaver
Necanicum	0.678	0.000	0.002	0.003	0.001	0.002	0.002	0.002	0.001	0.003
Nehalem	0.086	0.945	0.098	0.179	0.243	0.154	0.047	0.106	0.100	0.069
Wilson–Tillamook	0.010	0.025	0.754	0.017	0.006	0.011	0.159	0.007	0.009	0.009
Trask	0.003	0.001	0.002	0.679	0.001	0.002	0.003	0.002	0.001	0.003
Nestucca	0.005	0.001	0.003	0.007	0.709	0.003	0.004	0.006	0.004	0.013
Salmon	0.003	0.007	0.007	0.005	0.004	0.723	0.005	0.010	0.003	0.003
Devil's	0.003	0.001	0.003	0.004	0.002	0.002	0.683	0.003	0.002	0.003
Siletz	0.004	0.001	0.005	0.006	0.002	0.002	0.004	0.691	0.002	0.005
Yaquina	0.005	0.003	0.011	0.022	0.006	0.005	0.009	0.014	0.753	0.037
Beaver	0.004	0.000	0.003	0.004	0.001	0.002	0.003	0.002	0.001	0.676
Alsea	0.020	0.005	0.010	0.016	0.003	0.039	0.010	0.025	0.028	0.008
Yachats	0.004	0.001	0.002	0.004	0.001	0.002	0.003	0.002	0.001	0.003
Siuslaw	0.006	0.001	0.012	0.004	0.002	0.002	0.006	0.009	0.010	0.012
Lakes – lower Umpqua	0.019	0.002	0.007	0.006	0.004	0.005	0.016	0.016	0.015	0.030
Smith	0.018	0.001	0.008	0.005	0.002	0.008	0.005	0.006	0.003	0.004
Umpqua	0.104	0.002	0.039	0.021	0.005	0.024	0.015	0.069	0.004	0.033
Coos	0.021	0.003	0.027	0.008	0.003	0.006	0.017	0.025	0.053	0.081
Coquille	0.003	0.001	0.003	0.005	0.002	0.005	0.003	0.003	0.001	0.004
New	0.004	0.001	0.003	0.005	0.002	0.002	0.006	0.003	0.007	0.005
Rogue	—	0.001	0.003	0.004	0.002	0.003	0.003	0.004	0.002	0.004

Note: First column (Source) lists source of migrants (e.g., migration rate from Alsea to Beaver = 0.008).

onal) and tributaries of the Umpqua River (lower right inset).

Siletz	Salmon	New	Nestucca	Nehalem	Necanicum	Devil's	Coquille	Coos	Beaver	Alsea
0.013	0.014	0.034	0.008	0.016	0.012	0.030	0.030	0.018	0.013	—
0.009	0.014	0.033	0.008	0.014	0.014	0.026	0.041	0.025	—	
0.029	0.034	0.022	0.027	0.027	0.019	0.042	0.011	—		
0.043	0.048	0.022	0.041	0.049	0.033	0.058	—			
0.019	0.021	0.050	0.027	0.021	0.025	—				
0.010	0.008	0.038	0.010	0.013	—					
0.015	0.013	0.046	0.008	—						
0.009	0.008	0.038	—							
0.039	0.052	—								
0.009	—									
—										

Inset: Smith	South Umpqua	Calapooya	Elk	Main Umpqua	Cow Creek hatchery	
					0.004	Main Umpqua
				0.007	0.004	Elk
			0.008	0.012	0.020	Calapooya
		0.004	0.009	0.013	0.017	South Umpqua
	0.020	0.010	0.010	0.015	0.016	Smith
0.012	0.030	0.024	0.014	0.025	0.027	Lower Umpqua

BAYESASS (Wilson and Rannala 2003).

Alsea	Yachats	Siuslaw	Lakes – lower Umpqua	Smith	Umpqua	Coos	Coquille	New	Rogue
0.002	0.004	0.001	0.000	0.001	0.000	0.001	0.002	0.002	—
0.108	0.138	0.036	0.024	0.013	0.002	0.016	0.003	0.017	0.002
0.007	0.009	0.015	0.002	0.003	0.001	0.008	0.004	0.006	0.002
0.002	0.004	0.002	0.001	0.001	0.000	0.001	0.002	0.002	0.001
0.005	0.020	0.001	0.001	0.002	0.001	0.002	0.004	0.005	0.001
0.003	0.004	0.004	0.001	0.001	0.001	0.001	0.004	0.002	0.001
0.002	0.004	0.001	0.001	0.001	0.000	0.001	0.002	0.002	0.001
0.004	0.005	0.002	0.001	0.002	0.001	0.001	0.002	0.004	0.001
0.006	0.009	0.032	0.002	0.010	0.001	0.009	0.004	0.005	0.002
0.002	0.004	0.001	0.001	0.001	0.000	0.001	0.002	0.002	0.001
0.735	0.005	0.007	0.002	0.002	0.003	0.026	0.006	0.004	0.002
0.002	0.680	0.001	0.001	0.001	0.000	0.001	0.002	0.002	0.001
0.002	0.008	0.705	0.001	0.004	0.002	0.002	0.007	0.004	0.001
0.033	0.033	0.048	0.933	0.037	0.002	0.103	0.018	0.047	0.002
0.009	0.007	0.002	0.008	0.847	0.011	0.014	0.008	0.004	0.002
0.040	0.021	0.034	0.007	0.059	0.970	0.002	0.010	0.022	0.003
0.034	0.029	0.102	0.014	0.013	0.003	0.809	0.226	0.140	0.002
0.002	0.010	0.002	0.001	0.001	0.001	0.002	0.691	0.005	0.001
0.002	0.008	0.002	0.001	0.001	0.001	0.001	0.003	0.725	0.002
0.004	0.005	0.002	0.000	0.002	0.000	0.001	0.005	0.004	0.971