Lower Columbia Hatchery Steelhead Introgression Study Design Framework

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Introduction

Fish and wildlife managers have been translocating and artificially propagating animals for decades, generally for the purpose of conserving declining or depressed populations or species, or for supplementing a population that is exploited for commercial or recreational harvest (Frankham et al. 2002, Laikre et al. 2010, Naish et al. 2008). The use of hatcheries to increase the abundance of salmon and steelhead populations has been extensive, and our understanding of their effects on wild populations is growing (e.g., Araki et al. 2007, Christie et al. 2012, Hess et al. , Hilborn 1992, Laikre et al. 2010, Seamons et al. 2012, Theriault et al. 2011, Waples 1991, Zhivotovsky et al. 2012). These effects can be categorized as either ecological (e.g., competition), or genetic (e.g., domestication, hybridization) (Kostow 2009, Naish et al. 2008).

To moderate or eliminate the negative effects to wild populations the Hatchery Scientific Review Group (HSRG) recommended that every salmonid hatchery develop a genetic management plan, and every hatchery population be managed as either segregated from or integrated with the wild population(s) that spawn naturally within the basin (Mobrand et al. 2005). The intent of segregated hatchery programs is to keep separate the hatchery and wild populations, and they are managed so that only hatchery-origin individuals are used as broodstock, and hatchery-origin adults are restricted from spawning naturally, with the understanding that any natural spawning by hatchery-origin fish from the segregated program will impose potentially unacceptable risks to natural populations. Therefore, by design the hatchery and wild populations in segregated programs are genetically distinct, and the degree of genetic differentiation is a function of the source of the hatchery broodstock, hatchery founder effect, genetic drift, or domestication selection (Mobrand et al. 2005). Primarily, the purpose of segregated hatchery programs is to create harvest opportunities, and secondarily, to direct harvest away from wild populations of conservation concern (e.g., mark-selective fishery). However, because segregated hatchery programs assume that hatchery and wild populations will remain distinct, an unintended consequence of a segregated hatchery program is hybridization between hatchery-origin and wild fish that spawn naturally. Hybridization may be unavoidable if fishery managers lack the ability to restrict hatchery-origin fish from natural spawning grounds, and if spawning by hatchery-origin and wild fish is not segregated spatially or temporally (Naish et al. 2008, Waples 1991). Hybridization that is anthropogenic in origin has caused the extinction or decline of animal taxa either by the replacement of natural taxa by their hybrids, loss of fitness or adaptation through genetic mixing, loss of genetic diversity or change in population structure, and reduction in effective population size (Allendorf et al. 2001, Christie et al. 2012, Laikre et al. 2010, Ryman and Laikre 1991, Waples 1991). Despite fishery managers' intent to keep hatchery and wild populations segregated, introgressive hybridization occurs and may occur at a high rate (e.g., Seamons et al. 2012).

For the better part of half a century, hatchery production of steelhead trout (*Oncorhynchus mykiss*) in western Washington State has mainly propagated and outplanted two populations: winter-run from Chambers Creek and summer-run from the Washougal River (i.e., Skamania Hatchery) (Crawford 1979). The Chambers Creek hatchery stock was derived originally in 1945 from a now-extinct run in Chambers Creek, Tacoma, Washington, and cultivated at South Tacoma Hatchery (now Lakewood Hatchery). This broodstock was cultivated and used in segregated winter-run hatchery programs throughout Puget Sound, Washington outer coast, and

lower Columbia River. Thus, these hatchery programs have been spawning and releasing out-of-basin fish into streams that are home to indigenous natural populations since the mid-1950s.

Recreational harvest of winter and summer steelhead in the Lower Columbia River (LCR) region has been supported by domesticated hatchery stocks which have been planted into watersheds in the region since the 1950's (Washington Department of Fish and Wildlife 2012). Sources of smolts released across the region are in some cases hatched, reared, and released all within a single watershed where a hatchery maintains its own brood line of domesticated steelhead stocks whereas in other cases steelhead are transferred as eggs, fry, pre-smolts, or smolts for release in non-natal basins, both with and without facilities to trap un-harvested returning adults; all of which are factors potentially affecting the homing patterns of hatchery steelhead and availability to hybridize with comingled wild populations (Schroeder et al. 2001, Slaney et al. 1993; also see Table 1).

The Lower Columbia wild steelhead Distinct Population Segment (DPS) was listed as Threatened under the Endangered Species Act (ESA) in 1998 by NOAA Fisheries and hatchery practices were identified as a factor contributing to their decline. In its Statewide Steelhead Management Plan, WDFW stated that "Steelhead management shall place the highest priority on the protection of wild steelhead stocks to maintain and restore stocks to healthy levels" (WDFW 2008). In order to meet this objective and to receive coverage from NOAA Fisheries under section 4(d) of ESA for the take of listed species resulting from operation of its steelhead hatcheries in the LCR region, WDFW has crafted Hatchery Genetic Management Plans (HGMPs) detailing how it will operate its hatcheries to minimize negative impacts on wild populations. These HGMPs specify that the operation of segregated steelhead hatcheries will result in no greater than 5% hatchery origin spawners in the wild environment (PHOS)—meeting HSRG recommendations or 2% gene flow from segregated hatchery steelhead stocks into wild populations. Currently, limited PHOS or gene flow data exist within the LCR because systematic genetic sampling of wild populations to determine gene flow has not occurred, and field surveys are unable to identify the proportion of hatchery and wild spawners because unlike salmon, steelhead carcasses are unavailable to collect information on origin type since steelhead do not die after spawning. Thus in order to determine whether existing programs meet gene flow and PHOS goals, and in order to modify them to do so, HGMPs relied on modeled estimates of PHOS and gene flow based on the outputs of the HSRG's AHA model and the gene flow model proposed by Scott and Gill (2008). Due to the paucity of available data, parameterizing these models required making assumptions about the harvest rates and reproductive success of hatchery steelhead and the degree of overlap between hatchery and wild spawners in LCR watersheds.

In order to verify that existing steelhead programs are in fact meeting conservation objectives related to hatchery operations, a more rigorous evaluation of gene flow, PHOS and ecological risks posed by steelhead hatcheries in the LCR is needed.

Objectives

The intent of this study is to determine the extent to which the segregated Chambers Creek winter and Skamania summer steelhead populations have hybridized with wild steelhead populations within the Lower Columbia River region; specifically:

1. To obtain spatially unbiased estimates of hatchery introgression in wild steelhead populations resulting from segregated hatchery steelhead spawning in the wild in the LCR region.
2. To characterize the spatial structure of introgression and determine whether it may be explained by differences in hatchery practices, wild population spatial structure, habitat features, or other factors.
3. To create a model that will predict spatial patterns and magnitude of introgression in unmonitored watersheds, allowing for adaptive management of hatcheries to meet gene flow objectives.

Study Design

Introgression vs. Gene flow

In order to quantify genetic interactions between wild and segregated hatchery steelhead populations, we chose to distinguish gene flow from introgression. We define introgression (introgressive hybridization) as hybridization that results in the establishment of exogenous alleles within a population; here, the presence of hatchery alleles within a wild population. We differentiate introgression (a state; the instantaneous proportion of hatchery-origin genetic material present in a wild steelhead population) from gene flow (a process; the rate at which hatchery origin alleles are contributed to the wild population over time). That is, gene flow is the process that gives rise to the state of introgression.

Measurements of introgression made at juvenile life stages may be higher than measurements of introgression in adults because fish with hatchery origin genetic material may experience a survival deficit relative to pure wild fish during successive life stages (e.g., Kostow et al. 2003). Therefore, measuring introgression in age-0 steelhead, before differential mortality has occurred (the maximum measurable level of introgression in a cohort), represents a more risk-averse approach to monitoring hatchery-wild genetic interactions than adult sampling. It also enables, based on the relative abundance of hatchery and wild juveniles, an assessment of the magnitude of potential ecological interactions between naturally spawned hatchery steelhead and native wild steelhead juveniles during the freshwater rearing phase (e.g., Kostow and Zhou 2006). For these reasons, we have chosen to use collections of age-0 steelhead as our primary measure genetic interactions between hatchery and wild steelhead in the LCR region.

In addition to monitoring introgression in age-0 steelhead, we will measure introgression in smolt, and adult life stages. Introgression measurements made at the adult stage represent the survival of hatchery and hybrid origin individuals to adulthood and thus the potential for them to pass on their hatchery-origin genetic material to the next generation, and thus represent an important alternative to measurement of introgression in juveniles.

Study Watersheds

Within the LCR region, the Lower Columbia steelhead DPS (ESA-Threatened) contains 17 winter and 6 summer steelhead populations, of which 14 winter and 5 summer populations are wholly or partly located in Washington State. In addition, 3 unlisted winter populations occurring between the Cowlitz River and the Columbia River mouth are part of the Coastal Washington DPS. Across these watersheds, WDFW operates segregated steelhead hatcheries with a variety of operational configurations that may influence the potential for impacts on wild populations (Table 1). Due to the large wide variety of hatchery configurations and unique characteristics of each wild population and watershed, designing an introgression study which explicitly accounted for every unique combination of factors potentially affecting introgression would be difficult without conducting monitoring in every watershed in the region. Because such a study design would be cost-prohibitive, a series of study watersheds were chosen in order to represent the greatest range of hatchery configurations, wild population types, and potential introgression from segregated hatcheries.

In order to identify our study watersheds, we developed a conceptual framework, including simplifying assumptions, in order to narrow the watershed list to a subset containing the greatest range of *a priori* factors thought to potentially influence introgression:

Conceptual Framework for Selecting Study Watersheds based on Factors Affecting Introgression

This conceptual framework was developed in order to determine which watersheds to focus new tissue collections in to capture the greatest breadth of potential introgression rates and correlate these rates with hatchery practices. The framework is linear in that we start with an initial hypothesis which is subsequently modified by simplifying assumptions to narrow our candidate watershed list based on study design factors.

Initial Hypothesis: The proportion of spatial and temporal overlap between hatchery and wild origin spawners weighted by their relative abundances determines the level of genetic introgression (e.g. geneflow equation, Scott and Gill 2008).

Assumption 1: Segregated hatchery summer (Skamania) and winter (Chambers Creek) steelhead have similar spawn timing (December-January peak)

Assumption 2: Steelhead have similar spawn timing among basins in the LCR (hatchery—December-January peak, wild—April-May peak).

Intermediate Hypothesis: Differences in spatial overlap and relative abundance of hatchery and wild spawners will be the primary determinants of *between-basin differences* in introgression.

Assumption 3: Hatchery harvest rates are similar between basins

Assumption 4: Marine survival rates are similar between basins

Final Hypothesis: Two factors will be the primary determinants of *between-basin differences* in introgression:

1) The relative number of hatchery versus wild fish escaping fisheries and remaining uncollected
    a. Number of hatchery versus wild fish returns resulting from both production and marine survival rates

   b. Presence of (effective) collection facilities to remove non-harvested hatchery adults
2) The spatial distribution of hatchery versus wild spawners
   a. Rearing on- versus off-station?
   b. For off-station rearing, is there acclimation within the watershed?
   c. Life history of hatchery plants (summer versus winter)

Our conceptual framework eliminated many of the potential factors affecting introgression provided assumptions are reasonably satisfied. The resulting hypothesis suggested that the relative abundance of hatchery and wild spawners and their overlap in space would likely account for most between-basin variability in introgression. We thus considered the factors affecting these attributes in selecting our subset of study watersheds:

1) Relative abundance of hatchery and wild steelhead spawners
2) Presence of adult collection facilities
3) Rearing on- or off-station
4) Presence of acclimation facilities
5) Origin of hatchery plants (summer vs. winter)

Table 2 compares study watersheds with respect to five factors influencing introgression. Our study watershed set includes watersheds with (1) "large" (Elochoman), "medium" (lower E.F. Lewis), and "small" (Coweeman, upper E.F. Lewis) release numbers relative to wild population sizes, (2) with (Elochoman) and without (Coweeman, lower and upper E.F. Lewis) collection facilities, (3) with (Elochoman) and without (Coweeman, lower and upper E.F. Lewis) on-station rearing, and (4) with (Elochoman, Coweeman) without (lower and upper E.F. Lewis) acclimation facilities. Our study watershed subset includes (5) all segregated hatchery interaction types known to occur in the LCR region: hatchery summer and hatchery winter fish spawning in wild winter steelhead habitat (Elochoman, lower E.F. Lewis), hatchery winter fish spawning in wild winter steelhead habitat (Coweeman), and hatchery summer fish spawning in wild summer steelhead habitat (upper E.F. Lewis). Lastly, the Wind River will be included as a control watershed to validate molecular methods because it has had <1% hatchery origin spawners for more than 10 years, and should therefore show minimal or no introgression. The East Fork Lewis has been divided into upper and lower portions because seasonally impassable waterfalls naturally separate winter and summer steelhead populations within the watershed, as they similarly do in the Washougal and Wind Rivers.

Lower Columbia River Steelhead Genetic Baseline

Tissues will be collected from wild adult steelhead captured in weirs, traps, hatchery facilities, and by hook and line from all LCR populations as well as representative Chambers Creek and Skamania brood lines in Washington (ideally n = 100 per population). Additional samples will be collected from Oregon hatchery and wild populations thought to potentially contribute strays to Washington LCR rivers. These samples will be used to establish a genetic baseline representative of LCR steelhead populations. Recent work by WDFW has identified the problems with the ability of existing microsatellite markers to distinguish hatchery and wild

steelhead and have proposed development of Single Nucleotide Polymorphism (SNP) markers as an alternative that will provide sufficient power to confidently assign individuals (Warheit 2013). An SNP baseline will be developed for this introgression study.

Juvenile Introgression Sample Collections

Sampling of naturally produced age-0 steelhead for genetic analysis will occur throughout all study watersheds (Table 2) during the summer. Samples will be genotyped and compared with regional SNP steelhead baselines in order to classify individuals as wild, hatchery, or F1 (first generation) hybrid origin.

The spatial distribution of each hatchery, wild, and hybrid juveniles may differ because of different homing patterns exhibited by their parents. As a result, spatially biased sampling, such as sampling too close or far from hatchery facilities and imprinting sites could affect the composition of samples (i.e., proportion hatchery, wild and hybrid) at a site. To account for this problem and ensure an unbiased sample is obtained of the true proportions of hatchery, wild, and hybrid individuals, a spatially balanced sampling design (e.g., GRTS; Stevens and Olsen 2004) will be used. This will ensure that reaches are chosen for juvenile sampling in a manner that accounts for variation in the relative abundance of juvenile steelhead and spatial variation in introgression across watersheds.

Smolt and Adult Introgression Sample Collections

In a subset of LCR watersheds where spatially representative samples of smolts and adults are handled in ongoing monitoring activities, tissues will be genotyped in order to estimate introgression at these life stages. Genotyping adults and smolts from these watersheds also offers the potential for a faster initial assessment of introgression because WDFW already possesses tissue collections from several populations (e.g., Kalama summer and winter, E.F. Lewis summer, Washougal summer, Coweeman winter). Data from these watersheds will also be used to determine whether differential survival of hatchery, wild and F1 hybrids has occurred by comparing proportions of wild, hatchery, and hybrid individuals in fry, smolt, and adult samples. This information will help inform our understanding of the relationship between instantaneous measurements of introgression and long term gene flow.

Anticipated results

- Spatially representative and abundance corrected estimates of hatchery steelhead introgression into wild steelhead populations in representative study watersheds
- Quantitative information on natural and anthropogenic factors affecting the spatial structure and magnitude of hatchery introgression allowing for adaptive management of hatcheries throughout the LCR to meet conservation objectives
- Improved understanding of the relationship between introgression, PHOS, and gene flow across life stages.

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Tables and Figures

Table 1. Lower Columbia River Region steelhead populations by run type and listing status with the types of in-basin hatchery steelhead programs present and relevant operational considerations.

Population	Wild Stock Run	Listed	Hatchery Stock Run	Integrated/ Segregated	On-station Rearing	Acclimation	Collection Facility	Brood Collection	Known Into-Basin Strays
Grays	W	N	W	S	Y	Y	Y	N	S
Elochoman	W	N	W/S	S	Y	Y	Y	Y/N	
MAG[3]	W	N	W	I	Y	Y	Y	Y	S/W
Upper Cow	W	Y	W	I	Y	Y	Y	Y	
Cispus	W	Y	W	I	Y	Y	Y	Y	
Tilton	W	Y	W	I	Y	Y	Y	Y	
Lower Cow	W	Y	W/S	I/S	Y	Y	Y	Y	
SF Toutle	W	Y	S	S	N	Y	N	N	W
NF Toutle/Green	W	Y	S	S[1]	N	Y	Y	N	W
Coweeman	W	Y	W	S	N	Y	N	N	S
Kalama	W	Y	W/W/S/S	I/I/S/S[1]	Y	Y	Y	Y/Y/Y/N	
Kalama	S	Y	W/S/S	I/I/S[1]	Y	Y	Y	Y/Y/N	
NF Lewis	W	Y	W/W/S	I/S/S	Y	Y	Y	Y/Y/N	
NF Lewis[4]	S	Y	W/W/S	I/S/S	Y	Y	Y	Y/Y/N	
EF Lewis	W	Y	W/S	S	N	N	N	N	
EF Lewis	S	Y	S	S	N	N	N	N	
Salmon Cr.	W	Y	W	S	N	Y	N	N	
Washougal	W	Y	W/S	S	Y	Y	Y	Y	
Washougal	S	Y	S	S	Y	Y	Y	Y	
Lower Gorge	W	Y							S/W
Upper Gorge	W	Y	W	S[2]	N	Y	N	N	S/W
Wind	S	Y							

[1]Hatchery programs proposed to be discontinued
[2]Hatchery program proposed to replace White Salmon River programs, which were terminated due to Condit Dam Removal.
[3]Mill, Abernathy and Germany Creeks
[4]Extirpated wild population

Table 2. Study watersheds selected for LCR steelhead genetic introgression study and characteristics of wild populations and in-basin segregated hatchery steelhead releases.

Population	Wild Stock Run	Listed	Pop. Recovery Status*	Hatchery Stock Run	On-station Rearing	Collection Facility	Acclimation	Release Size Relative to Wild Pop.
Elochoman	W	N	C	W/S	Y	Y	Y	Large
Coweeman	W	Y	P	W	N	N	Y	Small
EF Lewis (L)	W	Y	P	W/S	N	N	N	Medium
EF Lewis (U)	S	Y	P	S	N	N	N	Small
Wind	S	Y	P	–	–	–	–	–
Kalama[1]	W/S	Y	P	W/S	Y	Y	Y	Large

*P=Primary, C=Contributing, S=Stabilizing

[1]The Kalama was not selected for new juvenile tissue collections based on our conceptual framework, however existing adult tissues may be used for analysis.