Genetic monitoring of hatchery-wild introgressive hybridization in Puget Sound steelhead

December 2 2014

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Introduction

In the Pacific Northwest, salmon and steelhead hatchery programs are a commonly employed approach to conserve declining populations and provide opportunities for commercial and recreational harvest. Through a variety of ecological and genetic processes, hatcheries can increase abundance and spatial distribution, but can also negatively impact productivity of sympatric wild populations (Araki et al. 2008; Naish et al. 2008; Kostow 2009). As part of the hatchery reform process aimed at balancing the inherent risks and benefits of hatchery programs, the Hatchery Scientific Review Group (HSRG) recommended that each hatchery program manage the hatchery stock as either segregated from or integrated with sympatric wild populations (Mobrand et al. 2005).

Under the segregated rearing strategy, hatchery managers aim to maintain reproductive isolation of the wild and hatchery populations[1]. Through broodstock management, specifically only spawning marked or tagged hatchery fish, hatchery managers can prevent gene flow from the wild into the hatchery. However, hatchery fish (commonly non-native for segregated programs) that do not return to the hatchery but instead spawn naturally may transfer maladaptive hatchery-origin genetic changes into the wild population through introgressive hybridization. For cryptically spawning fish such as steelhead trout, managers often cannot prevent hatchery-origin adults from reaching the natural spawning grounds, nor can they directly observe naturally-spawning of hatchery- or natural-origin fish. Genetic monitoring (Schwartz et al. 2007) offers a method of quantifying the extent to which hatchery-origin fish reproductively interact with wild fish, and thus monitoring the potential genetic risks of a hatchery program.

Natural-origin fish with hatchery-lineage may be maladaptive in the wild compared with fish of wild ancestry (Kostow et al. 2003). If wild by hatchery-origin spawning pairs yield fewer offspring than wild by wild pairs due to a survival deficit, introgressive hybridization would tend to contribute to the reduced productivity of the wild population, and may reduce the wild population's effective population size. Under this scenario, one would predict that the relative occurrence of hatchery-lineage and hybrid fish would be greater at younger life stages than older life stages. Therefore, comparing occurrence of hatchery-lineage steelhead and hybrids among multiple age classes of steelhead within a cohort permits a quantitative assessment of the consequences of introgression on the productivity of wild populations.

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[1] Fish whose origins are from a river, rather than a hatchery, are natural-origin fish. The parents of natural-origin fish can either be hatchery-origin, natural-origin, or mixed hatchery- and natural-origin. To avoid confusing the origin of a fish (i.e., where it was born), from its ancestry (i.e., where its parents were born), in this document we will refer to natural-origin fish with hatchery-origin parents as hatchery-lineage, natural-origin fish with natural-origin parents as wild-lineage (or simply wild), and natural-origin fish with one hatchery-origin parent and one natural-origin parent as a hybrid. The wild population consists of wild-lineage fish, the hatchery population consists of hatchery-origin fish spawned in a hatchery.

In Puget Sound, where steelhead are listed as *Threatened* under the Endangered Species Act, the Washington Department of Fish and Wildlife (WDFW) currently operates, in cooperation with the Puget Sound treaty tribes, segregated hatchery steelhead programs in five major river basins (Table 1). Similar to elsewhere in the state, in this region WDFW has primarily propagated and released fish derived from two segregated stocks: an early winter-run stock derived from Chambers Creek (Puget Sound) and a summer-run stock derived from the Washougal River and originally propagated at the Skamania Hatchery (Lower Columbia River). WDFW instituted major changes to some of these programs in the last five years intended to reduce genetic impacts on wild populations. Warheit (2014) described reproductive interactions between hatchery- and wild-ancestry fish associated with these programs in Puget Sound, finding evidence for hybridization in some, but not all, watersheds. However, definitive conclusions regarding the degree and consequences of hatchery-wild hybridization were limited by the sporadic temporal and spatial distribution of samples, and for some watersheds, the limited number of samples, which also varied in life stage.

Here we describe a ten year genetic monitoring program for Puget Sound steelhead intended to document the potential for hatchery introgression into a wild population by measuring the relative numbers of hatchery-lineage and hatchery-wild hybrids within a collection of natural-origin fish. Our overall goal is to determine if operation of Puget Sound segregated hatchery programs meet conservation objectives. We aim to institute a systematic sample collection approach that will reduce the uncertainty of the Proportion Effective Hatchery Contribution (PEHC) provided by Warheit (2014). In what follows, we use the phrase "introgressive hybridization" as shorthand for "occurrence of natural-origin (unmarked) hatchery-lineage and hatchery-wild hybrids."

Research questions

1. What are the trends through time of PEHC in Puget Sound wild steelhead populations?

2. Does PEHC change through the steelhead life-cycle? Is there differential survival of natural-origin fish with wild ancestry vs. natural-origin fish with hatchery ancestry?

Hypotheses

Question 1 – trends in hatchery-wild introgressive hybridization

The general hypothesis that we will address is that current hatchery operations maintain reproductive interaction between hatchery and wild fish, as measured PEHC, at or below the target threshold established by NOAA in their forthcoming Biological Opinion. However, the precise timeline at which we would expect to observe PEHC at or below this threshold will vary by river system according to hatchery program history. Target release numbers for early winter steelhead hatchery programs in three river systems (Nooksack, Stillaguamish and Dungeness) have remained relatively consistent in recent time periods. In a fourth watershed (Skykomish), release goals were elevated in 2014 relative to previous years. In all four of these river systems, we hypothesize that PEHC is currently below the target threshold, and our monitoring program is designed to confirm this expectation.

However, in two river systems, WDFW recently implemented major reductions in the number of early winter steelhead smolt releases. In the Green River system, the target release number was reduced from approximately 145,000 smolts in 2009 to approximately 80,000 smolts in 2010. In the Snoqualmie River system, the target release number was reduced from approximately 153,000 smolts in 2013 to 74,000 smolts in 2015. We would expect concomitant reductions in PEHC in the years following these reductions according to the steelhead life cycle (Table 2). Assuming two years of ocean rearing for hatchery steelhead, we would expect reduced reproductive interaction between hatchery and wild fish two years after reductions in smolt releases. However, reductions in PEHC would not be detected until we sampled the offspring from this natural spawning event. Assuming that naturally spawned steelhead primarily spend two years in freshwater and two years in the ocean, PEHC reductions in smolts would be detected four years after reductions in program size, and PEHC reductions in adults would be detected six years after reductions in program size (Table 2).

River	Hypothesis
Nooksack	PEHC currently maintained at or below target threshold established by NOAA in their forthcoming Biological Opinion
Stillaguamish	PEHC currently maintained at or below target threshold established by NOAA in their forthcoming Biological Opinion
Skykomish	PEHC currently maintained at or below target threshold established by NOAA in their forthcoming Biological Opinion
Snoqualmie	PEHC maintained at or below NOAA target threshold beginning in 2019 for smolts and 2021 for adults (see Table 2 for detailed timeline)
Green	PEHC maintained at or below NOAA target threshold beginning in 2014 for smolts and 2016 for adults (see Table 2 for detailed timeline)
Dungeness	PEHC currently maintained at or below target threshold established by NOAA in their forthcoming Biological Opinion

Question 2 – intensive, life-stage approach to introgression monitoring

This research will address the null hypothesis that there is no difference in the potential for introgression among juvenile parr, smolts, and adults in the Green River watershed, as measured by PEHC. This will be compared to the alternative hypothesis that introgression potential is higher at the parr stage than the smolt stage, and higher at the smolt stage than the adult stage. Support for this latter hypothesis would suggest lower survival of hatchery-lineage and hybrid steelhead compared to pure wild steelhead.

Assumptions

Our research rests on the following assumptions.

Question 1 – trends in hatchery-wild introgressive hybridization

1. Smolt traps obtain a representative sample of natural-origin hatchery-lineage, hybrid, and wild smolts in each watershed or population
2. There is no differential survival among hatchery-lineage, hybrid, and wild-lineage steelhead from the egg to smolt stage (this assumption will be tested via research question #2)

3. Genetic stock identification (GSI) can assign steelhead juvenile samples to population of origin in basins where smolt traps sample multiple independent populations (Nooksack, Stillaguamish, Snohomish). Alternatively, if GSI is not available, watershed-scale assessment of introgressive hybridization is sufficient for determining if hatchery programs meet conservation objectives in basins with multiple independent wild populations.
4. Genetic methods accurately identify hatchery-lineage, hybrid, and wild-lineage steelhead

Question 2 – intensive, life-stage approach to introgressive hybridization monitoring
1. Parr collections obtain a representative sample of the Green River population: samples are not biased with respect to spatial location or body size
2. Smolt traps obtain a representative sample of the natural-origin steelhead smolts in the Green River population
3. Adult sampling, pursued via collection of broodstock for the integrated program, obtains a representative sample of natural-origin adult steelhead in the Green River population
4. Genetic methods accurately identify hatchery-lineage, hybrid, and wild-lineage steelhead

Field sampling

For both research questions, it is important to recognize that introgressive hybridization may exhibit substantial spatial variation within a population due to homing behavior. For example, if stray rates of hatchery-origin fish decrease with distance from the hatchery, sample collection location may have a strong influence on the observed level of introgressive hybridization. We have designed our monitoring plan to obtain a representative sample of fish at either the population or watershed scale, and minimize bias due to sample collection location.

Question 1 – trends in hatchery-wild introgressive hybridization

We plan to monitor trends in introgressive hybridization primarily by collecting tissue samples from unmarked, natural-origin fish at tribally and WDFW operated smolt traps for two reasons. First, steelhead are scarce, elusive and difficult to sample, especially during winter flow conditions. Therefore, our monitoring plan builds on existing sampling infrastructure and ongoing efforts of the state and tribal comanagers to monitor Puget Sound steelhead. In all five basins containing segregated hatchery steelhead programs, either WDFW or a tribal co-manager operates one or more smolt traps.

Secondly, smolt traps likely obtain a representative sample of spawning and rearing habitats upstream of the trap. Assuming uniform vulnerability to the fishing gear, traps sample individual fish according to the proportionate contribution from upstream rearing habitats, minimizing spatial bias. Furthermore, when operated throughout the duration of the smolt migration, smolt traps minimize temporal sampling bias.

Smolt traps positioned downstream from multiple independent steelhead populations, as identified by the Puget Sound Technical Recovery Team (PSTRT 2013), will obtain a mixed sample at smolt traps. In these cases, monitoring introgressive hybridization at the population scale will rely upon genetic stock identification (GSI). Although genetic tools currently available may not permit assignments at this resolution, ongoing efforts to improve the Puget Sound genetic baseline by adding more single nucleotide polymorphisms (SNPs) will work toward this goal. If such tools are not available, introgressive hybridization will be measured at the watershed scale rather than at the population scale.

In the following sections, we describe a ten year plan for monitoring efforts within each of the five Puget Sound basins where WDFW operates segregated hatchery steelhead programs (Table 3). Our sampling plan rests primarily, but not exclusively, on collections from smolt traps (up to N = 100 annually from each basin). Additional adult samples (up to N = 50 annually) will be collected from high priority locations, in some cases on a rotating basis. Adult collections are proposed for populations that exhibited high PEHC values in Warheit's (2014) analysis (Snoqualmie winters), are needed for the life cycle assessment proposed by research question #2 (Green winters), contain unique summer run life histories whose genetic integrity is an essential component of Puget Sound steelhead evolutionary diversity (Deer summers, South Fork Nooksack summers), or are located downstream of smolt traps (Pilchuck winters). The periodicity of rotating adult sampling, once every three years, is designed to collect tissues at least once per steelhead generation. In some cases, especially with summer run populations, adult samples sizes will necessarily be small owing to small population size. Throughout this document, references to populations are those identified by the PSTRT. We acknowledge that these population designations are subject to revision pending further investigation.

Nooksack — Sampling from the Nooksack basin will occur from locations and life-stages based on availability. The PSTRT identified two populations in the Nooksack basin: Nooksack winter and South Fork Nooksack summer. The Lummi Nation operates a smolt trap downstream from both populations, providing a high priority source of samples. We propose to collect summer run adults from the South Fork Nooksack River on a rotating basis once every three years to provide additional genetic monitoring of this unique life history.

Stillaguamish — We anticipate collecting samples from the smolt trap operated by the Stillaguamish Tribe of Indians in the mainstem Stillaguamish River. This trap collects a mixed sample of the three steelhead populations in the basin: Stillaguamish winter, Deer Creek summer, and Canyon Creek summer. GSI will be necessary to resolve these samples to the population scale. Deer and Canyon creeks contain summer runs that are relatively unique within Puget Sound; minimizing genetic introgressive hybridization in these populations is important to conserving genetic diversity in the Puget Sound DPS as a whole. We propose to collect adult samples from Deer Creek on a rotating basis once every three years to represent the summer run life history in the Stillaguamish basin.

Snohomish — In the Snohomish basin, samples will be collected from two smolt traps operated by the Tulalip Tribes, or other locations if smolt trap samples are not available. A trap in the lower mainstem of the Snoqualmie River collects juvenile steelhead originating from the Snoqualmie winter and Tolt summer populations. A trap in the lower mainstem of the Skykomish River collects juvenile steelhead originating from the Snohomish/Skykomish winter and Skykomish summer populations. In recent years (2009 – 2012), both the Skykomish (average annual N = 55) and Snoqualmie (average annual N = 102) traps have caught sufficient unmarked steelhead to estimate introgressive hybridization (Kubo et al. 2013). In order to measure introgressive hybridization at the population scale, GSI tools would be needed to discriminate mixed samples at both traps. Both traps are located upstream of the mouth of the Pilchuck River, and thus will not provide samples from the Pilchuck winter population. To address this issue, adults from the Pilchuck River will be sampled on a rotating basis every three years. The Snoqualmie River populations merit special monitoring focus due to relatively large number of natural-origin hybrids and hatchery-lineage fish observed by Warheit (2014). Adult steelhead from the Snoqualmie River will be collected each year beginning in 2015.

Green — We plan to employ a more intensive, life-stage approach to introgressive hybridization monitoring on the Green River (see below for details).

Dungeness — WDFW operates a smolt trap near the mouth of the Dungeness River, providing a representative sample from the single wild steelhead population in the Dungeness watershed. Adult samples will be collected opportunistically in the Dungeness watershed and compared to the number of hybrid and hatchery-lineage fish observed in smolts.

Question 2 – intensive, life-stage approach to introgression monitoring

We propose to test the hypothesis that hatchery-lineage and hatchery-wild hybrid fish have lower survival than wild fish by monitoring introgressive hybridization at multiple life stages. If hatchery-lineage and hybrid steelhead have lower survival than wild steelhead, their numbers would decline at subsequent life stages within a cohort. To test this hypothesis, we plan a more intensive sampling effort within the Green River, where we will collect samples from three life stages: pre-smolt age-0 or age-1 juveniles, smolts and adults (Table 3).

We selected the Green River as the focal watershed for three reasons. First, ongoing sampling efforts provide existing opportunities to sample smolts and adults. WDFW operates a smolt trap at river kilometer 55 and handles adults via collection of natural-origin broodstock for an integrated hatchery program. Second, it is feasible to sample juvenile steelhead from the Green River given the basin's moderate size, and relatively good road access. Third, Warheit (2014) found a higher proportion of hatchery-lineage and hybrids among the juvenile steelhead than adults in the Green River, a result not observed in other basins of Puget Sound (due in part to a lack of samples). Thus, to the extent that hybridization poses a threat to Puget Sound wild steelhead, the Green has a high likelihood that we will detect, describe, and understand the pattern of introgressive hybridization.

To complement the ongoing smolt and adult sampling, we propose to collect tissue samples from juvenile *O. mykiss* from locations throughout the watershed during the summer. To minimize any accumulated lifetime mortality differential between wild and introgressed juveniles, we will target the youngest life-stage possible, age-0 or age-1. If logistically feasible, juvenile fish will be sampled in a spatially-balanced study design (i.e., GRTS: Stevens Jr. and Olsen 2004) to minimize bias due to collection location.

Genetic methods

A series of steps involving the formation and analysis of modeled populations combined with laboratory analyses of steelhead samples will produce measures of hatchery-wild introgressive hybridization. Two primary analysis tools will be used: (1) the program Structure (Pritchard et al. 2000, Falush et al. 2003), and (2) a likelihood-based procedure that adjusts Structure results to account for the close phylogenetic relationships (i.e., recency of common ancestry) between the hatchery and wild populations (Warheit and Knapp, in prep). The modeled populations provide data to objectively establish thresholds to assign individuals to Structure groups or categories, and measures of assignment errors. We will include the model-based assignment errors into the likelihood procedure to produce final estimates of population composition and hybridization. For a detailed methodology, see Warheit (2014). WDFW and other laboratories are currently using next-generation sequencing methods to discover and map thousands of SNPs from hatchery- and natural-origin steelhead genomes. These methods may produce molecular markers and analytical tools that will improve our ability to identify hatchery-lineage, hybrid, and wild steelhead. If these markers and analytical tools become available during the scope of this monitoring plan, we will propose to use these tools as our primary laboratory and analytical methods.

Expected results

The proposed monitoring program will address the fundamental management need to ensure that operation of segregated hatchery steelhead programs in Puget Sound meet the conservation objectives of WDFW's (2008) Statewide Steelhead Management Plan. This work will build on Warheit's (2014) initial assessment of introgressive hybridization in Puget Sound steelhead by implementing a systematic sampling regime and reduce the uncertainty associated with current estimates. This monitoring program will determine if management strategies aimed at minimizing hatchery introgression are effective.

We also anticipate that our results will increase our understanding of the factors that increase the risk of hatchery introgression in wild steelhead. The watersheds under investigation encompass a range of habitat quality, hatchery program sizes and wild population demographics. Comparing the rates of introgressive hybridization across watersheds varying in these attributes will offer insight into the process of gene flow from hatchery stocks into wild populations.

The focused study in the Green River will improve our understanding of the genetic effects of hatchery ancestry on survival patterns and fitness. Few studies of relative reproductive success have isolated the genetics effects of hatchery breeding from the environmental effects of hatchery rearing (Araki et al. 2008). In this case, we will test for genetic effects, as all of the unmarked fish under investigation will have been spawned and reared in the natural environment. A decline in introgression from younger to older life stages would indicate that genetic background, and specifically hatchery ancestry, may influence survival through ontogeny.

Tables and figures

Table 1. Summary of Puget Sound segregated hatchery steelhead programs. We separate releases for the Skykomish and Snoqualmie rivers, noting that both are components of a single hatchery program for the Snohomish basin.

		Rearing and release sites 2013		
Watershed	Program	N	Location	Average total release 2010-2013
Nooksack	Segregated winter	1	Kendall Creek	138,366
Stillaguamish	Segregated winter	1	Whitehorse Ponds	110,999
	Segregated summer	1	Whitehorse Ponds	286,304
Skykomish	Segregated winter	2	Wallace Hatchery, Reiter Ponds	166,291
Skykomish	Segregated summer	1	Reiter Ponds	191,574
Snoqualmie	Segregated winter	1	Tokul Creek	159,010
Green	Segregated winter	2	Soos Creek, Icy Pond	72,846
	Segregated summer	1	Soos Creek	62,621
	Integrated winter	2	Soos Creek, Flaming Geyser Pond	21,468
Dungeness	Segregated winter	1	Dungeness Hatchery	9,731

Table 2. Hypothesized changes in Proportion Hatchery Effective Contribution (PEHC) following reductions in program size in the Green and Snoqualmie rivers. The black cells represent the first calendar year we would hypothesize a reduction in each value, with the gray cells denoting subsequent calendar years of sustained reductions relative to the period prior to each hatchery's reduction in program size. These hypotheses assume two years of ocean rearing for hatchery steelhead and natural-origin steelhead smolt at age 2. Although there is significant variation age at smolting and adult maturity steelhead, these assumptions are useful to diagram our expectations of changes in PEHC.

River	**Value reduced**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**
Green	Number of hatchery smolts released														
	Number of hatchery adult returns														
	Unmarked smolt PEHC														
	Unmarked adult PEHC														
Snoqualmie	Number of hatchery smolts released														
	Number of hatchery adult returns														
	Unmarked smolt PEHC														
	Unmarked adult PEHC														

Table 3. Sample collection plan for Puget Sound steelhead genetic monitoring program.

Life stage	Location	N	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Pre-smolt juveniles	Green	up to 400	X	X	X	X	X	X	X	X	X	X
Smolts	Nooksack	up to 100	X	X	X	X	X	X	X	X	X	X
	Stillaguamish	up to 100	X	X	X	X	X	X	X	X	X	X
	Skykomish	up to 100	X	X	X	X	X	X	X	X	X	X
	Snoqualmie	up to 100	X	X	X	X	X	X	X	X	X	X
	Green	up to 100	X	X	X	X	X	X	X	X	X	X
	Dungeness	up to 100	X	X	X	X	X	X	X	X	X	X
Adults	Green winters	up to 50	X	X	X	X	X	X	X	X	X	X
	Snoqualmie winters	up to 50	X	X	X	X	X	X	X	X	X	X
	Deer summers	up to 50	X			X			X			X
	Pilchuck winters	up to 50		X			X			X		
	SF Nooksack summers	up to 50			X			X			X	

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