

NPAFC

Doc. 1027

Rev. _____

Rev. Date: _____

A Plan for a Range-wide Genetic Baseline for Steelhead (*Oncorhynchus mykiss*)

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Submitted to the

NORTH PACIFIC ANADROMOUS FISH COMMISSION

by the

UNITED STATES OF AMERICA

April 2007

THIS REPORT MAY BE CITED IN THE FOLLOWING MANNER:
Young S.F, Bartlett, H.R., Hawkins, D.K., and Warheit, K.I. 2007. A Plan
for a Range-wide Genetic Baseline for Steelhead (*Oncorhynchus mykiss*).
NPAFC Doc. 1027. 9 pp.
(Available at <http://www.npafc.org>)

Abstract

Steelhead (*Oncorhynchus mykiss*) are listed as either threatened or endangered under the United States Endangered Species Act in 11 of 15 Distinct Population Segments in the western United States south of Canada. Despite recovery efforts in freshwater habitats returns of adults from the North Pacific have remained low. This is a plan for developing a range-wide genetic baseline of steelhead encountered in the surveys in the North Pacific Ocean. We will use existing microsatellite markers and develop new nuclear and mitochondrial single nucleotide polymorphism (SNP) assays to characterize populations throughout the Pacific Rim and develop the ability to identify the stock-of-origin of steelhead in the North Pacific Ocean.

Introduction

In the mid-1990s, responding to petitions to protect steelhead (*Oncorhynchus mykiss*) populations under the Endangered Species Act (ESA), the United States government reviewed the status of steelhead in Washington, Oregon, Idaho, and California and identified 15 Distinct Population Segments (DPS) in those states (Busby et al. 1996). The federal government considers 11 of those population segments to be either threatened or endangered (Good et al. 2005) (Table 1). In response to those listings, state, federal, tribal, and local resource managers and motivated citizen volunteers have expended considerable effort and money to meet recovery goals set under the ESA. Despite those efforts, adult returns in a number of areas recently have been lower than expected. Some steelhead populations in Canada and Russia also are at low abundance and are afforded legal protection.

Pacific salmon (*Oncorhynchus spp.*) and steelhead have iconic status in western North American culture, and these species are the source of substantial economic activity within the region. They are familiar subjects of indigenous art throughout the region and are key elements of tribal culture in the North American Pacific Northwest. Steelhead populations in western North America are targets of Native American ceremonial and subsistence, and some commercial fisheries. Recreational fisheries generate substantial economic activity in many communities. Native American and all-citizen fisheries on steelhead populations in the Columbia Basin alone in 2000 generated \$16 - 18 million in income to local communities (Independent Economic Analysis Board 2005). The freshwater sport fishery generated more than two-thirds of that economic activity. Population recovery efforts consume large amounts of public and private funding and the legal protection of the fish and their habitats in the United States under the ESA results in loss of substantial economic activity.

Current recovery efforts for natural *O. mykiss* populations focus primarily on addressing limiting factors during the freshwater phases of the life cycle (restoring and maintaining freshwater migration, holding, and spawning areas for adults and freshwater rearing habitat and migration routes for juveniles); however, anadromous salmonid populations can experience substantial mortality after leaving freshwater and entering the North

Pacific Ocean (Brodeur et al. 2003). Steelhead production in the North Pacific Ocean likely is influenced by climatic conditions and numerous biotic factors, however, we know little about how steelhead stocks are distributed in the North Pacific and how they respond to changing climatological and ecological factors. Steelhead juveniles appear to move offshore soon after entering the ocean in spring or early summer and are rarely encountered in nearshore sampling of Pacific salmon juveniles (Brodeur et al. 2003). Few steelhead populations are coded-wire tagged (CWT) but occasional recoveries in the North Pacific of those that are tagged reveal that Pacific Northwest steelhead are broadly distributed south of the Bering Sea. McKinnell et al. (1997) reported, however, that tagged steelhead that were released from hatcheries near each other at similar times were recovered together on the high seas up to three years later, at a rate that was greater than would be expected by chance, suggesting that the ocean migrations of some regional groups of tagged (hatchery) steelhead are somehow coordinated. Aside from the few CWT recoveries, all from hatchery populations, few stock-specific marine distribution data exist for steelhead; consequently, we know very little about the oceanic distributions of specific stocks of concern. Improving our understanding of the marine distribution of specific steelhead stocks might allow us to identify environmental correlates in the North Pacific with high or low growth or survival rates. Stock-specific knowledge of distribution from saltwater entry through oceanic rearing to return to natal rivers for spawning would allow scientists to elucidate the effects of changes in climate and the North Pacific ecological community on steelhead production and abundance. Resource managers might use stock-specific abundance estimates in the North Pacific to improve forecasts of spawning run sizes and to factor-in the effects of oceanic conditions when they evaluate the effectiveness of freshwater actions intended to benefit steelhead.

Genetic methods to identify donor stocks in population mixtures are in widespread use in salmonid management and research (for example: Shaklee et al. 1999, Beacham et al. 2004, Banks 2005). These methods rely on among-population genetic diversity and substantial isolation of spawning populations. In genetic mixed stock analyses, genotypes encountered in a sample from a stock mixture are analyzed relative to a set of reference genotypes from potential donor stocks. When multiple genetics laboratories are involved in an analysis, or when multiple laboratories perform independent analyses that will be compared, it is important for all to use a common set of reference populations, common markers and standardized allele calling protocols.

We propose to develop a standardized, range-wide reference population data set that will include mitochondrial and nuclear genetic data. Sato et al. (2004) used the geographical distribution of mitochondrial DNA variation to distinguish Asian and North American populations of chum salmon (*O. keta*) during the oceanic portion of their life cycle. Microsatellite and nuclear single nucleotide polymorphism (SNP) variation allows finer-scale resolution of populations, sometimes within river systems. The combination of nuclear and mitochondrial markers should provide robust stock discriminatory power and might enhance our understanding of evolutionary relationships among stocks. This standardized genetic baseline data set should allow us to identify the stock-of-origin of steelhead caught during high seas salmonid sampling surveys and to estimate population

membership of steelhead recovered from illegal, unreported, unregulated (IUU) fishing activity.

The participation of multiple laboratories in constructing large, shared, standardized genetic baselines will be beneficial in several ways. It allows the workload of baseline construction to be distributed so that no single laboratory needs to direct large portions of its resources to the task; it requires communication among laboratories and promotes technology sharing; it allows scientists in separate laboratories and agencies to perform analyses using common reference data and avoid a potential source of conflicting results; and it reduces the risk that changing priorities at a single laboratory might render the data set incomplete and thus useless to the interested scientific community.

The steelhead genetic baseline we propose will add to our scientific understanding of the roles of anadromous fish in the North Pacific ecosystem and is consistent with the North Pacific Anadromous Fish Commission (NPAFC) Science Plan 2006-2010 (available at [http://www.npafc.org/new/publications/Science_Plan/SciPlan\(2006-2010\).pdf](http://www.npafc.org/new/publications/Science_Plan/SciPlan(2006-2010).pdf)). That plan identifies two broad scientific questions:

- 1) What are the current status and trends in marine production of anadromous stocks; and how are these trends related to population structure (spatial and temporal) and diversity of anadromous stocks in marine ecosystems of the North Pacific?

- 2) How will climate and climate change affect anadromous stocks, ecologically related species, and their North Pacific marine ecosystems?

The baseline should allow scientists to estimate the continent and region of origin of steelhead that are encountered during North Pacific Ocean research activities, to map the distributions of regional stock aggregates, and to partition production estimates by region of origin. Improved ability to identify stock-specific ocean distributions will allow us to study stock-specific vulnerabilities to climate changes.

Methods

We propose to construct the genetic baseline in stages. During the first stage, we will subdivide the species range into biogeographic population aggregates and genotype three population samples, each of 50 adults to represent each bio-geographic aggregate. The aggregates in the United States of America south of Canada will be DPSs identified in the ESA steelhead status review (Busby et al. 1996, Good et al. 2006; Table 2). We will construct aggregates from Canada, Alaska, and Russia with the intent to represent biological diversity that is roughly equivalent to that within DPSs (Table 2). A steelhead DPS can include substantial life history variation and that variation should be included in the genetic baseline. We will evaluate the power of an initial set of candidate genetic loci to resolve population membership among those samples. In the second stage, we will search for existing microsatellite or SNP markers to add to the locus suite to improve population resolution where it is weak or lacking. If we are unable to find existing markers that will provide adequate resolution, we will develop new SNP markers using ascertainment panels that target populations that are difficult to resolve with the existing

markers. In the third stage, we will add populations from each bio-geographic population aggregate to represent the physiographic and life history variation present in those population aggregates.

Genetic locus evaluation and selection

There currently is no broadly standardized set of molecular markers and scoring protocols for steelhead population genetics studies. The Conservation Genetics Laboratory at Canada Department of Fisheries and Oceans' (DFO) Pacific Biological Station used 13 microsatellite loci to analyze 51 steelhead populations from British Columbia, Canada through Washington, Oregon and Idaho in the United States. Washington Department of Fish and Wildlife (WDFW), National Marine Fisheries Service Northwest Fishery Science Center (NWFSC), U.S. Fish and Wildlife Service Abernathy Fish Technology Center (USFWS-Abernathy), Idaho Department of Fish and Game (IDFG), and the Columbia River Inter-tribal Fisheries Commission (CRITFC) have established a common set of 18 microsatellite loci and standard allele calling protocols (the Stevan Phelps *Oncorhynchus mykiss* Allele Nomenclature, or SPAN, standards) to use in steelhead genetics studies within the Columbia Basin. Unfortunately the DFO and SPAN suites have only four loci in common so that data collected using the SPAN standards are not compatible with the data collected by DFO (Table 3). Single nucleotide polymorphism assay development for *O. mykiss* is underway in several laboratories, and we will evaluate their use in resolving problematic stocks within the baseline.

We anticipate that neither the DFO suite nor the SPAN suite will provide enough resolving power by itself to distinguish all populations of interest in high seas mixtures. The resolving power of loci in mixture analyses is a function of allele frequency differences in the reference populations. The most polymorphic loci do not necessarily provide the most power for identifying closely related populations; however, our reference data set will include many populations, and we have no prior knowledge of allele frequencies in many of those stocks. We expect that highly polymorphic loci likely will provide informative allele frequency differences between more population pairs than would loci with fewer alleles. We propose initially to use a set of the most polymorphic microsatellite loci from the combined DFO and SPAN suites, evaluate their power to identify populations of concern, and then decide whether we should add more loci. We will incorporate a set of SNP markers in the evaluation.

Standardizing allele calling

We will assemble microsatellite allelic ladders or sets of allelic standards to share with other genetics laboratories that want to collect compatible data and contribute to and use the shared database. Periodic genotyping of common sets of extracted genomic DNA by all contributing laboratories will promote the continuing compatibility of the data in the database. We will provide sequence data for primers and probes used in SNP assays and identify the strand orientation used for naming SNP alleles.

Reference populations

Construction of the baseline data set will require cooperation with scientists from Canada, Alaska and Russia, either as participating laboratories or as sources of tissue

samples or extracted genomic DNA. We will develop a list of candidate populations for the steelhead genetic baseline in consultation with scientists and managers throughout the species' range. Within the State of Washington, state and tribal steelhead managers have identified 137 natural-origin steelhead stocks, but they have been grouped into seven DPSs. Our initial goal will be to achieve DPS-like resolution throughout the species' range. We will rely on local experts to identify likely population groupings that will identify potentially important ecotypes (e.g. freshwater residents, half-pounders, summer run, winter run) and allow us to evaluate the genetic uniqueness of those types.

Power analysis

We will genotype three populations per bio-geographical population aggregate throughout the species' range on a scale similar to the DPS clusters and evaluate the genetic variance within clusters and among clusters. We will use a leave-one-out (jackknife) analysis routine developed by Dr. Kenneth Warheit, WDFW Molecular Genetics Laboratory, to assess the resolving power of the loci for the reference population set. The power analysis will tell us if we can expect the suite of genetic markers to identify the constituent populations when they are present in a mixed stock sample.

Database accessibility

Non-genetic biological data (i.e. life history type, age, gender), multilocus genotypes, and sampling data for every individual analyzed will be stored in an electronic database. We will work with collaborating laboratories to identify a public host with internet accessibility where the database can reside to insure that the data remain accessible to all parties to the Convention.

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Table 1. Steelhead Distinct Population Segments in the United States south of Canada and their legal status

Distinct Population Segment	Endangered Species Act status
Puget Sound	Proposed Threatened
Olympic Peninsula	Not listed
Southwest Washington	Not listed
Snake River Basin	Threatened
Upper Columbia River	Threatened
Middle Columbia River	Threatened
Lower Columbia River	Threatened
Upper Willamette River	Threatened
Oregon Coast	Species of concern
Klamath Mountains Province	Not listed
Northern California	Threatened
Central California Coast	Threatened
South-Central California Coast	Threatened
Southern California	Endangered
California Central Valley	Threatened

Table 2. Provisional baseline bio-geographic population aggregates.

United States south of Canada
Southern California
South-Central California Coast
Central California Coast
California Central Valley
Northern California
Klamath Mountains Province
Oregon Coast
Upper Willamette River
Lower Columbia River
Middle Columbia River
Upper Columbia River
Snake River Basin
Southwest Washington
Olympic Peninsula
Puget Sound
Canada
Lower Fraser River
Thompson River
Upper Fraser River
South British Columbia Coast/Vancouver Island
Central British Columbia Coast
Nass & Skeena summer run
Nass winter run
Canada/UnitedStates
SEAK/BC Trans-boundary Rivers
Alaska
SEAK Islands
Cape St. Elias-Yakutat
Copper River
Southcentral AK Coast
Kodiak Island
Alaska Peninsula-south side
Alaska Peninsula-north side
Russia
Kamchatka Peninsula

Table 3. Microsatellite locus suites used by DFO and SPAN labs have only four loci in common. Data collected with one suite are incompatible with data collected with the other suite.

Locus	DFO	SPAN
Ocl-1		x
Ogo-4	x	x
Oke-4	x	x
Oki-10	x	x
Oki-23MMBL		x
Omm-1070		x
Omy-1001		x
Omy-1011		x
Omy-325	x	
Omy-77		x
Omy-7iNRA		x
One-101	x	
One-102		x
One-111	x	
One-114	x	
One-14		x
Ots-1	x	
Ots-100		x
Ots-108	x	
Ots-2	x	
Ots-3M		x
Ots-4		x
Ots-9	x	
Ssa-197	x	
Ssa-289		x
Ssa-407		x
Ssa-408	x	x