

FINAL REPORT
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Northern California steelhead DPS-Configuration Review-Panel Report

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Introduction

On November 15, 2018, the National Marine Fisheries Service (NMFS) received a petition from the Friends of the Eel River to separate summer-run steelhead in the Northern California Steelhead Distinct Population Segment (DPS) into a new DPS, and to then list this DPS as Endangered under the US Endangered Species Act (ESA). On April 22, 2019, NMFS determined that the petition presented substantial information indicating that the petitioned action may be warranted (a “positive 90- day finding”) and published the finding in the Federal Register (84 FR 16632), pursuant to 50 CFR 424.14. The Federal Register notice also announced the opening of a 60- day public comment period to solicit information from interested parties that is relevant to the status of Northern California (NC) summer-run and winter-run steelhead. This public comment period ended on June 21, 2019.

To address this petition, the NMFS West Coast Region (WCR) asked the Southwest Fisheries Science Center (SWFSC) to convene an expert panel to inform the WCR whether summer-run steelhead warrant designation as a separate DPS from winter-run steelhead. Specifically the WCR asked the SWFSC to compile and review the best available scientific and commercial information relevant to evaluating the DPS structure of summer-run steelhead in northern California, including information presented by the petitioners, and use the criteria in the DPS Policy (61 FR 4722) to evaluate the claims of the petition and write a report on their findings. The DPS Policy describes three elements to be considered in a decision regarding the status of a possible DPS as endangered or threatened under the ESA. These are applied similarly for addition to the lists of endangered and threatened wildlife and plants, reclassification, and removal from the lists:

1. Discreteness of the population segment in relation to the remainder of the species to which it belongs;
2. The significance of the population segment to the species to which it belongs; and
3. The population segment's conservation status in relation to the ESA's standards for listing (i.e., is the population segment, when treated as if it were a species, endangered or threatened?).

Discreteness: A population segment of a vertebrate species may be considered discrete if it satisfies either one of the following conditions:

1. It is markedly separated from other populations of the same taxon as a consequence of physical, physiological, ecological, or behavioral factors. Quantitative measures of genetic or morphological discontinuity may provide evidence of this separation.

2. It is delimited by international governmental boundaries within which differences in control of exploitation, management of habitat, conservation status, or regulatory mechanisms exist that are significant in light of section 4(a)(1)(D) of the ESA.

Significance: If a population segment is considered discrete under one or more of the above conditions, its biological and ecological significance will then be considered in light of Congressional guidance (see Senate Report 151, 96th Congress, 1st Session) that the authority to list DPS's be used "...sparingly" while encouraging the conservation of genetic diversity. Here we consider significance with respect to the listed taxonomic unit, the NC steelhead DPS, as opposed to the full species. In carrying out this examination, the panel considered the available scientific evidence of the discrete population segment's importance to the taxon to which it belongs. This consideration may include, but is not limited to, the following:

1. Persistence of the discrete population segment in an ecological setting unusual or unique for the taxon,
2. Evidence that loss of the discrete population segment would result in a significant gap in the range of a taxon,
3. Evidence that the discrete population segment represents the only surviving natural occurrence of a taxon that may be more abundant elsewhere as an introduced population outside its historic range, or
4. Evidence that the discrete population segment differs markedly from other populations of the species in its genetic characteristics.

Because precise circumstances are likely to vary considerably from case to case, it is not possible to describe prospectively all the classes of information that might bear on the biological and ecological importance of a discrete population segment.

Thus, this panel was charged with two very specific tasks, first to "...evaluate if new genetic information indicates that summer-run steelhead warrant designation as a separate DPS." Then, if so, to next "...evaluate the status of summer-run steelhead and any other identified DPSs." These two tasks provide the boundaries within which the panel was directed to operate.

The tasks of this panel are in many ways parallel to those of the 2018 Upper Klamath and Trinity River Chinook Salmon ESU-Configuration Review-Panel (KTC ESU Panel; Anderson et al. 2018), leading to significant overlap in the issues addressed. Thus, we have attempted to provide consistency in terminology and structure between these reports and to avoid duplication of effort. For example, one issue that overlaps with the KTC ESU Panel report is the language used to discuss salmonid life-history phenotypes. Alternative run-timing phenotypes have been called different things in different salmonid species, i.e., spring- vs. fall-run in Chinook salmon, and summer- vs. winter-run in steelhead. However, in many ways these represent the same categories of life-history variants, with spring- and summer-run phenotypes sharing many characteristics, and fall- and winter-run phenotypes likewise similar. Because of this, previous authors have attempted to use a common language to describe them, such as

'stream-maturing' vs. 'ocean-maturing' (Busby et al. 1996; Micheletti et al. 2018), or 'premature' vs. 'mature' phenotypes (Quinn et al. 2016; Prince et al. 2017). Here, we follow the KTC ESU panel report in using these terms only when quoting others or in order to refer collectively to life histories in multiple species. In all other instances we will refer to summer-run and winter-run steelhead ecotypes.

There are also significant biological differences between the Klamath-Trinity Chinook ESU and the NC steelhead DPS, as well as differences between the ESU policy applied to most Pacific salmon, including Chinook, and the joint NMFS/USFWS DPS policy that is applied to *O. mykiss*. The implementation of the joint DPS policy for listing steelhead-only units has a complicated history (Gulesserian 1999; Alagona 2016), highlighting the importance and complexity of the species *O. mykiss*, "...a type of salmonid with characteristics not typically exhibited by Pacific salmon." (NOAA 2006). Although the DPS policy puts emphasis on "marked separation" in defining a DPS rather than on demonstrated reproductive isolation as in the ESU policy, it is clear that the intent of the DPS policy is to designate meaningful biological units. Here we review the application of the DPS policy to the NC steelhead DPS and consider the possibility of listing summer-run steelhead as a separate DPS within this geographic area.

Overview of the current NC steelhead DPS

The NC steelhead DPS extends from Redwood Creek (Humboldt County) in the north, southward to, but not including, the Russian River. Within this region, the Eel River is the largest watershed, with numerous tributaries that contain significant spawning habitat for steelhead. Importantly, the DPS contains populations of both the more widespread winter-run life history and scattered populations with summer-run steelhead, the largest of which is in the Middle Fork of the Eel River. The timing of river entry varies considerably among populations and ecotypes, both across the species range and within California (Busby et al. 1996). For California populations, summer-run steelhead typically enter freshwater in the spring or early summer (~March through June or July); however, these fish do not spawn until the following fall, winter, or spring. In contrast, winter-run steelhead enter freshwater at any time from the late summer through the following spring, and spawn sometime during that same period (Shapovalov and Taft, 1954; Puckett 1975; Busby et al. 1996).

Historically, where summer-run and winter-run steelhead co-occurred in a watershed they have been thought to be partially reproductively isolated, separated by differences in spawn timing and/or spawning habitat. However, the degree of isolation appears to vary greatly among specific watersheds. In southern British Columbia, summer-run steelhead typically spawn in the upper reaches of a basin that are temporally isolated by a migration barrier (Withler 1966). In general, Puget Sound and the Lower Columbia River streams with both summer-run and winter-run steelhead populations had identifiable cascades or waterfalls that provide spatial and temporal windows of separation for summer-run steelhead (Myers et al. 2006, Myers et al. 2015)[1], while in coastal rivers west of the Cascade Range without migrational barriers, only the winter-run life history is present. However, there are a number of coastal basins in western Washington, Oregon and California that support both summer- and winter-run steelhead,

and in many cases, the spawning distributions can overlap in the absence of a discernable migration barrier or spatial separation (e.g., Sauk River, Myers et al. 2006; see also Busby et al. 1994, KMP status review, p. 20).

Extant and historical summer- and winter-run steelhead populations in the Northern California DPS were identified by Bjorkstedt et al. (2005). Within the NC steelhead DPS area, winter-run are widely distributed across the landscape, but summer-run steelhead have very specific habitat requirements for parts of their life history, primarily the need for access to large pools with cool water in which they remain during the summer holding period (Nakamoto 1994; Nielsen et al. 1994). Puckett (1975) identified potential natural migrational barriers in the Middle Fork Eel River and Van Duzen River that provided some degree of separation between summer-run and winter-run steelhead spawning habitat, and recommended against removing migration barriers because it would likely result in increased mixing of the two run types. In the Mad River, a natural barrier apparently separating summer- and winter-run steelhead was identified by Knutson (1975) near Bug Creek. Roelofs (1983) suggested that summer-run spawning habitat is often characterized by limited accessibility, “ruggedness”, and intermittent flow. Thus, a combination of factors influencing river geomorphology and hydrology (e.g., precipitation, stream gradient, geology, etc.) likely limit the distribution of summer-run steelhead, but may be highly variable among years such that complete reproductive isolation is unlikely even in the presence of a strongly flow-dependent migration barrier.

During NMFS’ most recent 5-year status review (NMFS 2016a; Williams et al. 2016), data on summer-run steelhead populations were available for Redwood Creek, Mad River, Van Duzen River, Middle Fork Eel River, and Mattole River. Additional potential populations for which little information was available included Larabee Creek, North Fork Eel River, and South Fork Eel River (Williams et al. 2016). Although both life-history types were likely to have been negatively impacted by the recent drought in California, Williams et al. (2016) concluded that there was “*no strong evidence to indicate conditions for winter-run populations in the DPS have worsened appreciably since the last status review (Williams et al. 2011).*” However, they also noted that “*Summer-run populations continue to be of significant concern. The Middle Fork Eel River population has remained remarkably stable for nearly five decades and is closer to its viability target than any other population in the DPS.... Although the time series is short, the Van Duzen River and Mad River appears to be supporting populations numbering in the low hundreds. However, the Redwood Creek and Mattole River populations appear small, and little is known about other populations including various tributaries of the Eel River (i.e., Larabee Creek, North Fork Eel, and South Fork Eel).*” (Williams et al. 2016). Furthermore, Spence et al. (2008) defined representation and redundancy criteria to specifically account for persistence of major life-history types in assessing ESU (DPS) viability, and considered it “*highly likely that, at a minimum, the representation and redundancy criteria are not being met for summer-run steelhead.*”

Note that, by definition, all steelhead DPS listings exclude *O. mykiss* in habitats that are not currently accessible to anadromous fishes (71 FR 859), despite the close evolutionary relationships between populations above and below barriers to anadromy (Clemento et al. 2009; Pearse et al. 2009, 2014). In the case of the NC steelhead DPS, all *O. mykiss* above Matthews Dam (Ruth Reservoir) on the Mad River and Scott Dam (Lake Pillsbury) on the upper Eel River, as well as above all natural waterfall barriers within

these watersheds are excluded from the DPS boundaries (Clemento 2006; NOAA 2006). Furthermore, the areas above Matthews Dam and Scott Dam are among those that are excluded from critical habitat designation, as were tribal lands in the Spy Rock, NF Eel River, Eden Valley, and Round Valley areas (70 FR 52529). However, while the 2006 listing of the NC steelhead and other DPSs included only populations downstream of natural and man-made barriers, both viability criteria and recovery criteria explicitly considered areas upstream of dams as potential parts of the DPS, as dam removal could reconnect those habitats (NMFS 2016b; Spence et al. 2008).

[1] Populations (Demographically independent populations, DIPS) were identified on the basis of demographic separation and not reproductive isolation (see McElhany et al. 2000).

Summary of Points Raised in the 2018 NC steelhead Petition

The petition submitted by the Friends of the Eel River requests the creation of a Northern California summer-run steelhead DPS separate from winter-run steelhead in this region, and that this new summer-run-only DPS be designated Endangered. The petition states that Northern California summer steelhead “...*plainly meet the criteria to be considered a separate DPS, and the conservation status of that DPS is obviously endangered as the ESA defines the term*”. In support of this, the petition cites Moyle et al. (2017), who characterized NC summer steelhead as discrete from winter steelhead, and as having a high or critical risk of extinction, with a particular emphasis on the increased risks associated with climate change.

The primary new information on which the petition is based comes from a recent paper on the adaptive genomic basis of ecotypic differentiation in steelhead and Chinook salmon (Prince et al. 2017; see detailed description below). The petition suggests that the data presented in Prince et al. (2017) demonstrate that “...*summer steelhead are genetically distinct in profound ways from winter steelhead in the same watersheds*”, and that “...*the key differences between Northern California summer and winter steelhead in run timing, behavior, and physiology can be attributed to genetic differences in the GREBIL gene*”. Based on this, the petition asserts that the existing understanding of the relationships between these ecotypes is flawed, and that they should be designated as separate ‘species’ (DPSs) under the ESA, based on the fact that a single gene of major effect is associated with this life-history variation.

Finally, the petition reviews the relevant aspects of the DPS policy, including the discreteness and significance of the taxon, as well as the criteria under which a listed unit should be characterized as Endangered. The petition concludes by listing a number of actions necessary for the protection of summer steelhead in the NC steelhead DPS.

Summary of Existing and New Information Sources

The petition draws almost exclusively on two sources in describing the rationale for delineating summer- vs winter-run steelhead populations and defining ESU and DPS

boundaries: Moyle et al. (2017) and Prince et al. (2017). However, the study of population genetic relationships among steelhead populations, including those with sympatric summer- and winter-run life-history types, has a long history, and there are a number of published studies that are highly relevant to this issue that were not cited in the petition. Here we review the key results from the existing literature and summarize the current best available information on the relationships between summer and winter life-history types in the NC steelhead DPS.

Neutral population genetic studies

A number of published studies have examined neutral population genetic structure among summer- and winter-run steelhead populations at varying geographic scales. These studies have consistently found that, for presumably neutral genetic loci distributed throughout the genome and thus reflective of the ancestral relationships among populations, genetic variation is distributed among steelhead populations almost entirely based on their geographic proximity. This is consistent with the long-standing concept of isolation by distance, in which genetic differences reflect the balance between migration (gene flow) and drift (random change in small populations) both within and among watersheds and life-history types. We summarize these studies as follows:

Chilcote et al. 1980: Allozyme study of summer and winter steelhead within the Kalama River, WA. Concluded no genetic differentiation between life-history types within the watershed.

Thorgaard 1983: Analyzed chromosomal variability in winter- and summer-run steelhead from the Quinault River in Washington and the Rogue River in Oregon. Chromosome number differed between the two river systems but was similar in summer- and winter-run steelhead within each river system, consistent with ongoing genetic exchange.

Nielsen and Fountain 1999: Analysis of microsatellite data focused on population structure of samples from the Eel River, based on very limited samples of adult and juvenile summer- and winter-run steelhead from the Eel River and Mendocino coast winter-run. Showed that summer- and winter-run steelhead within the Middle Fork Eel River were more closely related to each other than to Mendocino Coast winter-run steelhead.

Clemento 2006: Analysis of microsatellite data focused on population structure of samples from the Eel River, including summer steelhead adults sampled in the Middle Fork, as well as juveniles sampled in the Middle Fork and other Eel River tributaries. Found differentiation followed geography, with Middle Fork Eel summer-run nested within the diversity of winter-run populations, as well as structure among presumptive winter-run samples.

Papa et al. 2007: Analysis of microsatellite data of Klamath River *O. mykiss* samples from returning adults migrating below Klamath-Trinity confluence. Found genetic

differentiation between Early (summer) and Late (winter) returning fish, but this result is confounded by unknown population structure within the samples. (see further discussion of this issue below in Discussion section “*What does monophyly of two genetic groups mean?*”).

Arciniega et al. 2016: Population genetic study of summer- and winter-run steelhead populations from California to Washington based on combined microsatellite and SNP data. Found a clear pattern of parallel evolution of summer steelhead populations at a broad scale, consistent with a model in which pairs of summer- and winter-run populations in a river system are closely related to each other rather than to other populations of the same life-history type in different rivers.

Finally, as noted in the petition, Prince et al. (2017) used genome-wide reduced representation (RADseq) data and found that, with the exception of the genomic region around *Greb1L*, their data were entirely concordant with previous studies showing that summer- and winter-run populations from within a watershed are each others’ closest relatives over the entirety of their genome.

These studies represent the best available data on the relationships among sub-populations of coastal *O. mykiss*, and strongly confirm a model of repeated parallel evolution of multiple summer-run life-history forms through a combination of shared genome-wide ancestry and ongoing gene flow with neighboring local populations (Waples et al. 2004). This conclusion has previously been reached by Busby et al. (1996), who stated that the genetic data available at that time “...do not support the hypothesis that winter- and summer-run steelhead are separate monophyletic units” but that this “...does not mean that there cannot be genetic differences between summer and winter steelhead in any given drainage.” Thus, all existing neutral population genetic data support the current understanding of the evolutionary relationships between the run-timing ecotypes in *O. mykiss*.

Run-timing and the Greb1L genomic region

In considering data related to variation in the *Greb1L* genomic region, it is critical to recognize that this region encompasses approximately 200,000 base pairs of genomic sequence on chromosome Omy28, and contains a great deal of variation in addition to the *Greb1L* gene itself. Furthermore, the distribution of variation within this region has not yet been characterized in detail, and the actual functional variation in *Greb1L* or elsewhere in this region that is associated with run-timing is not known. Thus, all existing data is based on assays that are presumed to be in genetic linkage disequilibrium with some unknown SNP(s) or other polymorphism (e.g., insertion/deletion, duplication, etc.), and these individual assays vary in their informativeness with respect to run-timing. Figure 1 shows the relative positions of SNPs in the *Greb1L* region from published and unpublished sources, including SNP and microhaplotype assays used by the SWFSC, mapped to the best available assembly of the *O. mykiss* genome (Pearse et al. 2018; https://www.ncbi.nlm.nih.gov/assembly/GCF_002163495.1/). In addition, whole genome

resequence data for *O. mykiss* collected from individuals throughout the native range (Gao et al. 2018) provided a resource for detailed information on all variation in the Greb1L region.

Published papers on the genomic basis of run-timing in O. mykiss

Because of the emphasis in the petition on the results of Prince et al. (2017), we quote here the summary of this paper written by the KTC ESU panel in its entirety:

“This study reports a survey of genetic variation between populations of spring- and fall-run Chinook salmon and of summer- and winter-run steelhead (anadromous O. mykiss). The authors used a reduced representation sequencing method called Restriction-site Associated DNA sequencing (RADseq; Andrews et al. 2016) to obtain information from small segments of DNA spread throughout the genome. DNA was sequenced from eight collections of steelhead and 16 collections of Chinook salmon in California and Oregon. In locales where early- (spring and summer) and late-migrating (fall and winter) fish inhabit the same basin, fish were chosen to represent the extremes of the run-timing distributions to be included in different run type groups.

The authors first used all the genomic data to assess genetic relationships between the run types in both species. These results confirmed previous studies, showing that premature-migrating fish are typically more closely related to mature-migrating fish in the same basin or tributary than they are to premature-migrating fish in different basins or tributaries. Subsequently, however, the authors performed genome-wide association study (GWAS) analysis to detect regions of the genome at which specific variation was associated with premature migration.

In steelhead, two different analyses were performed: one on summer- and winter-migrating fish from the Eel River and the other on fish of the two run types in the Umpqua River. Each GWAS found significant associations between some of the same SNPs within the GREB1L region and migration ecotype, and nowhere else in the genome. In Chinook salmon, a single GWAS was performed to compare spring-run and fall-run fish from all the different populations, using river basin as a covariate to account for geographic population structure. This GWAS also found spring migration to be associated only with SNPs within the GREB1L region.

The authors then resequenced about 1500 base pairs of DNA from three fragments of the genome near the associated SNPs in the GREB1L region in many of the steelhead samples. The sequences were used to infer a tree representing the relationship between those sequences using a maximum parsimony criterion. The resulting tree separated the groups of sequences into two different major branches. One branch included sequences from summer-run steelhead and the other included sequences from winter-run steelhead. Resequencing data were not obtained from the Chinook salmon samples, so the authors investigated the allele frequencies at SNPs associated with migration ecotype in Chinook salmon. They

concluded that there was a pattern of allele frequency changes in a consistent direction between paired groups of spring- and fall-migrating ecotypes in a number of different basins.

On the basis of the steelhead resequencing data and the allele-frequency-shift data in Chinook salmon, the authors concluded that an allele carrying a polymorphism causative for premature migration evolved only once in the history of steelhead and once in the history of Chinook salmon, and that this allele was spread via migration to now be shared by the premature-migrating fish in all the river basins they studied.

The authors also undertook a reanalysis of data from steelhead in the Klickitat River (Hess et al. 2016), a study that included samples from throughout the migration period, rather than only during early and late periods. They found that the same region near GREB1L was associated with migration timing, and that fish heterozygous at the migration-associated SNPs migrate at a time that is, on average, intermediate to homozygous fish. On the basis of this observation, they concluded that variation in the GREB1L region is not recessive with respect to run timing, and, as a consequence, heterozygotes, with intermediate migration timing, might be less fit than either homozygous category, and thus will be lost through natural selection.”

(Anderson et al. 2018)

In addition to the paper by Prince et al. (2017), two other papers have been published as of June 2019 using RADseq and/or targeted assay data that examine the distribution and/or association of variation in the Greb1L genomic region with respect to summer- and winter-run steelhead. These papers can be summarized as follows:

Hess et al. 2016: This paper was the first to describe the association between the Greb1L genomic region and run-timing in steelhead, and found that three SNPs in this region explained 46% of trait variation in populations of summer- and winter-run steelhead in the lower Columbia River basin. Furthermore, these three SNPs combined with 15 additional SNPs in other parts of the genome together explained up to 60% of the variation in run-timing.

Micheletti et al. 2018: Used a single SNP (Omy28:11613335; Figure 1) associated with run-timing by Hess et al. (2016) to characterize variation in a geographic survey throughout the interior Columbia River basin. Despite the fact that all interior Columbia River steelhead are considered to be summer-run, they found high polymorphism at this SNP, and this variation was associated with differences in timing of arrival at spawning tributaries, but not with significant differences in reproductive timing. Micheletti et al. (2018) attributed the maintenance of this polymorphism to temporal and spatial balancing selection, with environmental conditions favoring the early-arriving ecotype in some years, and late arrival in others. Micheletti et al. (2018) also used pooled whole genome sequencing to characterize the Greb1L genomic region and found that the strongest association with run-timing was not with Greb1L, but with the intergenic region between that and a neighboring gene, ROCK1.

Overall, the published studies summarized above concordantly show that variation in the Greb1L region is strongly associated with run-timing ecotypic variation in *O. mykiss*. The strength of this association varies across a large genomic region (>200,000 base pairs) that contains at least four genes, including Greb1L and ROCK1, as well as potentially important intergenic variation. The specific genetic changes (i.e., base substitution(s), insertions or deletions, recombination) that are the direct targets of selection within this region are not known. Thus, while we and other authors refer to ‘the Greb1L genomic region’ as if it is a single locus, here represented by SS, SW, and WW genotypes, we recognize that a great deal of additional variation is carried within it that could potentially vary in its adaptive potential to affect life-history variation. It is also important to note that there is very little evidence at the individual, as opposed to population, level of the effects of variation in this region on phenotypic expression in *O. mykiss*.

Finally, two other papers, Thompson et al. (2019) and Narum et al. (2018), have examined Greb1L region variation in Chinook salmon, but not in steelhead. Thompson et al. (2019) was extensively reviewed by Anderson et al. (2018), so we do not consider it further here. Narum et al. (2018) conducted pooled whole genome sequencing and other analyses and concluded that the strongest association with run-timing was not with Greb1L, but with the intergenic region between that and a neighboring gene, ROCK1. The SWFSC has also conducted an extensive analysis of Chinook salmon whole genome shotgun sequence data, with a detailed focus on the genomic structure and phenotypic effect of the Greb1L region (Anderson and Garza 2019). Collectively these studies substantiate the finding that the Greb1L region is strongly associated with the early and late freshwater-entry migratory phenotypes in Chinook salmon, and improve our understanding of the distribution of genetic variation within this region in that species.

New Unpublished Data Available to the Panel

Prior to receiving the petition, the Molecular Ecology and Genetic Analysis team at the Fisheries Ecology Division of the NMFS Southwest Fisheries Science Center had multiple studies underway to investigate the genetic basis of life-history variation in salmonid fishes. Results from these efforts relevant to the present report include two general data types, (1) targeted SNP assays designed around published and unpublished SNPs located in the Greb1L region, genotyped using both SNP genotyping assays and microhaplotype amplicons to estimate alternate allele frequencies in geographically and temporally distributed samples, and (2) whole genome shotgun sequence data collected from individuals throughout the native range of *O. mykiss* (Gao et al. 2018). All of these data were aligned to the *O. mykiss* genome assembly (Pearse et al. 2018), which provides fine-scale resolution of the placement of SNP variation around the Greb1L gene region (Figure 1). See Appendix B for details.

Public Comments

During a 90-day comment period ending June 21, 2019, NMFS received public comments from five organizations and one individual commenter. These public comments are summarized in Appendix A.

Evaluating New Information with Respect to NC steelhead DPS Structure

The panel assessed the possible delineation of a separate Northern California summer steelhead DPS, distinct from the winter steelhead distributed within the same geographic range. This assessment was based directly on the two criteria for delineating a DPS:

Criterion 1: Discrete from Other Con-Specific Populations: *A group of organisms is discrete if it is “markedly separated from other populations of the same taxon as a consequence of physical, physiological, ecological, and behavioral factors” (61 FR 4722).*

Criterion 2: Significant to its Taxon: *Considerations in evaluating the significance of a discrete population segment include: (1) persistence of the discrete population segment in an unusual or unique ecological setting for the taxon; (2) evidence that the loss of the discrete population segment would cause a significant gap in the taxon’s range; (3) evidence that the discrete population segment represents the only surviving natural occurrence of a taxon that may be more abundant elsewhere outside its historical geographic range; or (4) evidence that the discrete population segment has marked genetic differences from other population segments of the species.”*

Considerations for Criterion 1: Discrete from Other Con-Specific Populations

Northern California summer- and winter-run steelhead are only physically distinguishable for a short, albeit important, part of their life-cycle, i.e., during adult freshwater migration following return from the ocean and summer holding in freshwater. No consistent differences have been documented over the rest of their life-history, including during the juvenile rearing, smolting, and sub-adult marine phases. Furthermore, while the redds and juveniles of the summer and winter ecotypes may be somewhat spatially and/or temporally partitioned, the extent of this partitioning is highly variable among specific spawning tributaries as well as among years. The degree of this separation is dependent on changes in geomorphology, rainfall patterns, temperatures, and other climate variables, leading to incomplete and fluctuating separation at all stages of their life-cycle, as well as mating between life-history types when conditions limit their separation (see below). Importantly, the high variability in the natural hydrograph of the Middle Fork Eel River and other coastal rivers that support Northern California summer steelhead is unlike those in the snow melt-driven streams of the interior Columbia or Sacramento Rivers, which may separate early- and late-migrating adults in a more predictable manner. This suggests that there will be a larger amount of variation among years in the degree to which a particular natural flow barrier temporally separates migrating adult steelhead in coastal watersheds.

Consistent with this, the available genetic data on neutral genetic structure among populations within the NC steelhead DPS support the view that, on average, summer- and winter-run populations are no more or less divergent from each other than are populations

within each ecotype separated by similar geographic distances. Further, while summer-run steelhead may be spatially segregated during the summer freshwater holding period, they at least occasionally interbreed with, and thus are most closely related to, the winter-run steelhead in the same stream, tributary, or watershed over a range of spatial scales. This conclusion is consistent with numerous studies on population genetic structure in *O. mykiss* and other salmonid species that display similar ecotypic variation.

Do the new data on the association between the Greb1L genomic region and run-timing in *O. mykiss* change our understanding of the discreteness of summer and winter steelhead? If variation in the Greb1L-region was to be used to separate summer- and winter-run steelhead into two DPSs, it would first be important to understand the distribution of individuals present in this geographic area representing different genotypic categories under consideration. Data collected by the SWFSC clearly show that many *O. mykiss* collections in California contain individuals with all three Greb1L-region genotypes present at a given place and time (i.e., SS, SW, and WW; Figures 2-4). Furthermore, Greb1L-region variation is distributed broadly among populations, including the widespread occurrence of heterozygotes and the presence of both ‘summer’ and ‘winter’ Greb1L variants in many populations without documented current expression of the summer-run steelhead ecotype (e.g., above-barrier populations and polymorphic winter-run steelhead populations; Figure 2). This demonstrates that this haplotypic variation is not uniquely partitioned into summer-run and winter-run steelhead ‘populations’, but is broadly distributed across a range of populations with variable phenotypes. This conclusion is further supported within the NC steelhead DPS by analyses provided as a public comment to the petition review panel, showing the distribution of both neutral genetic data and Greb1L-region variants throughout the Eel River system (S. Kannry, public comment). Notably, Prince et al. (2017) did not observe this because the populations included in that study were chosen to represent only the most divergent examples of these life-history types, including the summer-run samples from the Middle Fork Eel and winter-run from the upper mainstem Eel (Van Arsdale Fisheries Station), rather than to encompass the diverse distribution of habitats used by *O. mykiss*. Populations with less clearly defined summer- or winter-run phenotypes were intentionally excluded from Prince et al. (2017) because they represented intermediate phenotypes (e.g., “fall-run” steelhead in the South Fork of the Trinity River; Figures 2 and 3b). As a result, the data presented by Prince et al. (2017) are not informative with respect to questions involving the temporal or geographic distribution of genetic variation in the Greb1L region, the relative frequency, dominance, or relative fitness of Greb1L-region genotypes in different populations, or the extent of gene flow between these ecotypes.

Beyond these population-level data, the observation of nearly complete overlap in the return timing at Van Arsdale of individuals with heterozygous and winter-run Greb1L genotypes, as well as some SS individuals apparently migrating during the typical winter-run migration period (e.g., Figures 3c and 4), indicates that matings between parents with alternate Greb1L-region genotypes (e.g., SWxWW, SWxSW, SSxWW) must occur, resulting in full-sibling families with a mix of Greb1L genotypes. In fact, some such situations were directly observed in the parentage data from the upper Eel River evaluated by the SWFSC (Appendix B). Thus, designation of separate summer- (i.e., SS) and winter-run (WW) DPSs would both ignore the contribution of SW heterozygous

individuals to these populations and potentially create situations in which full-siblings of heterozygous matings would be divided into different ‘species’ under the ESA. This is clearly an untenable situation biologically.

While our understanding of the specific genomic basis of these run-timing phenotypes is improved by the data presented in Prince et al. (2017), these new genetic data do not substantially change our understanding of the biology of summer- and winter-run ecotypes, as these phenotypes had been recognized as ‘proxies’ for the underlying genetic variation for decades (Dizon et al. 1992; Waples 2006). We knew that there was a genetic basis for these traits long before we could say exactly what that basis was (Clemente 2006; Pearse 2016). In addition, it is likely both that additional polygenic variation contributes to these phenotypes (Abadia-Cardoso et al. 2013), and that different parts of the genome contain adaptive genomic variation associated with other, unknown, traits important to local adaptation within the NC steelhead DPS. Thus, despite the finding that variation in the *Greb1L* region is strongly associated with the summer- and winter-run phenotypes in steelhead, our understanding of the evolutionary dynamics of this and other ecotypic variation is not fundamentally altered by this knowledge. Taken together, the available data on neutral and adaptive genetic variation continue to support a model in which summer-run steelhead phenotypes evolve from standing genetic variation segregating among locally polymorphic populations dominated by winter-run phenotypes, where and when the ecological conditions capable of supporting the summer-run life history exist (Arciniega et al. 2016).

Overall, while summer- and winter-run steelhead are nominally recognizable as distinct life-history types, they occupy dynamic and partially overlapping habitats incompletely separated by waterfalls, dams, or other barriers to migration. It is also clear that there is variable but active and ongoing gene flow between these life-history types over ecological and evolutionary timescales. The lack of complete physical or political barriers separating summer- and winter-run within the range of the NC steelhead DPS and the fact that they are indistinguishable for much of their life-cycle further suggest that they cannot be managed separately, just as all juvenile *O. mykiss* below barriers to anadromy are de facto considered to be steelhead due to their “similarity of appearance” (Hey et al. 2005; NMFS 2006). Thus, splitting these interrelated groups would create a similar situation to the one that was rejected by the *Alsea* decision (*Alsea Valley Alliance v. Evans*, No. 99-6265-HO, Sept. 10, 2001), which ruled against splitting below the ‘species’ level under the ESA. This interpretation is also consistent with an earlier review of a petition to list summer steelhead in Deer Creek, Washington, that concluded that they should not be considered a separate species under the ESA (59 FR 59981).

Considerations for Criterion 2: Significant to its Taxon

The success of the species *O. mykiss* both in its native range and globally is due at least in part to the resilience it gets from being able to express a diverse array of life-history strategies. These strategies can include adult steelhead run-timing variation and other variation such as juvenile migratory behavior (Hayes et al. 2011; Moore et al. 2014), adult age-at-return, within-season variance in spawn timing (Abadia-Cardoso et al. 2013), and the half-pounder life history (Roelofs 1983; Hayes et al. 2016), as well as a diversity

of non-anadromous life histories (freshwater adfluvial and resident life histories; Hayes et al. 2011). This diversity allows different individuals in the species to maximize their fitness by taking advantage of the habitat conditions present in a particular place and time. Given the importance of inter-annual variation in this geographic area and its effect on the ability of streams in the NC steelhead DPS geographic range to support salmonids (Power et al. 2015), this diversity clearly adds resilience to the NC steelhead DPS and supports its continued survival. Life-history variants that do best in one year may not have the highest fitness in a different year, but collectively they can maintain a robust population size and high genetic diversity (i.e., the portfolio effect: Schindler et al 2015; Moore et al. 2014; Brennan et al. 2019). Thus it is critical not to underestimate the contribution of the many diverse life-history forms to the resilience of *O. mykiss*.

With respect to the significance of the summer-steelhead ecotype to the Northern California steelhead DPS, this ecotypic diversity is already recognized by its explicit inclusion in the recovery and viability documents developed for salmon and steelhead in this area (Spence et al. 2008; NMFS 2016b; Williams et al. 2016). The recovery plans were based on viability criteria, which in turn were based on the viable salmonid population (VSP) concept (McElhany et al. 2000). The VSP concept recognizes that life-history diversity is (1) a key parameter and (2) hierarchical in nature (from populations on up to species). These summer-run populations have been explicitly identified with viability criteria based on their shorter-term demographic independence and the need to maintain the appropriate building blocks for recovery (i.e., population units capable of persisting in relative isolation of other units). Having summer-run populations as substrata within diversity strata (and essential for viability) provides the umbrella under which longer-term evolutionary processes are maintained. However, it is also important to keep in mind that all of the other life histories described above in the species *O. mykiss* are likely to be of equal if not greater significance to the resilience of the species as the variation in adult migration timing associated with the Greb1L region. Thus, there is no clear basis for deciding that adult migratory timing variation should be prioritized more highly than the other, similarly important and diverse characteristics of this highly variable species, or that separating any of these life histories into separate management units would provide a benefit given their interdependent and dynamic relationships.

DISCUSSION

The panel concluded that summer- and winter-run steelhead should remain together in a single Northern California steelhead DPS representing both ecotypes. The available data indicated that summer-run steelhead cannot be listed as a separate DPS from winter-run steelhead, as the two groups maintain an ongoing and interconnected genetic legacy[2]. However, retention of both ecotypes in a single DPS does not indicate a lack of recognition that the summer-run steelhead life history is an important component of the DPS or suggest that measures should not be taken to protect and improve habitat, including access to upstream habitats through dam removals, fish passage programs, reduced water diversions, etc. Rather, it is an acknowledgment that the diverse life histories of *O. mykiss* are fundamental parts of the listed unit as a whole and cannot be separated from each other. They need to be protected together. As noted above, this is

explicitly addressed in the NMFS status reviews and recovery plans through recognition of the need to focus protection on and consider populations of both of these ecotypes in assessing recovery status (NMFS 2016a,b; Spence et al. 2008). Below we review some of the key factors that the panel considered in reaching these conclusions.

[2] Waples defined the evolutionary legacy as the “genetic variability that is the product of past evolutionary events and that represents the reservoir upon which future evolutionary potential [of the species] depends.” (see Waples 2006)

--The importance of considering evolutionary timescales.

In evaluating the foreseeable future[3] for the summer- and winter-run ecotypes, it is important to consider an appropriately long evolutionary timescale. This is because these ecotypes have evolved and continue to interact in ways that reflect their long evolutionary history, so understanding how both neutral and adaptive genetic variation is distributed among them requires consideration of this timescale. For example, there is evidence that a landslide dammed the Eel River downstream of the Middle Fork confluence for a period of time approximately 22k years ago (Mackey et al. 2011). If true, this event would put an upper bound on the time frame over which all population genetic structure and local divergence among populations upstream of this, including the evolution of the extant population of summer steelhead in the Middle Fork Eel River, must have occurred. Similarly, distinctive summer- and winter-run life histories evolved in Puget Sound, Washington, rivers on a time scale of less than 15k years (Crandell 1965). More importantly, it highlights the fact that, over evolutionary timescales, it is inevitable and expected that some local populations of steelhead will decline or go extinct, while others increase and new populations are established through colonization in natural or improved and restored habitats. Thus, the individuals in extant populations represent the descendants of the mix of colonizers that re-established breeding populations when the habitat recovered from the flood, landslide, drought, dam, fire, or glacier that extinguished it in the first place, as well as the ongoing migrants that continue to move between basins, tributaries, and populations. To the extent that a colonized habitat favors one life history over another, the distribution of genomic variation associated with these traits will change (evolve), reflecting the relative reproductive success of individuals expressing alternative life-history ecotypes associated with it. This process of natural selection is of fundamental importance to the interconnected network of populations in the NC steelhead DPS.

Looking forward in evolutionary time, it is not only likely that one or more populations within a metapopulation will experience an extinction-recolonization event at some point, it is nearly certain. This dynamic extinction-recolonization pattern among local populations within the larger metapopulation is an important part of both their history and foreseeable future, and will result in exactly the patterns of genetic relationship we see among neutral and adaptive genetic variants, i.e., concordant

geographic and genetic relationships, with adaptive variants at increased frequencies in habitats favorable to the phenotypes they influence, assuming sufficient standing genetic variation exists to maintain a diversity of life-history forms within the overall metapopulation.

The flip side of extinction and recolonization is the development of local adaptation, both at the watershed and local population levels, which is known to be critical to salmonid populations. For example, Mobley et al. (2019) demonstrated a 3-9 fold difference in reproductive success between local and migrant Atlantic salmon within a watershed. This difference occurred over a similar spatial scale as that of the NC steelhead DPS, highlighting the importance of fine-scale genetic structure and local adaptation even in the absence of any detectable phenotypic differences among populations. Thus, both spatial and temporal scales are important to consider in defining the boundaries of any distinct population segment in salmonids, and the distribution of adaptive genetic variation associated with life-history ecotypes is not independent of the evolutionary factors that affect the rest of the genome (Pearse 2016).

[3] In providing guidance for review of petitions under the ESA, NMFS (2017) defines the term “foreseeable future” as describing: *“the extent to which the Secretary can, in making determinations about the future conservation status of the species, reasonably rely on predictions about the future (Department of the Interior Solicitor’s Memorandum M-37021, “The Meaning of ‘Foreseeable Future’ in Section 3(20) of the Endangered Species Act”(Jan. 16, 2009)). Those predictions can be in the form of extrapolation of population or threat trends, qualitative analysis of how threats will affect the status of the species, or assessment of future events that will have a significant new impact on the species. The biologist (or Team) should consider the life history of the species, habitat characteristics, availability of data, kinds of threats, ability to predict threats and their impacts, and the reliability of models used to forecast threats over that “foreseeable future” in determining the time period that constitutes the foreseeable future.”*

--What does monophyly of two genetic groups mean?

Unlike the consideration of ESU designation undertaken by the recent Upper Klamath-Trinity Chinook salmon petition panel (Anderson et al. 2018), there is no criterion of monophyly required in defining a DPS. Nonetheless, the question remains of how could one consider all NC summer-steelhead to be ‘distinct’ from all winter-run fish given that they co-occur in multiple basins throughout the DPS boundaries, and are clearly interconnected and integral to each others’ evolutionary persistence? Fundamental to this discussion is the difference between monophyly of a single gene sequence, which derives from its mutational history at the level of individual DNA base pairs, and the monophyletic grouping of populations based on their overall shared genetic ancestry. As discussed by Anderson et al. (2018), the apparent monophyly of the early/premature *haplotypes* found in the Greb1L region within steelhead supports the hypothesis that the extant genetic alleles have evolved only once in this species, but not that the *phenotype* of early/summer-steelhead emerged only once. In fact, it is clear from available genome-wide data that summer-run steelhead phenotypes have arisen multiple times (Arciniega et

al. 2016). This parallel adaptation from standing genetic variation is similar to the change in frequency of the Omy05 inversion haplotypes seen in isolated resident populations above barriers to anadromy (Pearse et al. 2014; Leitwein et al. 2017). While such processes result in populations that have experienced similar selective environments sharing a high frequency of the same adaptive allele, this does not make these populations monophyletic with respect to the rest of the genome, over which ancestry is shared with other geographically-proximate populations..

Directly related to the above, the current petition to create a separate summer-run steelhead DPS and list it as Endangered quotes this passage from Moyle et al. 2017: “*Genetic analyses support two discrete, separate monophyletic units of migrating populations based primarily on timing of freshwater entry and resulting maturation (Papa et al. 2007), correlating with run timing for the ocean-maturing (winter) and stream-maturing (summer, fall) ecotypes (Prince et al. 2015 [sic]). (Moyle 2017, pp. 270-71)*”. However, as is clear from the above discussion, this statement incorrectly interprets the meaning of ‘monophyletic units’, which cannot be inferred in this context from the samples and data examined in the Papa et al. (2007) study, as they were collected as mixed temporal samples from migrating fish, without regard for the different tributary populations to which they were returning. More importantly, SWFSC analysis of the samples from the Papa et al. (2007) study using two SNP loci located in the Greb1L region that are strongly associated with the summer- and winter-run phenotypes clearly show a continuum of allele frequencies during the migratory season in which adult steelhead are entering the river, with overlap between individuals carrying summer- and winter-run variants (Figure 3a). Such a scenario is likely mirrored in the Eel River, in which putative winter steelhead are known to migrate from September through April or May, and so overlap with summer-run individuals that typically migrate between March and June (Clemento 2006; Busby et al. 1996).

As genomic data continue to provide increasingly fine-scale resolution of the taxonomic relationships among lineages, individuals, and specific genes regions, our view of the importance of variation in historical and ongoing gene flow among semi-isolated species, subspecies, populations, and life-history ecotypes is changing, confounding simple dichotomous views such as monophyly and requiring a more nuanced understanding of biodiversity (Coates et al. 2018; NAS 2019). Similarly, the detailed genomic data now available to us is changing our view of the adaptive genomic basis of biodiversity (see next section). However, such genomic data should be interpreted conservatively to avoid misguided efforts to protect specific genetic variants that have been identified as ‘important’, at the expense of protecting the evolutionary framework in which they developed in the first place.

--Potential Challenges of Designating a DPS on the Basis of Single Genomic Regions

There is an active and ongoing discussion in the scientific literature about the potential for the incorporation of adaptive genomic variation into conservation unit delineation (e.g., Stapley et al. 2010; Allendorf et al. 2010; Funk et al. 2012; Pearse 2016; Coates et al. 2018; Kardos & Shaffer 2018; Waples and Lindley 2018). These papers provided important background that the panel considered in discussing the distribution of variation in the Greb1L region in summer- and winter-run steelhead. In general, this work suggests

a conservative approach to delineating conservation units based on adaptive genomic variation, and highlight concerns about over-emphasizing the importance of a single genomic region relative to the rest of the genome for identifying units for conservation. In part, this is because there will always be uncertainty in our understanding of the evolutionary and ecological significance of any adaptive trait under consideration. Moreover, in addition to specific genetic changes (mutations), there is an increasing awareness of the importance of polygenic variation, as well as changes in patterns of gene expression and potential epigenetic effects (Baerwald et al. 2015), all of which can influence phenotypes. In light of this complexity, the finding of an association with a single region on Omy28 described by Prince et al. (2017) represents just the first step in understanding the significance of the heritable underpinnings of this trait, and is far from providing a complete understanding of the effects of this genetic region on life-history variation in steelhead (Waples and Lindley 2018).

Another important consideration is the potential for environmental dependence of the effects of adaptive genetic variants on phenotype. This may be particularly important in salmonid species like *O. mykiss* that inhabit a large latitudinal range and experience a broad range of environmental variables such as temperature. For example, Pearse et al. (2018) investigated the effects of a large inversion on chromosome Omy05 on expression of resident rainbow trout and anadromous steelhead life histories and found that, while variation in this region is strongly associated with residency and anadromy in California populations, in other parts of the species range this association breaks down, likely due to an interaction with environmentally dependent effects of temperature. This interaction apparently drives a strong latitudinal cline in the frequencies of alternate alleles in the Omy05 inversion. Given that the specific relationship between variation in the Greb1L region and steelhead phenotypes in diverse habitats is unknown (e.g., above waterfalls and dams, across a large geographic range), it would not be appropriate to direct conservation actions based primarily on variation in this genomic region.

For the NC steelhead DPS in particular, it is notable that while the *O. mykiss* isolated above barrier waterfalls in two tributaries to the Middle Fork Eel share genome-wide ancestry with Middle Fork Eel summer steelhead (Clemento 2006), they are fixed for both the ‘resident’ haplotype of the Omy05 inversion (Pearse et al. 2014) and the summer-run associated variant in the Greb1L region (Figure 2). This paradoxical result clearly illustrates the inherent conflicts that will arise from attempts to define population units based on single genes: listing all NC summer-run steelhead populations as a single, separate unit, connected by their shared phenotypic and single-gene characteristics, but disconnected both geographically and in their ancestry and adaptation over the rest of the genome, amounts to conflating an allele with a population (see discussion in Anderson et al. 2018). Given the disconnect already observed in *O. mykiss* between these two adaptive genomic variants, Omy05 and Greb1L, an important question that arises is “What would happen if/when yet another significant genomic variant is discovered that explains additional life-history, or other adaptive, variation in the species?” Would that then need to be incorporated as an additional layer of management, and which variant would take precedence? It is clear that this type of single-gene approach to conservation could quickly become very complicated and intractable. Such a hypothetical scenario is not unlikely, as numerous studies are being published that identify associations between particular genes or genomic regions and traits important to conservation and management

(Matala et al. 2014; Barson et al. 2015; Barrio et al. 2016; Veale and Russello 2017; Parker et al. 2019), suggesting that this issue will be a challenge going forward for conservation decisions in many taxa. Moreover, while some such studies have identified single genes of major effect, most highlight the potential for complex genetic architectures of important biological traits and the extreme difficulties that could be encountered in trying to manage such genetic loci on an individual basis (Pearse 2016). Thus, while in principle there could be situations in which variation at individual adaptive loci might be managed in a supplemental manner within the ESU/DPS framework (Funk et al 2012), such an approach should not change the criteria for delineation of the ESU or DPS boundaries themselves. These conservation units are intended to capture the full range of intra-specific diversity and adaptive potential of the interconnected set of populations within its boundaries. This approach to management is consistent with recognition of the dynamic interconnectedness of populations as well as the web-of-life (WOL) conservation model (vonHoldt et al. 2018), rather than supporting the further subdivision of management units within species.

Conclusions and Recommendations

The panel arrived at a final conclusion regarding DPS configuration using a voting method often called the FEMAT method. As noted by the BRT evaluating the 2011 petition,

“This approach, described in Good et al. (2005), has been used in all status review updates for anadromous Pacific salmonids since 1999 and allows for BRT members to express any uncertainty they may have concerning ESU structure” (Williams et al. 2013, p. 11).

Each of the four panel members was given 10 votes to apportion between the two ESU configurations: 1) summer-run and winter-run steelhead should remain together in a single Northern California steelhead DPS, or 2) summer-run and winter-run steelhead in Northern California should be separated into two distinct DPSs according to run-time phenotype.

FEMAT vote, June 26, 2019:

- 1) 38
- 2) 2

Thus, the panel came to a strong consensus that the NC steelhead DPS should remain as a single inclusive unit, protecting both summer- and winter-run steelhead within its boundaries. The data presented in the petition did not fundamentally change our understanding of the importance of preserving the evolutionary processes that connect populations, allowing them to maintain genetic diversity at both neutral and adaptive loci and preserve their evolutionary potential.

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Appendix A: Summary of Public Comments

Organization/ Commenter	Summary of Comments
L. Arguello, National Park Service	<ul style="list-style-type: none"> ● Provide data from Redwood Creek snorkel surveys 1981-2017; In 20 years from 1999-2018, average 10 ss, range 1-22.
N. Gayeski, Wild Fish Conservancy and The Conservation Angler	<ul style="list-style-type: none"> ● Provide excerpt from comments on Draft Puget Sound Steelhead Recovery Plan. ● Provide published papers and reports on summer steelhead populations in Western Washington, including information on Deer Creek summer steelhead.
S. Greacean, Friends of the Eel River	<ul style="list-style-type: none"> ● Offer additional data on the status of summer steelhead within the NC steelhead DPS. ● Suggest management actions.
S. Kannry, UC Davis graduate student (individual)	<ul style="list-style-type: none"> ● Presented genetic analysis of Eel River summer steelhead within the Middle Fork and Van Duzen, including neutral, Greb1L, and Omy05 variation.
K. Lackey, Humboldt Redwood Company	<ul style="list-style-type: none"> ● Emphasized the forest management practices that HRC undertakes to protect steelhead habitat. ● Offer support for further monitoring of the status of summer steelhead on HRC landholdings.
E. Schilling, National Council for Air and Stream Improvement, Inc.	<ul style="list-style-type: none"> ● Emphasize that existing forest management practices already protect steelhead habitat.

Appendix B: NOAA Unpublished Greb1L genetic data analysis

New and unpublished data available to the panel review included two general data types:

- 1) Targeted SNP assays designed around published (Prince et al. 2017) and unpublished SNPs located in the Greb1L region and used to estimate alternate allele frequencies in geographically and temporally distributed samples.
- 2) An improved assembly of the *O. mykiss* genome (Pearse et al. 2018) and whole genome shotgun sequence data collected from 18 individuals throughout the native range of *O. mykiss* (Gao et al. 2018), as well as more than 100 additional individual whole genomes sequenced by the SWFSC and colleagues (Ben Koop).

Targeted SNP assay data

The SWFSC SNP assay data analysis used both Fluidigm and microhaplotype amplicons to target 7 and 9 SNPs in the Greb1L region, respectively (Figure 1). Among these, the analysis presented here focused on two SNPs that overlap between these data sources within the Greb1L region (Omy28: 11667915 and 11667954). These are the two SNPs that most strongly associated with run-timing in the Eel River in Prince et al. (2017), as well as in analyses conducted by the SWFSC, and one of these SNPs was also used in the analysis provided as a public comment by S. Kannry. Thus these loci provide a common basis for comparison among studies. This does not imply that these SNPs represent the causative polymorphisms responsible for this phenotypic variation or that other variation in the region is unimportant, or that. However, based on the best available information at this time, these tightly-linked SNPs provide the clearest single markers of the adaptive haplotypic variation in this region. In all analyses, we show these genotypes as summer homozygote (SS, yellow), heterozygote (SW, brown/dark orange), and winter homozygote (WW, purple).

The targeted assay data can be divided into three distinct data sets:

- 1) Geographically distributed population samples (Table 1) genotyped using SNP assays identified in Figure 1. Results in Figure 2.
- 2) Temporally-sampled individuals genotyped using SNP assays to provide information on the relative run-timings of alternative Greb1L genotypes (SS, SW, WW). Note that these samples were collected at different stages of migration, and in different rivers, so are not directly comparable. Results in Figure 3a,b,c.
 - a. Papa et al. (2007) samples collected from in-river fisheries in the lower Klamath River.
 - b. Samples collected at Willow Creek Weir on the Trinity River.

- c. Van Arsdale steelhead samples haphazardly selected from among collections in 2003-2005, and 2013.
- 3) A large dataset of samples collected from adult steelhead passing Van Arsdale fisheries station; 2,898 adults sampled returning to the upper mainstem of the Eel River in 2009-2017 and genotyped at microhaplotype loci in the Greb1L region targeting the same two informative SNPs on mhap8 that show near-complete linkage in this dataset (Figure 4). Key results:
- a. The distributions of WW and SW Greb1L genotypes overlap completely (Figure 4).
 - b. There are 40 SS homozygotes in this dataset, of which 35 arrived at Van Arsdale in early August 2011 (18) or late September 2013 (17). The other five returned in February or March of 2013, 2014, or 2015, overlapping with the distribution of SW heterozygote and WW homozygote return-timing.
 - c. Parentage analysis shows that these loci segregate as expected in identified offspring of SW heterozygote x WW homozygote matings (12 heterozygotes produced 23 offspring that returned in subsequent years, with Greb1L genotypes consistent with mendelian inheritance).

Specimen collection and DNA extraction

For the large dataset of steelhead ascending the VAFS fishway, fish were sampled from 2009 to 2016 (2,898 specimens). Small pieces of fin tissue were taken, placed on blotting paper and stored in coin envelopes. Tissue was extracted with the DNeasy Blood and tissue kit and a BioRobot 3000 following the manufacturer's specifications (Qiagen, Inc.).

Genotyping and parentage analysis

The Van Arsdale steelhead samples were genotyped with 96 SNPs (Aguilar & Garza, 2007; Abadia-Cardoso et al., 2011) using the Fluidigm system. These SNPs have been shown to be suitable to reconstruct parent-offspring trios (Abadia-Cardoso et al., 2013). Parentage analysis using SNPPIT (Anderson 2012) allowed for the reconstruction of parent-offspring trios for this time period. These and other steelhead sampled were also genotyped at two informative SNPs loci using microhaplotype amplification assays (Omy28: 11667915 and 11667954, Figure 1) using the GTseq method (Campbell et al., 2015) and sequencing on a Miseq (Illumina). Sequences were mapped to a reference and variants were called using FreeBayes (Garrison & Marth, 2012).

We identified that steelhead with both homozygous genotypes ascended the VAFS fishway, as well as individuals that were heterozygous. The vast majority of individuals were WW winter-run homozygotes that arrived between December and March (N=1,726). However, thirty-five SS summer-run steelhead were observed ascending the fishway during the summer-run migration period in two years (18 in early August 2011 and 17 in late September 2013). In addition, five SS homozygous steelhead were observed ascending the fish way in February across multiple years (Figure 4). A total of 71 SW heterozygote fish were observed in the dataset, all ascending the fishway

during the winter-run migratory period (Figure 4). Twelve of these individuals were identified as parents of 23 offspring returning to VAFS in subsequent years. All heterozygotes mated with winter run partners and produced offspring that were heterozygous at both SNPs or homozygous for the winter run alleles.

Whole genome sequence data

An improved assembly of the *O. mykiss* genome was released in June 2017 (Pearse et al. 2018; https://www.ncbi.nlm.nih.gov/assembly/GCF_002163495.1/). In addition, whole genome shotgun resequence data collected from 18 individuals throughout the native range of *O. mykiss* (Gao et al. 2018), as well as more than 100 additional individual whole genomes sequenced by the SWFSC and colleagues (Ben Koop) were aligned to the improved genome assembly. This provided accurate placement of the SNP loci used in published and unpublished studies of the Greb1L region (Figure 1). While a complete analysis of the whole genome sequence data is beyond the scope of this report, visual evaluation of these data provided insight into linkage among individual SNPs in this region and the strength of the association between this genetic variation and the summer- and winter-run ecotypes.

Table 1: Geographically distributed population samples included in Figure 2.

Basin	Pop_ID	Tributary	Life Stage	Mig Type	Years	Months	N
Columbia River	ColWash	Washougal River	A	S	2008	*-----	23
Columbia River	ColWiley	Willamette River	J	U	1997	-----*---	23
Columbia River	ColStill	Sandy River	J	U	1998	-----*---	24
Columbia River	ColLShee	Snake River	A	S	2011	--***-----	47
Columbia River	ColSSanti	Willamette River	A	W	2009	---*-----	47
Klamath River	KlaPapa	Klamath River	A	S,W	2000-02	***_*****_*	186
Klamath River	TriSF	Trinity River	A	W	1993-94	-----***	47
Klamath River	TriWCW	Trinity River	A	S,W	1991-95	-----***_	94
Klamath River	TriNew	Trinity River	A	S	1994	---****---	47
Eel River	EelBB	Middle Fork Eel	J	R	2002	----*-----	24
Eel River	EelCutf	Middle Fork Eel	J	R	2002	----*-----	23
Eel River	EelWill	Middle Fork Eel	J	R	2002	----*-----	24
Eel River	MFE	Middle Fork Eel	A	S	1998-04	---****---	95
Eel River	VanArs	Eel River	A	W	2003-13	****-----*	92
Sacramento River	COLM	Battle Creek	A	W	2014	*-----	24
Sacramento River	FRHM	Feather River	A	W	2013	-----*	24
Sacramento River	AmNFI	American River	J	R	2014	-----*---	23
Sacramento River	AmRR	American River	J	R	2014	-----*---	23
Sacramento River	NIMM	American River	A	W	2013	-----*	23
Sacramento River	TuoClav	Tuolumne River	J	R	2015	----**-----	47
Sacramento River	MKHM	Mokelumne River	A	W	2013-14	*-----*	23
San Lorenzo River	SanLorenzo	San Lorenzo River	A	W	2003	-**-----	23
Salinas River	TassaJa	Arroyo Seco Creek	J	R	2003	-----*--	18
Salinas River	TassaJe	Santa Margarita Ck	J	R	2003	-----*--	29
Salinas River	Nacimiento	Nacimiento River	J	R	2003	-----*---	24
Santa Ynez River	SYSantaCruz	Santa Cruz Creek	J	R	2004	----**-----	24
Santa Ynez River	SYNFJuncal	North Fork Juncal	J	R	2003	-----*--	23
Santa Clara River	SCLionCan	Sespe Creek	J	R	2003	-----*--	24
Santa Clara River	SCFrenchmen	Piru Creek	J	R	2003	-----*--	23
Santa Clara River	SCGoldHill	Piru Creek	J	R	2003	-----*--	23

Figure 1

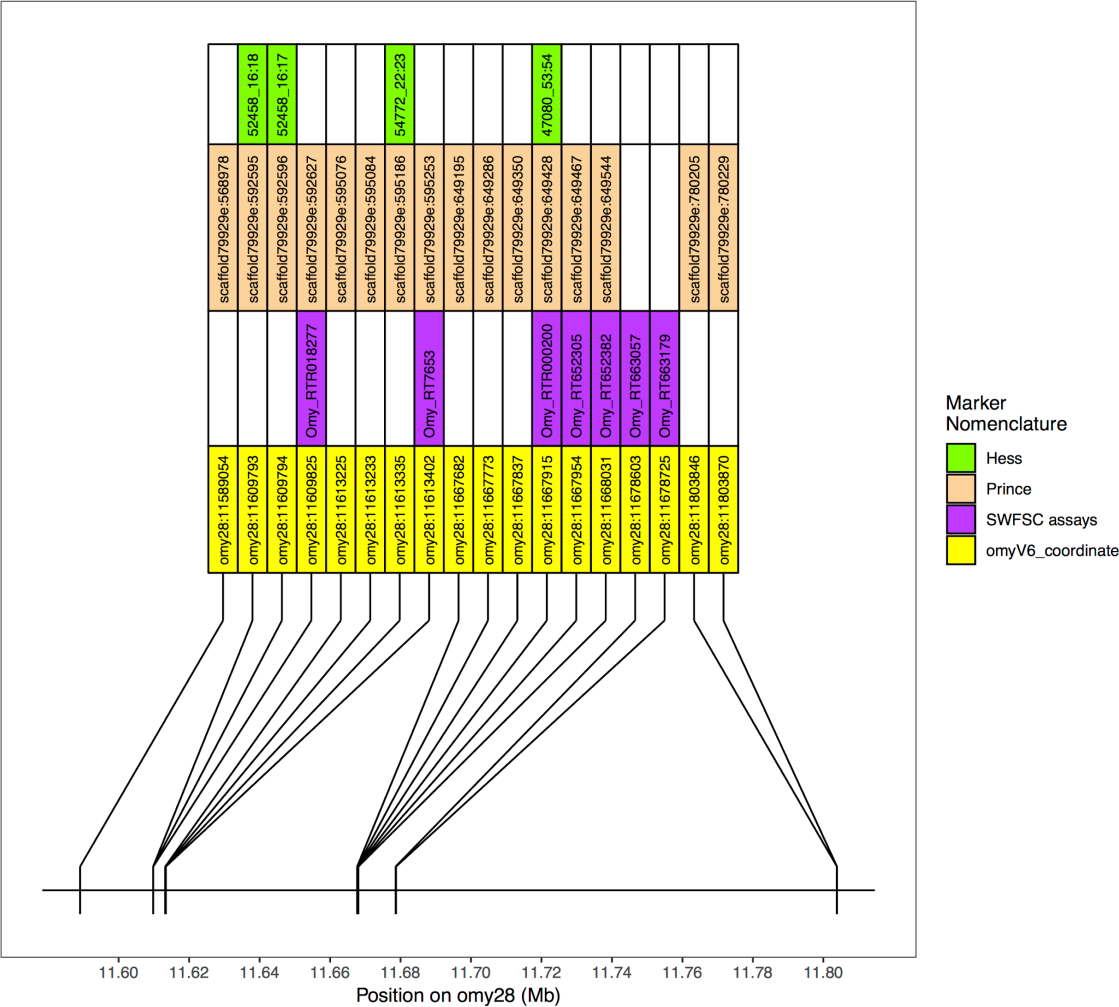
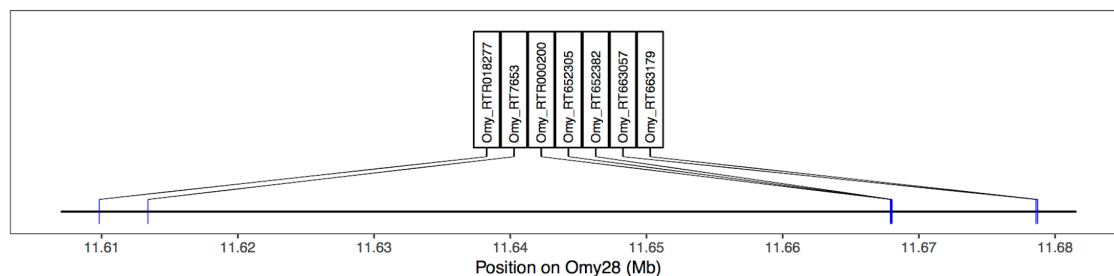


Figure 1: Single nucleotide polymorphisms (SNPs) used in published and unpublished datasets that were considered by the panel and their locations within the Greb1L region on chromosome Omy28 in the *O. mykiss* genome.

Figure 2

(a)



(b)

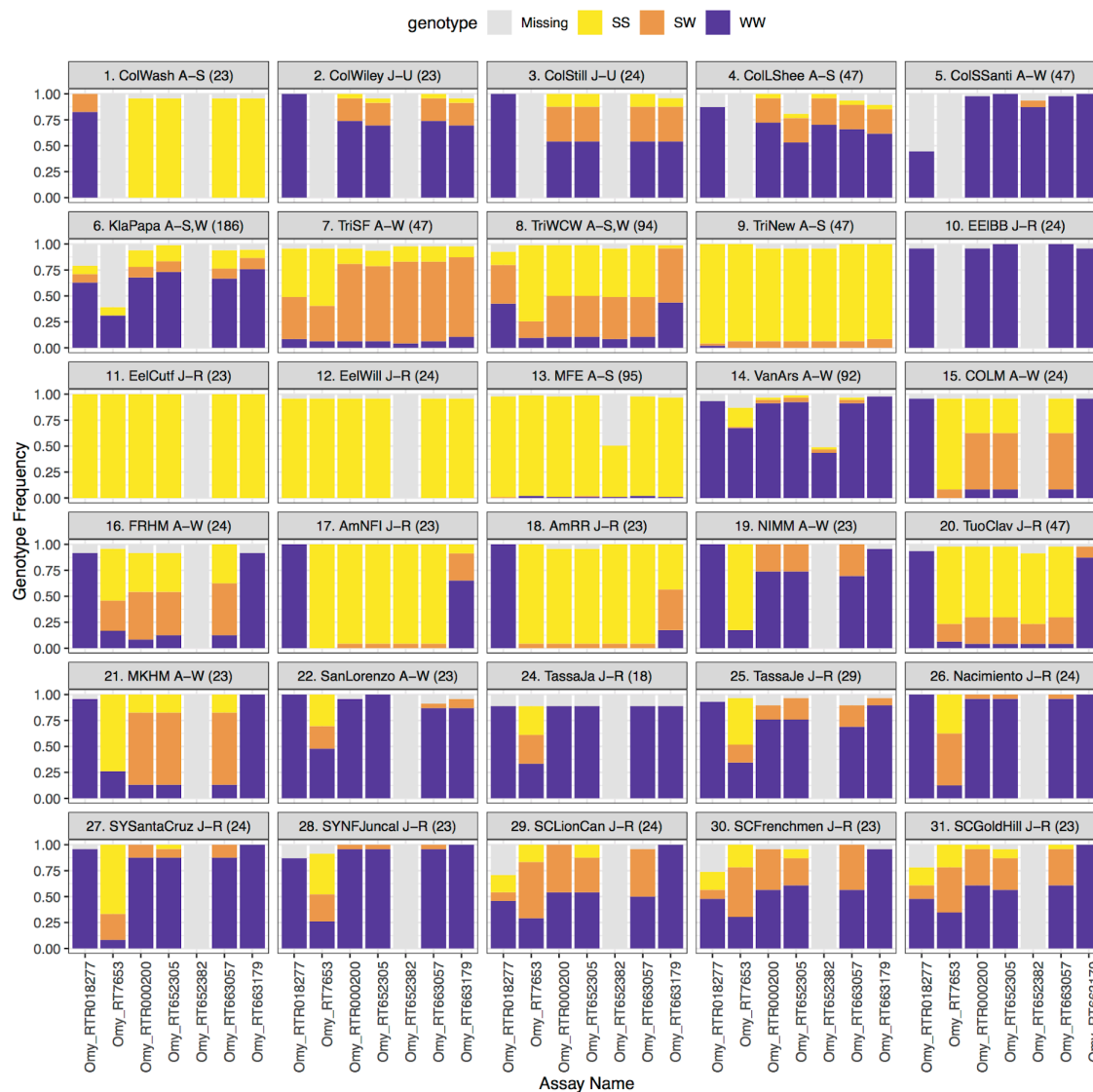


Figure 2: **a)** Alignment of the seven SNPs in the Greb1L region targeted using Fluidigm assays (see Figure 1). These SNPs are distributed across >200,000 base pairs of DNA sequence, and vary in their association with the summer- and winter-run ecotypes. **b)** Frequencies of summer-run homozygote (yellow), heterozygote (orange), and winter-run homozygote (purple) genotypes in 31 populations of *O. mykiss* in California and in Columbia River tributaries. For each plot, information is given as follows: Population number, name (Pop_ID in Table 1), life stage (Adult, Juvenile, or Unknown), life-history ecotype (Summer, Winter, Unknown, or above-barrier Resident rainbow trout), and sample size (N).

Figure 3

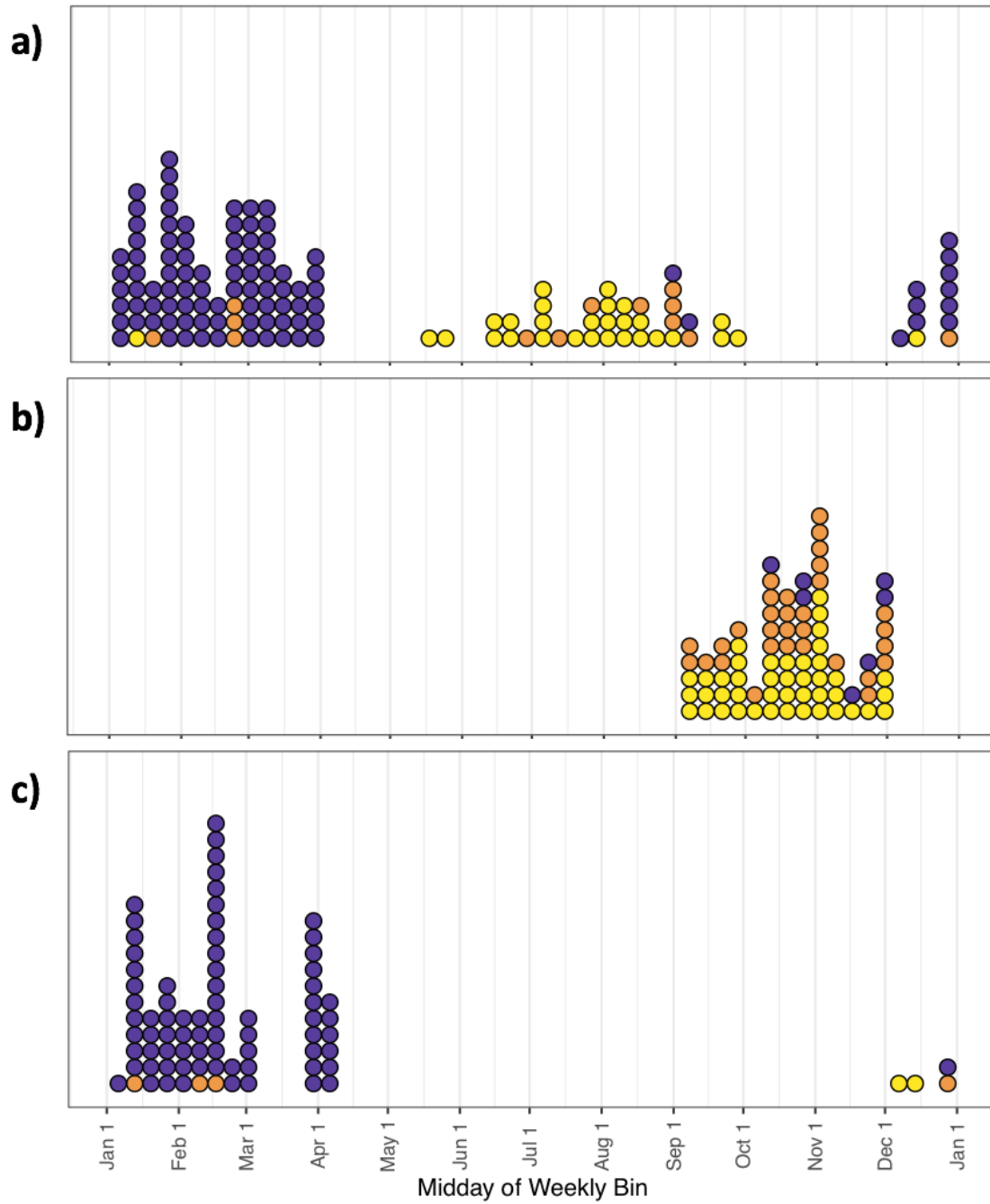


Figure 3: Plots showing number of *Greb1L*-region summer-run homozygote (SS, yellow), heterozygote (SW, orange), and winter-run homozygote (WW, purple) individuals among **a)** samples used in Papa et al. (2007), **b)** samples collected at Willow Creek Weir on the Trinity River, and **c)** samples collected at Van Arsdale Fisheries Station.

Figure 4

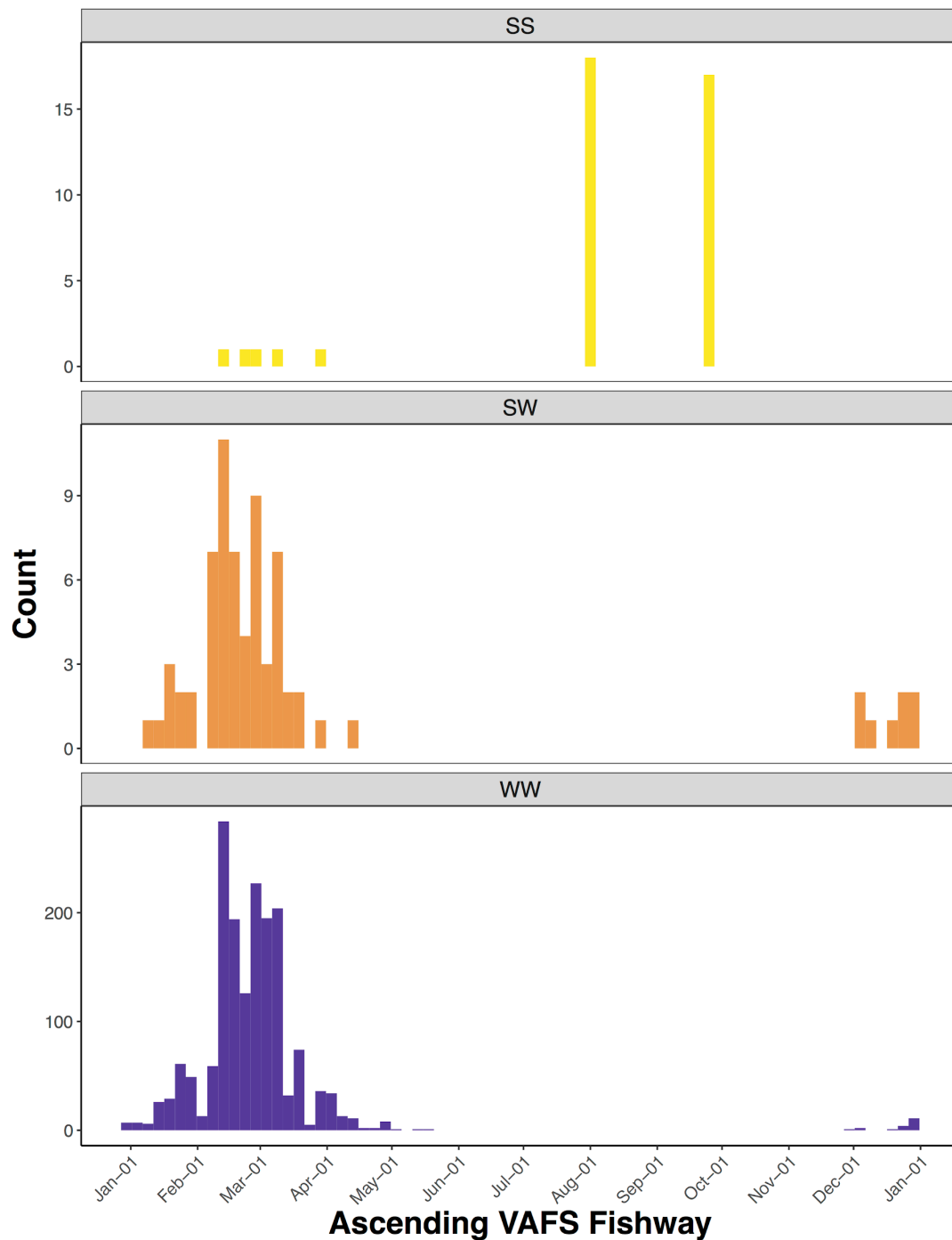


Figure 4: Plots showing the distribution of dates that a total of 1,837 steelhead (*O. mykiss* individuals >300mm for which date information was available), including 40 summer-run homozygote [yellow], 71 heterozygote [orange], and 1,726 winter-run homozygotes [purple], ascended the Van Arsdale Fisheries Station fishway between 2009 and 2016.