Report on a review of the Oregon coast and Southern Oregon Northern California Coastal spring Chinook salmon ESU configuration

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Introduction

On September 24, 2019, the National Marine Fisheries Service (NMFS) received a petition (OCC petition) from the Native Fish Society, Center for Biological Diversity, and Umpqua Watersheds to identify an Oregon Coast (OC) spring-run Chinook salmon Evolutionarily Significant Unit (ESU) and list that ESU as threatened or endangered under the Endangered Species Act (ESA). On April 13, 2020, NMFS announced a "90-day" finding on the petition, determining that the petitioned action may be warranted (85 FR 20476). On May 4, 2020, NMFS received a petition from Mr. Richard K. Nawa of Selma, Oregon, to evaluate Southern Oregon and Northern California Coastal (SONCC) spring-run Chinook salmon for listing under the ESA (SONCC petition). On May 1, 2020, the NMFS West Coast Region (WCR) requested that the Northwest Fisheries Science Center (NWFSC) conduct an analysis and review of the OCC petition's claim that Oregon Coast spring-run Chinook salmon should be considered a separate ESU, and, if so, provide a description of the demographic risks to any new ESU described as a result of that evaluation. To conduct this evaluation, the NWFSC set up a review panel consisting of NWFSC and Southwest Fisheries Science Center (SWFSC) experts. On May 14th, 2020, the WCR requested that the review panel concurrently evaluate the SONCC petition's request that SONCC spring-run Chinook salmon should be considered a separate ESU. On March 16, 2021, NMFS announced a 90-day finding on the SONCC petition, finding the action may be warranted (86 FR 14407).

This report addresses only the first part of the WCR's request; that is, to evaluate whether OC and/or SONCC spring-run Chinook salmon should be considered ESUs. To make this evaluation, the panel compiled the best available scientific and commercial information, including consideration of information received in response to both 90-day findings.

Background information

NMFS ESU policy

The ESA allows listing of species, subspecies, and distinct population segments (DPS) of vertebrates. The ESA as amended in 1978, however, provides no specific guidance for determining what constitutes a DPS. Waples (1991) developed the concept of an ESU for identifying DPS of Pacific salmon. This concept was adopted by NMFS in applying the ESA to anadromous salmon species (ESU Policy, NMFS 1991). The NMFS ESU policy stipulates that a salmon population or group of populations is considered a DPS if it represents an ESU of the biological species. An ESU is defined as a population or group of populations that 1) is substantially reproductively isolated from conspecific populations, and 2) represents an important component in the evolutionary legacy of the species.

Information that can be useful in determining the degree of reproductive isolation[1] includes incidence of straying, rates of dispersal, degree of genetic differentiation, and the existence of barriers to migration. Insight into evolutionary significance or discreteness can be provided by data on genetic and life-history characteristics, habitat differences, and the effects of stock transfers or supplementation efforts on historical patterns of diversity (Waples 1991).

The majority of the ESUs for Pacific salmon were initially defined in the late 1990s as part of the coast-wide status review process undertaken by NMFS. In the intervening decades, the most marked change in population information has arguably been in the analysis of additional genetic variation. The majority of the genetic information available to the original status reviews in the 1990s was developed using starch-gel electrophoresis of allozymes, which typically involved surveying variation at <50 loci, with typically 2-3 alleles each. Increasingly in the early 2000s, the use of DNA microsatellite and single-nucleotide polymorphisms (SNPs) provided a wealth of additional genetic information. More recently, genomic methods, which survey variation to varying extents throughout the entire genome, have increased the amount of genetic information available by several orders of magnitude (thousands to millions of loci). Thus, the quantity and type of genetic information available to address the issue of ESU and DPS delineation has changed considerably since the time of the original ESA listings.

Description of the currently identified OC and SONCC ESUs

In the 1990s, NMFS undertook a series of coast-wide status reviews of Pacific salmon. These involved both identifying ESUs of salmon spawning in west coast (California to Washington) rivers and evaluating their ESA risk status (endangered, threatened, or not at risk). Myers et al. (1998) originally described two ESUs that included Chinook salmon spawning in Oregon coastal streams: an Oregon Coast ESU containing coastal populations of spring- and fall-run Chinook salmon from the Elk River to the mouth of the Columbia River, and a Southern Oregon and

[1] Note that the ESU policy was developed and applied to salmon populations of the same species that are physiologically capable of interbreeding. The term reproductive isolation refers to restricted gene flow for any reason, including, for example, geographic isolation or temporal differences in spawn timing.

California Coastal ESU containing all spring- and fall-run Chinook salmon spawning in coastal rivers from Cape Blanco south of the Elk River to the southern extent of the species range (Figure 1). Based on additional genetic information, the Southern Oregon and Coastal California ESU was divided into two separate ESUs, the SONCC ESU and a California Coastal ESU (NMFS 1999). The SONCC ESU included Chinook salmon spawning in rivers from Euchre Creek to the Lower Klamath River. The OC ESU and the SONCC ESU were determined to not be at risk of extinction either at the time of the review or in the foreseeable future and have not been listed under the ESA (Myers et al. 1998; NMFS 1999).

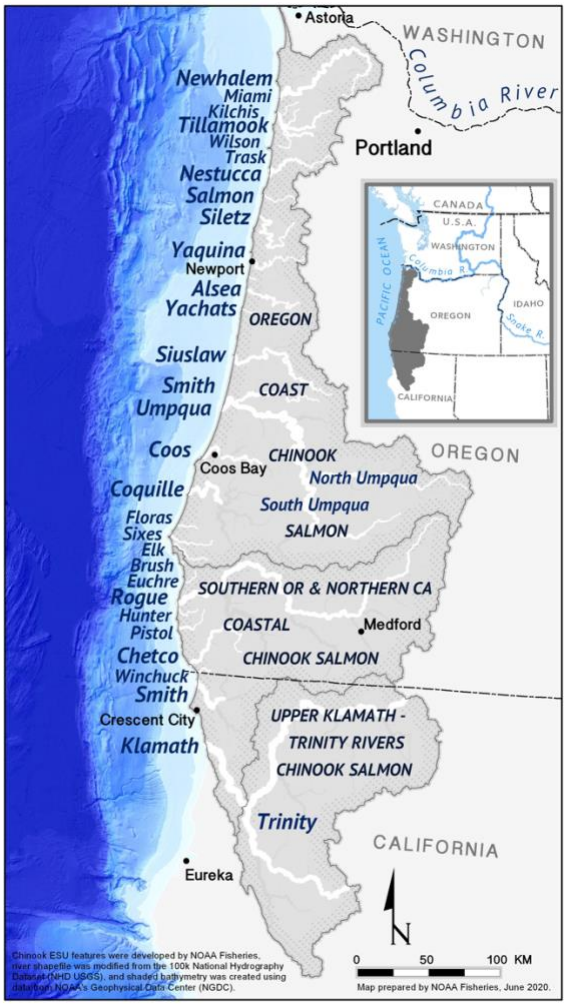


Figure 1 – Map of OC and SONCC Rivers

Consideration of adult migration (run) timing in the coastwide Chinook salmon status reviews

Adult migration (run) timing, along with multiple other life-history characteristics, was considered as an important factor in evaluating both prongs of the ESU policy (Waples 1991; Myers 1998; Waples et al. 2004). For coastal Chinook salmon ESUs[2] differences in run timing alone were not considered to be indicative of either substantial reproductive isolation or a significant component of the evolutionary legacy of the species, and all six coastal Chinook salmon ESUs contain populations that exhibit a range of adult run timing. There were three primary reasons the status reviews reached that conclusion. First, in some areas (Washington, Oregon, California coasts) the review noted that the relatively small size of many rivers limited the amount of spawning habitat, likely minimizing the spatial separation of fish with different run times (Myers et al. 1998, p. 55). The review did note that some rivers (the Rogue, Umpqua, and Chehalis rivers and multiple rivers in the Puget Sound and Lower Columbia River areas) were larger and did contain separate spring- and fall-run populations. Second, the review found that although coastal populations exhibited variation in adult run timing, this variation generally did not correspond to differences in other life-history traits. In particular, spring- and fall-run populations were characterized by similar patterns of ocean distribution, age structure, spawn time, and age at smolting (Myers et al. 1998). Finally, in coastal rivers, patterns of genetic variation were much more associated with geography than with run timing, such that spring-run populations were more genetically similar to nearby fall-run populations than to other spring-run populations spawning in geographically separated rivers (Myers et al. 1998; Waples et al. 2004).

In contrast, Chinook salmon ESUs in the Interior Columbia, Snake, and Sacramento rivers are largely concordant with differences in adult run timing, and these ESUs are also characterized by concordant differences in multiple life-history traits and patterns of genetic variation. For example, Upper Columbia River spring-run Chinook salmon spawning in the Wenatchee, Entiat, and Methow rivers are all genetically much more similar to each other than to the summer/fall runs in the same rivers, and are also characterized by marked differences in age at smolting and ocean distribution patterns (Myers et al. 1998; Waples et al. 2004). Consequently, the Upper Columbia spring-run and summer/fall-run Chinook salmon were determined to be different ESUs (Myers et al. 1998).

Summary of information considered

In this section we briefly summarize the primary information considered in this review. With regard to the ESU configuration question, the petitions focus on recently available genetic

[2] California Coastal, SONCC, OC, Lower Columbia River, Washington Coast, Puget Sound

information, so we also focus our review on genetic data that have become available over the last two decades since the original status reviews. For added context, we also briefly summarize the public comments received and several of the key documents cited in the 1998 status review.

A note on terminology: various studies and documents have employed a variety of terms associated with the seasonality of adult salmon migration from the ocean to freshwater. Chinook salmon returning early in the season are variously referred to as spring-run, summer-run, early-run, and premature migrating, whereas fish returning later are referred to as fall-run, late-run, or mature migrating. To complicate matters further, run timing designations in steelhead use overlapping terms, such as summer- and winter-run. In this report, we use the terms spring-run and fall-run to refer to fish returning early versus late in the adult migration season. Unless explicitly noted in the text, we consider these terms to be synonymous with terms premature/mature and early/late or other similar terms that are used in some of the studies cited and reviewed by both this report and the petitions.

Summary of OC spring-run Chinook salmon petition (Native Fish Society et al. 2019)

The petition summarizes the NMFS ESU policy, including a discussion of its use of genetic data. The petition then summarizes and discusses four recently published studies related to the genetic basis of run timing (Davis *et al.* 2017; Prince *et al.* 2017; Narum *et al.* 2018; Thompson *et al.* 2019a). Based on these studies, the petition notes that run timing in both Chinook salmon and steelhead is strongly associated with variation in or near the GREB1L genomic region and that spring-run alleles appear to have arisen as a result of a single evolutionary event in each species. The petition notes that the Prince et al. (2017) study found that variation throughout the genome was consistent with the current NMFS ESU designations, but that all coastal spring-run Chinook salmon studied by Prince et al. (2017) clustered together in a single group based on variation at the GREB1L region, separate from the fall-run Chinook salmon samples, and that Narum et al. (2018) found a similar pattern for spring- and fall-run Chinook salmon in the Columbia River Basin. The petition notes that the Davis et al. (2017) study found that spring- and fall-run Chinook salmon in the Siletz River on the Oregon coast were genetically and phenotypically distinct and spawned in different parts of the watershed.

The petition also summarizes the 1998 NMFS status review that included Oregon Coast Chinook (Myers et al. 1998), and concludes that the new information (not available at the time of the earlier status review) indicates that Oregon Coast spring-run Chinook salmon should be considered a distinct ESU. In particular, the petition concludes that the new studies indicate spring-run Chinook salmon are distinct and that fall-run Chinook salmon would be unable to re-establish spring-run populations or traits should the spring-run fish be extirpated. The petition concludes that

> "…the genotypic basis for premature migration meets at least two criteria of importance in ESU determination: 1) It confers a unique element of diversity to the species as a

whole by way of gaining access to specialized habitats, and increasing species-level diversity of migration times and other life history factors; 2) it reinforces its own distinct evolutionary lineage, because access to special habitats results in the effective natural reproductive isolation of a substantial fraction of spring-run from the fall-run Chinook that co-occur in the same river systems. The genomic capacity for premature migration, and the dispersal into headwater habitats that it supports, also enhance the ecological diversity of Chinook salmon. For example these expand the time and locations at which salmon are available to predators, as well as to freshwater fisheries, and the timing and locations of subsidy of marine-derived nutrients to inland ecosystems." (p. 10).

The petition also notes that the Oregon Department of Fish and Wildlife (ODFW) considered spring- and fall-run Chinook salmon on the Oregon Coast to be in two different Species Management Units.

After concluding that spring-run Chinook salmon on the Oregon coast should be considered to be an ESU separate from fall-run Chinook in the same area, the petition summarizes a study by Thompson et al. (2019a) that evaluated changes in adult run timing and variation at the GREB1L region in Chinook salmon in the Rogue River. The petition cites results of that study that indicate that the spring-run-associated GREB1L variant is not maintained at high frequencies in fall-run populations and the current habitat conditions have resulted in a loss of spring-run variation. The petition also cites results that conclude that the spring-run life-history is important to the species as a whole by allowing access to habitat not accessible to fall-run fish, and the threats to the spring-run therefore represent a threat to the species as a whole. The remainder of the petition summarizes demographic and environmental threats to the spring-run populations.

Summary of SONCC petition (Nawa 2020)

The petition summarizes the NMFS ESU policy and the history of status reviews of SONCC Chinook (Myers et al. 1998, NMFS 1999). The petition concludes that new information (the same four studies summarized by the OC petition) suggests that spring-run SONCC Chinook salmon are a separate ESU from the fall-run fish. The petition then summarizes these new studies. The description of these studies and their conclusions is essentially the same (using nearly identical wording) as the OC petition, and we therefore do not repeat it here.

Summary of public comments

NMFS received comments on the OC petition from 21 individuals or organizations. Most comments either expressed support or opposition to a potential ESA listing, in some cases citing existing scientific literature or agency reports. Several comments included contemporary or historical data related to spring-Chinook salmon existence, abundance, or habitat requirements. One issue raised by several commentators was the possible influence of hatchery releases of spring-run fish on the occurrence of natural spring-run fish in several rivers.

None of the comments contained genetic data or citations to genetic data not already cited by the petitions or otherwise available to the review team.

NMFS received comments on the SONCC petition from 11 individuals or organizations. None of the comments contained genetic data or citations to genetic data not already cited by the petitions or otherwise available to the review team.

Summary of prior status reviews of OC and SONCC Chinook

Myers et al. (1998) – coastwide Chinook salmon status review report

This review reported spring populations of OC Chinook salmon in the Trask, Nestucca, Siletz, Alsea, Umpqua, and Rogue rivers (Figure 1), based on an extensive ODFW report (Nicholas & Hankin 1988). The report also cited another ODFW report (Kostow 1995) identifying 11 spring, 1 summer, and 33 fall-run populations on the Oregon coast. The Myers et al. report cited spring-run populations in several SONCC rivers, including the Rogue, Klamath and possibly the Smith (see citations in report). The report noted that life-history information for Chinook salmon in smaller rivers in the SONCC region is "extremely limited" (p. 119). The report cited an earlier genetic study that noted that only 0.9% of genetic variation in Chinook salmon was due to run timing differences (Utter et al. 1989). The report also summarized new genetic data (based on 31 allozyme loci) analyzed for the status review. These new data included samples from numerous OC and SONCC populations, but only two collections from spring-run fish from OC and one from the SONCC (Table 1). The review team unanimously concluded the OC Chinook salmon ESU was not at risk of extinction nor likely to become so in the foreseeable future. The team unanimously concluded that the then-defined Southern Oregon and California Coastal ESU was likely to become at risk of extinction in the foreseeable future (p. 247). The team was particularly concerned about the status of spring-run populations. Subsequently, the originally defined Southern Oregon and Coastal California ESU was split into separate Coastal California and SONCC ESUs (NMFS 1999).

The status report described a history of hatchery programs for OC Chinook salmon that may have influenced patterns of genetic variation in multiple watersheds. Spring- and fall-run Chinook salmon from multiple sources, including the SONCC, the lower Columbia River, the Willamette River, and the Snake River and its tributaries, were released into most major OC watersheds over a period of multiple decades from the early 1900s until the late 1980s (Appendix D in Myers et al. 1998). Releases in SONCC watersheds, in contrast, were primarily from sources derived from within the ESU (Appendix D in Myers et al. 1998).

Table 1 -- Summary of genetic studies. Samples identified as spring-run (SP) are in bold; fall-run (FA) are in plain text.

Study	OC samples	SONCC samples	Type and number of loci	Comments

Myers *et al*. 1998	Euchre Creek FA n=57; Elk R FA n=400; Sixes R FA n=268; Coquille R FA n=100; Bandon H FA n=59; Millicoma R FA n=100; Morgan Cr H FA n=100; Noble Cr H FA n=100; **Rock Cr H SP n=300 (1981, 1985, 1995)**; Rock Cr H FA n=100; Siuslaw R FA n=160; Alsea R FA n=181; Fall Cr H FA n=300; Trask H FA n=300; **Nehalem R SU n=53 (1996)**	Rowdy Cr H FA n=112; Smith R FA n=99; Winchuck R FA n=170; Chetco R FA n=343; Pistol R FA n=200; Hunter Cr FA n=100; **Cole R H SP n=263 (1981, 1985, 1995)**; Applegate R FA n=181; Rogue R FA n=100	31 allozyme loci	
(Waples et al. 2004)	**Rock Cr H SP n=300**; **Trask R SP n=300 (1981, 1985, 1995)**; Elk R FA n=100; Sixes R FA n=268; Coquille R FA n=100; Smith R FA n=80; Alsea R FA n=181; Trask R FA n= 33; **Nehalem F/SU n=53**	Winchuck R FA n=170; Chetco R FA n=343; **Cole R H SP n=263;** Applegate R FA n=181; Rogue R FA n=100	32 allozyme loci	Same spring-run samples Myers et al. 1998 with addition of Trask R Spring
(Beacham et al. 2006a; Beacham et al. 2006b)	**Trask H SP n=48 (1997)**; Trask H FA n=100; Umpqua R FA n=93; Elk R FA n=69; **Nehalem R SU n=53 (1996);** Siuslaw R FA n=37	**Cole R H SP n=50 (1995)**; Hunters Cr FA n=100; Winchuck R FA n=80; Lobster Cr FA n=48; Pistol R FA n=100; Euchre Cr FA n=57	13 microsatellite loci	Some samples likely the same as earlier studies
(Seeb et al. 2007)	Coqille FA; Siuslaw FA, **Umpqua SP (2004)**; Alsea FA; Nehalem FA; Siletz FA	Chetco FA; Applegate FA; **Cole R H SP** (2004)	13 microsatellite loci	Umpqua SP is from Rock Creek hatchery (Paul Moran, pers. comm.)
(Narum et al. 2008)	Nestucca H FA n=88; **Umpqua H SP n=95**; Elk H FA n=93	**Cole R H SP n=91**	13 microsatellite loci, 37 SNP loci	Same samples as Seeb et al. 2007
(Moran et al. 2013)	Necanicum H FA n=77; Nehalem R SU/FA n=151; Wilson R FA n=139; Kilchis R FA n=58; Trask R FA n=162; Nestucca H n=130; Salmon R FA n=102; Siletz R FA n=165; Yaquina R FA n=136; Alsa R FA n=168; Siuslaw R FA n=159; Coos R FA n=50; S Umpqua H FA n=134; Coquille R FA n=141; Sixes R FA n=124; Elk R H FA n=141	**Cole R H SP n=142**; Applegate Cr n=143; Chetco R FA n=137	13 microsatellite loci	Same samples as Seeb et al. 2007
(Clemento et al. 2014)	Coquille R n=47; **Umpqua R SP n=137**; Siuslaw R n=93; Nestucca H n=48; Alsea R n=131; Nehalem R n=93; Siletz R n=93;	Smith R n=159; Chetco R n=94; **Cole R H SP n=141**; Applegate Cr n=92	96 SNPs	Same samples as Seeb et al. 2007
(Hecht et al. 2015)	Nestucca R FA n=43; **Rock Cr SP n=48 (2010)**	**Cole R H sp n=46 (2010)**	19,703 SNPs	
(Davis et al. 2017)	Siletz R FA n=565; **Siletz R sp n=258 (2011, 2012)** (n = 94 FA and 94 SP for SNPs)		21 microsatellites and 96 SNPs	
(Prince et al. 2017)	**N Umpqua SP n=24 (2013)**; Siletz R FA n=4; **Siletz R SP n=4 (2014)**; S Umpqua FA n=24; **S Umpqua SP n=8 (2009, 2012)**	Rogue R FA n=16; **Rogue R SP n=16 (2014);**	215,354 SNPs (posterior prob >.8 in >50% inds)	
(Thompson *et al.* 2019a)	none	**Rogue R FA and SP n=460 (2014)**	2 run time associated SNPs in the GREB1L region	

(Anderson & Garza 2018)	**Siletz R SP n=89 (2011, 2012)**; Siletz R FA n=65	none	9 run time associated SNPs in the GREB1L region	Same sample as Davis et al. 2017
(O'Malley et al. 2020a)	none	**Rogue R SP and FA n=162 (2019)**	2 run time associated SNPs in the GREB1L region	
(O'Malley et al. 2020b)	none	**Rogue R FA and SP n=445,485,485 (2016-2018). 1575 Cole Rivers Hatchery spring in 2018.**	2 run time associated SNPs in the GREB1L region and 298 SNPs	

Updated Biological Review Team report (NMFS 1999)

This was an updated status review report that included additional SONCC genetic samples. Based on the analyses described in the report, NMFS divided the original (1998) Southern Oregon - California Coast ESU into two separate ESUs: SONCC and California Coast. The SONCC ESA was evaluated to be at low risk of extinction, while the California Coast ESU was determined to be likely to be at risk of extinction in the foreseeable future.

Summary of genetic studies

Studies focused on patterns of 'neutral' variation that is not functionally associated with run timing

Waples et al. (2004) – This paper analyzes largely the same genetic data as Myers et al. (1998). It includes two OC spring Chinook salmon populations – Rock Creek (Umpqua spring-run) and Trask (Tillamook) spring-run. OC and SONCC samples each formed well-supported genetic clusters, although the Rock Creek sample clustered with the SONCC samples, similar to what Myers et al. (1998) had observed previously. One of the main conclusions of this paper was that in coastal Chinook salmon ESUs, run timing does not correspond to distinct evolutionary lineages. Instead, these data supported a pattern in which spring- and fall-run Chinook salmon spawning in the same rivers were genetically more similar to each other than to spawners of either run-type in more geographically distant rivers (Figure 2).

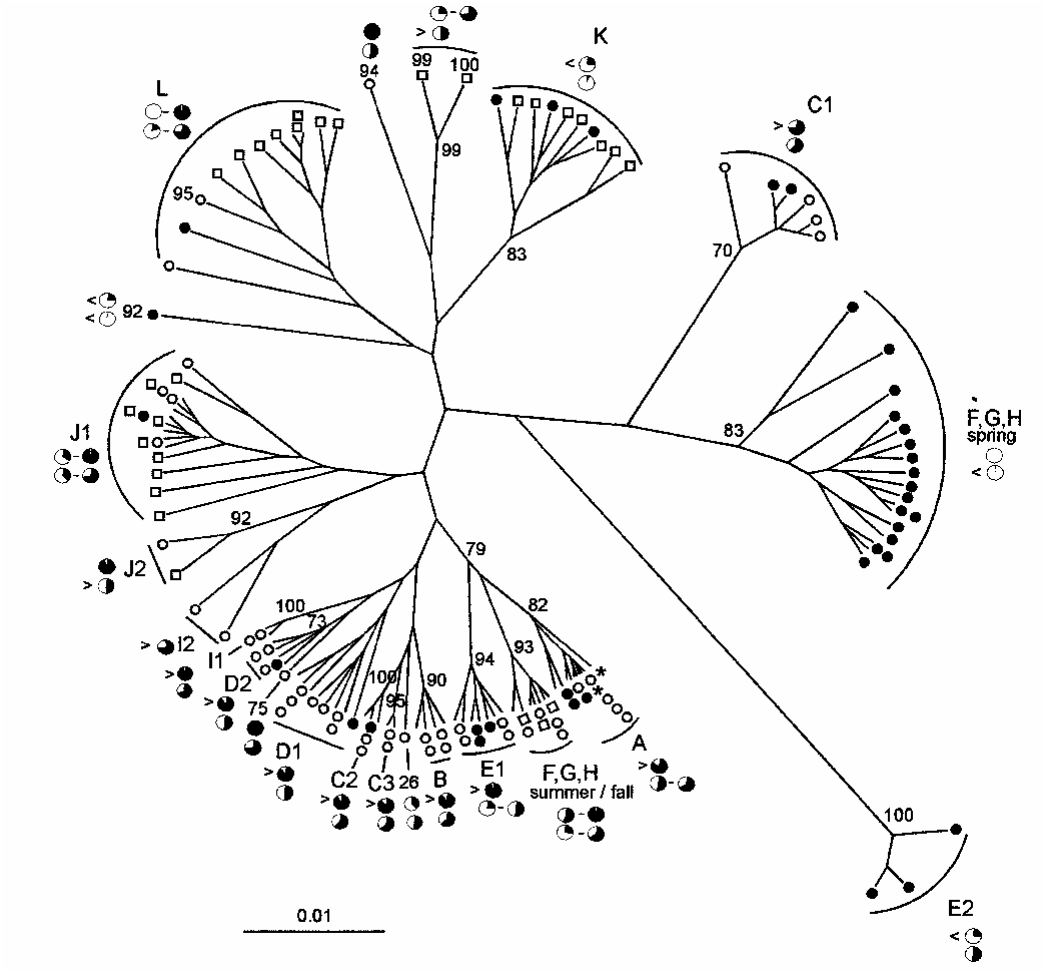


Figure 2 -- UPGMA phenogram of genetic distances (Cavalli-Sforza and Edwards 1967) among 118 Chinook salmon populations. Population symbols indicate adult run timing: closed circle, spring; open square, summer; open circle, fall; asterisk, winter. Pie diagrams show the range of other life-history trait values (upper: percent subyearling smolts; lower: marine harvest rate). Numbers at branch points indicate bootstrap support >70%. Strong bootstrap support also exists for nodes within some labeled clusters but is not shown. A = California Central Valley; B = Northern California coast; C = Klamath Mountain Province (C2 and C3 are SONCC); D1 = OC; D2 = Washington coast; E1 = Lower Columbia River; E2 = Upper Willamette River; F,G,H = Interior Columbia and Snake Rivers; I = Olympic Peninsula and W. Vancouver Island; J = Georgia Basin; K = Interior Fraser; L = Central British Columbia. Reproduced from Waples et al. (2004).

Beacham et al. (2006)

This study reports on a large coastwide microsatellite dataset for 325 Chinook salmon population samples. It includes 7 OC populations, including one spring-run (Trask Hatchery) and a Nehalem summer-run population (Table 1). Similar to Waples et al. (2004), the paper found that genetic patterns for coastal populations are structured by geography and not by run timing.

Seeb et al. (2007)

This study also reports on a large coastwide microsatellite data set for 110 Chinook salmon population samples. It includes 4 OC fall-run and 1 OC spring-run population and 2 SONCC fall-run and 1 SONCC spring-run populations (Table 1). Similar to Waples et al. (2004), the

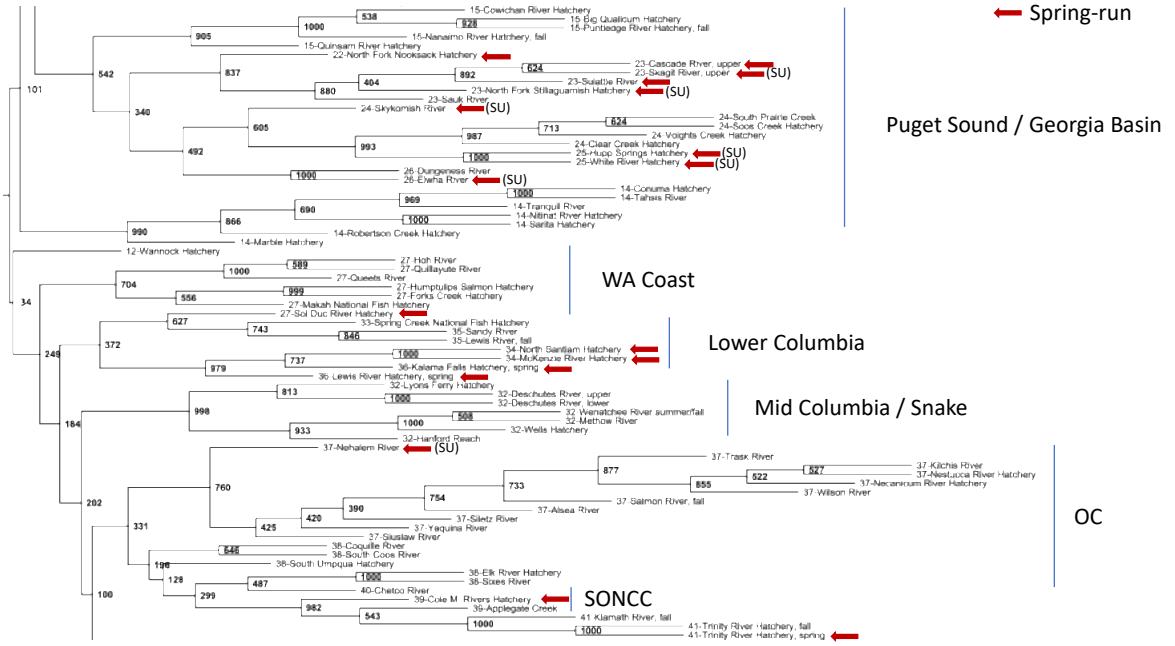
study found that genetic patterns for coastal populations are structured by geography and not by run timing. Similar to Myers et al. (1998) and Waples et al. (2004), the single OC spring-run sample (Rock Creek Hatchery, Umpqua) clustered with SONCC samples rather than other OC samples.

Narum et al. (2008)

This study reports on a coastwide dataset that examined variation at 37 SNPs and 13 microsatellite loci for 29 Chinook salmon population samples. It includes 3 OC samples and 1 SONCC sample (Table 1). Many samples, including all the spring-run OC and SONCC samples, were the same individual fish that were used in Seeb et al. (2007). Similar to previous studies, the Rock Creek Hatchery (Umpqua) samples were genetically more similar to the other SONCC samples than they were to the OC samples.

Moran et al. (2013)

This study reports on a coastwide dataset that examined variation at 13 microsatellite loci for 144 Chinook salmon population samples, including many of the same samples previously analyzed by Seeb et al. (2007). No OC spring-run samples were included, and the study includes only 1 SONCC spring-run population sample (Table 1). Similar to Waples et al. (2004), genetic patterns for coastal populations are structured by geography and not by run timing (Figure 3).



Moran et al. (2013)

Figure 3 – Reproduction of supplemental figure from Moran et al. *(2013), with spring-run samples from coastal Chinook salmon ESUs identified by red arrows. Note that, similar to Waples* et al. *(2004), spring-run samples from coastal ESUs are genetically most closely related to the fall-run populations from the same areas and do not form a distinct evolutionary lineage.*

Clemento et al. (2014)

This study reports on a coastwide dataset that examined variation at 96 SNPs. It included seven OC populations, including the Rock Creek (Umpqua) spring-run population and four SONCC populations (Table 1). Similar to Waples et al. (2004), genetic patterns for coastal populations are structured by geography and not by run timing.

Davis et al. (2017)

This paper described a detailed study of genetic variation among spring- and fall-run Chinook salmon in the Siletz River using largely neutral markers (the study also included some possibly functional genetic markers, but not the GREB1L markers). This study found evidence for two genetically differentiated groups (SNP F_{ST} = 0.009, microsatellite F_{ST} = 0.02) within the Siletz River, corresponding to the spring- and fall-run samples.

Hecht et al. (2015)

This study reported on a coastwide RAD-seq dataset, including samples from Nestucca, Rock Creek (Umpqua), and Cole Rivers (Rogue) hatcheries (Table 1). The primary purpose of the study was to examine genomic associations with environmental co-variables, but the study also reported overall patterns of population structure.

Studies focused on the genetic basis of run timing variation in Chinook salmon

O'Malley et al. (2008)

This study examines coastwide variation in circadian "clock" genes, including spring-run and fall-run samples from the Umpqua, Siletz, and Rogue rivers. The study finds clinal patterns in variation at these genes, but no differentiation between spring- and fall-run samples from the same rivers.

Prince et al. (2017)

This study contains information that has been cited in several recent status reviews and was a prominent paper cited by the Petitions. A recent NMFS report (Anderson et al. 2018) reviewing a petition to consider spring-run Chinook salmon in the Upper Klamath and Trinity rivers as an ESU provided an extensive summary of this paper. We have reviewed and agree with this summary, which we quote here.

> This study reports a survey of genetic variation between spring- and fall-run Chinook salmon from multiple locations and of summer- and winter-run steelhead (anadromous *O. mykiss*) from a distinct set of locations. 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 from the extremes of the run-timing distributions to represent 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 migration ecotype.

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[3] to account for geographic population structure. This GWAS also found migration ecotype 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 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

[3] This statement was subsequently found to be incorrect. They actually used the first 15 PCs from a PCA as the covariate to account for population structure.

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.

Samples from the OC included North Umpqua River spring-run (n = 24), Siletz River spring- and fall-run (n = 4 each), and South Umpqua River spring- and fall-run (n = 8 each). Samples from SONCC included the Rogue River spring- and fall-run (n = 16 each).

Thompson et al. (2019a)

This paper has also been cited by recent status reviews, and was previously summarized by Anderson et al. (2018). We have reviewed and agree with this summary, quoted below:

> The study first investigates a reported rapid shift in run-timing phenotype in Rogue River Chinook salmon following the construction of Lost Creek Dam in 1977. The authors employed a capture-array laboratory approach to resequence the GREB1L region in 64 spring- and fall-run Chinook salmon used in Prince et al. (2017). From this higher-resolution data they were able to identify SNPs that were more strongly associated with migration timing than those found in Prince et al. (2017). They developed assays for two of these new SNPs and typed them in 269 fish sampled during three different periods (early, middle, and late), each of approximately one week, during the migration season. They found a strong association between run timing and genotype, with samples from the early week being composed mostly of homozygotes for the spring-run-associated alleles, the middle week mostly heterozygotes, and the late week mostly homozygotes of the fall-run-associated alleles. Lower in the watershed, closer to the point of freshwater entry, 38 adult fish were sampled in September (the late part of the migration season for Chinook salmon in that basin) and were found to consist entirely of fish homozygous for the fall-run-associated allele. With data from the three weekly sampling periods, the authors fit a model to estimate the frequency of each genotype during the migration period and extrapolated that to run-count data (i.e., escapement) to estimate the number of fish of each genotype category passing the Gold Ray fish-counting station each day in 2004.
>
> Using the observed change in frequency [estimated using the above-described model] from 1976 (before Lost Creek Dam) to 2004 of the spring-run-associated alleles at the two strongly associated SNPs, the authors estimated selection coefficients against the spring-run-associated allele under three models in which the effect of the allele is recessive, codominant, or dominant. High selection coefficients were estimated for each model. Those estimates were then used to predict how long the allele might persist in the face of such selection, and they estimated that, under such selection scenarios, the allele could be lost from the Rogue River population within 50 to 100 years after the

construction of Lost Creek Dam, unless the allele acts in a recessive manner (i.e., if heterozygotes have the same fitness as homozygotes for the fall-run-associated allele).

The authors also analyze samples from the Klamath River at the two newly developed SNP markers. From ancient DNA samples (n = 9) with a range of ages (from ~100 years to several thousand years) homozygotes for both the spring-run- and fall-run-associated alleles were observed in the upper Klamath Basin, upstream of the sites of four dams that are scheduled to be removed within the next three years. From over 800 contemporary samples of juvenile Chinook salmon in the Scott River and the Shasta River (two major tributaries within the UKTR [Upper Klamath Trinity River] basin), only four total individuals carrying the spring-run-associated alleles were found, all of them heterozygous. The authors note that this low frequency suggests that the alleles are not being maintained, in the absence of spring-run Chinook salmon, in the Scott or Shasta rivers, and that the alleles are susceptible to complete loss from the two sub-basins.

O'Malley et al. 2020a,b

O'Malley et al. (2020a) describe patterns of variation in two GREB1L-region SNPs assayed in 162 natural-origin Chinook salmon sampled from fisheries in the lower Rogue River from late March to early July 2019. The SNPs assayed were the two SNPs reported in Thompson et al. (2019a) that were most highly associated with run timing. Of the 158 samples that were successfully genotyped, 115 were homozygous for the 'spring' variant, two were homozygous for the 'fall' variant, 32 were heterozygous (one 'spring' allele and one 'fall' allele) and nine had 'discordant' genotypes (a contrasting pattern at the two SNPs).

O'Malley et al. (2020b) report on variation at the same two GREB1L-region SNPs in unmarked (presumed natural-origin) Chinook salmon carcasses sampled in the upper Rogue River (upstream of the Gold Ray counting station) in 2016-2018 (n = 442, 485 and 484, respectively) and 1575 fish used as broodstock at the Cole Rivers Hatchery in 2018. Results from one of the SNPs (snp640165) indicated that in 2018 the Cole Rivers Hatchery broodstock were primarily homozygous for the 'spring' variant (~89%). Natural spawning samples contained a mixture of all three genotypes, with homozygous 'spring' genotypes the most common (Figure 4).

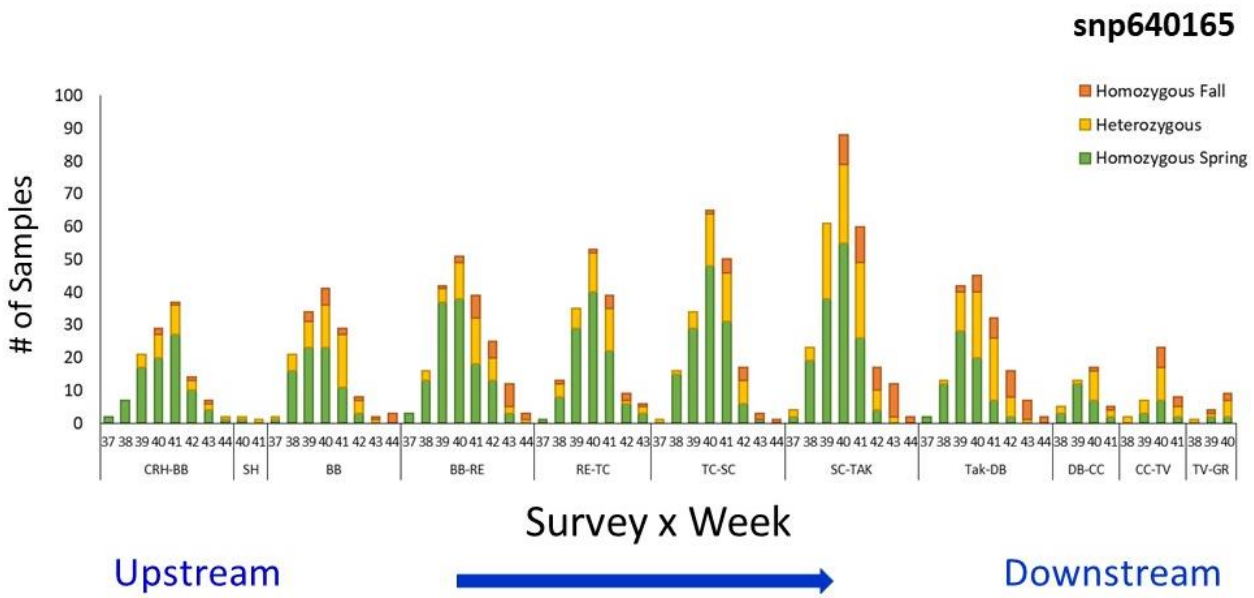


Figure 4 – Reproduced from K O'Malley's section of Ford et al. *(2020). "Distribution of* GREB1L *SNP1 genotypes across survey locations and time with all three years combined (2016 - 2018).* Greb1L *SNP1 (snp640165) is more diagnostic of adult migration phenotype in Rogue River Chinook salmon than SNP2 (snp670329) (T. Thompson, pers. comm.). The Julian week when carcass samples were collected is on the x-axis and ranges from 37 (Sep 10th – 16th) to 44 (Oct 28th – Nov 4th), grouped by survey location. The most upstream survey location is Cole Rivers Hatchery (CRH) and the furthest downstream location is the old Gold Ray Dam site (GR). Number of carcass samples collected is on the y-axis. Figure courtesy K. O'Malley."*

Anderson and Garza (2018) and Thompson et al. (2020)

Anderson and Garza (2018) (subsequently published as Thompson et al. (2020)) describe results from a study based on a combination of whole genome sequencing of 160 Chinook salmon sampled from the Sacramento River and Klamath/Trinity River watersheds followed by genotyping of nine SNPs that characterize the two main evolutionary lineages in the GREB1L region and that were found to be highly associated with run-timing-in the Klamath River, and with run-type designation of samples from 10 populations in California and the Siletz River in Oregon. These results were also summarized by E. Anderson in Ford et al. (2020). Samples from the Siletz River that had been labelled "spring-run" according to their time and place of collection (n = 89) consisted of 67% homozygotes for the "spring" variant, 3% for the "fall" variant, and 29% were heterozygotes. The Siletz River fall-run samples (n = 65) were nearly entirely "fall" homozygotes, a pattern qualitatively similar to that seen in the UKTR Chinook salmon samples (see Table 2, reproduced from Table 3 of Anderson and Garza (2018)).

Table 2 -- Frequency of RoSA [GREB1L region] genotypes across multiple collections within California and on the Oregon coast. Reproduced from Anderson and Garza (2018).

Collection	Ecotype	ESU	EE	EL	LL	n
Siletz River spring	spring-run	OC	0.67	0.29	0.03	89
Siletz River fall	fall-run	OC	0.00	0.02	0.99	65
Iron Gate hatchery fall	fall-run	UKTR	0.00	0.00	1.00	375
Salmon River spring	spring-run	UKTR	0.87	0.13	0.00	23
Salmon River fall	fall-run	UKTR	0.17	0.33	0.50	6
Salmon River unknown	mix	UKTR	0.14	0.29	0.57	256
Trinity River hatchery spring	spring-run	UKTR	0.60	0.34	0.06	94
Trinity River hatchery fall	fall-run	UKTR	0.00	0.02	0.98	93
Trinity River unknown	mix	UKTR	0.26	0.23	0.51	160
Eel River fall	fall-run	CC	0.00	0.00	1.00	45
Russian River fall	fall-run	CC	0.00	0.00	1.00	46
Sacramento River winter	winter-run	SRWR	0.96	0.04	0.00	23
Coleman hatchery late-fall	late fall-run	CVFLF	0.00	0.00	1.00	44
Butte Creek spring	spring-run	CVSR	1.00	0.00	0.00	73
Butte Creek fall	fall-run	CVFLF	0.00	0.14	0.86	21
Butte Creek unknown	mix	CVSR and CVFLF	0.80	0.02	0.18	89
Mill-Deer Creek	mix	CVSR and CVFLF	0.34	0.13	0.53	243
Feather River hatchery spring	spring-run	CVSR	1.00	0.00	0.00	34
Feather River hatchery fall	fall-run	CVFLF	0.15	0.28	0.57	67
Feather River unknown	mix	CVSR and CVFLF	0.11	0.13	0.76	276

ESUs:
OC = Oregon Coast; **UKTR** = Upper Klamath-Trinity Rivers; **CC** = California Coastal; **SRWR** = Sacramento River Winter Run; **CVFLF** = Central Valley Fall-run and Late-Fall-run; **CVSR** = Central Valley Spring Run

Recent status review reports on ESU/DPS issues related to the genetics of run timing

Anderson et al. (2018) (Upper Klamath-Trinity River spring-run Chinook salmon)

This report evaluates whether the spring-run Chinook salmon in the Upper Klamath and Trinity River basins are an ESU. The report was part of NMFS's response to a 2017 petition from the Karuk Tribe and the Salmon River Restoration Council to list Upper Klamath-Trinity Rivers (UKTR) Chinook salmon as threatened or endangered, or to create a new ESU consisting of UKTR spring-run Chinook salmon and list it as threatened or endangered. The report evaluates only the question of whether UKTR spring-run Chinook salmon are an ESU, separate from fall-run fish inhabiting the same watershed. The report evaluates information from many of the same sources cited by the OC and SONCC petitions, notably the studies by Prince *et al*. (2017) and Thompson *et al*. (2019a). The report also considers the information in Anderson and Garza (2018).

The report analyzes and evaluates the new genetic information with respect to both the reproductive isolation and evolutionary legacy criteria of the NMFS ESU policy, and also extensively discusses the potential problems of using only small and non-random portions of the genome to identify conservation units. Based on these analyses, the report concludes that spring-run Chinook salmon in the UKTR meet neither prong of the NMFS ESU criteria:

In summary, the panel finds that new data and analyses available since the previous (2011) petition do not substantially change our understanding of evolutionary relationships of Chinook salmon in the Upper Klamath-Trinity Rivers Chinook Salmon ESU and do not support the separation of the spring-run ecotype from the fall-run ecotype into a new ESU. We find that this new information provides an interesting addition to our understanding of the heritable basis of phenotypic variation in salmonid fishes, but the details of how this genomic region influences Chinook salmon phenotype remain unclear. The new information further confirms that spring-run Chinook salmon in the Klamath River are extremely closely related to fall-run Chinook salmon in their respective sub-basins, and that they therefore share the majority of their evolutionary history and its legacy with them.

In other words, these spring-run ecotypes do not form a monophyletic group and have a long history of gene flow with their fall-run counterparts, which likely leads to substantial shared local adaptation. The panel did not find that the newly available data demonstrated that either the substantial reproductive isolation or unique evolutionary legacy criteria for ESU delineation under the NMFS ESU policy were met for spring-run Chinook salmon in the Klamath River basin.

Further, the petition and Prince et al. (2017) argue that the more recent common ancestry of a single, small genomic region in the vicinity of the GREB1L gene is sufficiently important that it should take precedence over the pattern of ancestry of the vast majority of the genome in designating these spring-run Chinook salmon in the Klamath River basin as a distinct species under the ESA. This would represent a major departure from the scientific consensus on delineation of ESUs and other management units based on shared ancestry. We find such a departure to lack justification and note that it would lead to several intractable problems in both the present case and in wider application. (p. 26)

Pearse et al. (2019) (Northern California Coastal steelhead report)

This report was part of the NMFS response to a November 2018 petition from the Friends of the Eel River to separate summer-run steelhead in the Northern California Steelhead DPS into a new DPS and then list it as endangered under the ESA. The petition, and the report, consider many of the same new genetic studies cited in the OC and SONCC petitions and also described in this report (e.g. Prince et al. 2017).

The report evaluates a variety of new genetic studies, focusing on both genome-wide and GREB1L-specific data. Similar to Anderson et al. (2018), the report finds evidence for substantial interbreeding, gene flow, and locally shared ancestry among individuals with different run timing and GREB1L-region genotypes. The report concludes 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." (p. 15). Similar to Anderson *et al*. (2018), the report also discusses some of the challenges of designating conservation units on the basis of single genomic regions.

Agency reports

ODFW 2005 (Oregon native fish status report)

This report identified nine populations of OC spring-run Chinook salmon in the Coastal Species Management Unit (SMU) including spring-run populations in the Tillamook, Nestucca, Siletz, Alsea, Siuslaw, South Umpqua, North Umpqua, Coos, and Coquille rivers. The Siuslaw and Coos populations were determined to be extirpated. Population existence was inferred from historical records of commercial landings, although the report noted that a more thorough review "may determine that some populations defined here, especially the extinct or presumed extinct populations, were not historic populations." (p. 142).

ODFW 2014 (Coastal multi-species conservation and management plan (CMP))

This plan focuses primarily on OC non-listed salmonids. In contrast to the 2005 report, the CMP identifies only two independent spring-run OC Chinook salmon populations, both in the Umpqua River. An additional 6 populations (Nehalem, Tillamook, Nestuca, Siletz, Alsea, and Coquille) were determined to have both spring- and fall-run components within each population. The rationale for considering the two run types to be parts of the same populations in these coastal rivers was "a) there are fewer isolating mechanisms between the two life history components; b) these basins are not naturally conducive to independent spring or summer Chinook populations (as evidenced by both the lack of snow-fed summer water and the limited presence and scope of early Chinook runs); and c) existing data do not strongly support a bi-modal distribution in returns." (p. 10). The report notes that new genetic or demographic data could change this determination.

Review papers or reports on the use of genomics in conservation

Over the past decade, there have been several reviews of the use of genomic data to identify conservation units. In a broad review of the use of genomic data for conservation genetics, Allendorf et al. (2010) notes that genomic approaches are useful for evaluating the amount of adaptive divergence among conservation units, but cautions that "there are pitfalls in focusing on individual adaptive loci rather than neutral patterns of genome-wide averages" (p. 704) in defining conservation units. Funk et al. (2012) also cautions against identifying ESUs on the basis only one or a few loci, and suggests instead that both neutral and adaptive variation should be considered together.

Waples and Lindley (2018) review some of the recent studies on the genetic basis of run timing variation in Pacific salmon and steelhead (e.g., Prince et al. 2017) and raise several questions

related to how this information could be considered in making conservation decisions. One issue of particular concern this paper raises is the potential difficulty in reestablishing the spring-run trait in populations from which is has been lost, noting that the new data suggests that the only source of the genetic material required for this trait would be other populations in which the "spring" allele still exists.

Finally, Ford et al. (2020) summarize the results of an expert workshop held in February 2020 that reviewed and discussed the latest information available on the genetics of run timing in Chinook salmon and steelhead. Among the conclusions of the report was that variation in the GREB1L/ROCK1 region on chromosome 28 is highly associated with adult run timing in multiple Chinook salmon and steelhead populations. The report also concludes that conservation units should generally be defined on the basis of variation throughout the genome, rather than on the basis of small genomic regions associated with specific traits, but that evaluation of risk needs to consider current knowledge of the genetic basis of adult run timing variation.

Analysis of ESU question

Reproductive isolation criterion

Patterns of neutral genetic diversity

As discussed above, for coastal Chinook salmon, the 1998 coastwide status review did not consider differences in run timing alone to be indicative of substantial reproductive isolation. This conclusion was due in part to the patterns of genetic variation at allozyme loci, in which spring- and fall-run fish spawning in the same or nearby rivers were genetically similar to each other and more similar to each other than to populations of either run type spawning in geographically distant rivers (Myers et al. 1998; Waples et al. 2004; Figure 2). Subsequent genetic studies of randomly sampled genomic variation at small numbers of microsatellite or SNP markers have confirmed these patterns, as have a smaller number of studies that have examined thousands of SNPs (Table 1). These studies clearly confirm the earlier allozyme-based findings that, as a group, coastal spring-run Chinook salmon are not a distinct evolutionary lineage within the species, but rather share their evolutionary history and most of their genetic variation with the fall-run Chinook salmon spawning in the same and nearby rivers (e.g., Figure 3). In other words, the patterns of genetic variation coastwide indicate that spring-run Chinook salmon spawning in different rivers are generally more differentiated from each other than they are to co-occurring fall-run Chinook salmon (Figure 2, Figure 3).

Although this pattern is apparent when viewed on a coastwide scale, it is important to note that most of the coastwide Chinook salmon genetic studies conducted over the past two decades had few samples from the OC and SONCC areas (Table 1). ODFW identified up to nine rivers in the currently defined OC Chinook Salmon ESU as having either spring-run populations or a spring- or summer-run component to a population, but no genetics study has included more than three spring-run or summer-run population samples, and early- (spring- or summer-) run

samples have only been analyzed for a total of four OC river systems (Nehalem (su), Trask (Tillamook), Siletz, Umpqua; Table 1). The SONCC area is somewhat more thoroughly sampled, particularly with respect to the large Rogue River system (Table 1).

Within the SONCC ESU, it is apparent that the close genetic relationship between geographically proximate spring- and fall-run Chinook salmon continues to be true when viewed at the within-ESU scale. In particular, in several studies, spring- and fall-run samples from the Rogue River are more genetically related to each other than either are to samples from other rivers in the SONCC ESU. In other words, within the currently defined SONCC Chinook salmon ESU, spring- and fall-run fish spawning in the Rogue River appear to reproduce more with each other than either does with fall-run fish spawning in other rivers in the ESU. This pattern is similar to what has been reported in the Upper Klamath and Trinity Rivers (Anderson and Garza 2018), and is also apparent in the Puget Sound and Lower Columbia Chinook ESUs (Figure 2, Figure 3).

The patterns of genetic relationship between spring- and fall-run Chinook salmon within the currently defined OC Chinook ESU are not informed with as much data. Only a few studies (Table 1) have included spring- and fall-run Chinook salmon sampled from the same river system, and based on these limited samples spring- and fall-run population samples do not necessarily cluster closely together in the resulting tree diagrams. In particular, Umpqua River spring-run (sampled from the Rock Creek hatchery) tend to cluster with SONCC samples of both run types in a number of studies rather than with Umpqua fall-run samples or other OC fall-run samples (Myers et al. 1998, Waples et al. 2004, Seeb et al. 2007; Narum et al. 2008; Clemento et al. 2014; Hecht et al. 2015; note that some studies used the same set of samples so these data are not all independent – Table 1). This pattern could indicate that Umpqua River spring-run Chinook salmon are in fact historically more closely related to SONCC Chinook salmon, or could be a result of past broodstock transfers from the Rogue River (and elsewhere) into the Rock Creek Hatchery (Myers et al. 1998, Appendix D). In addition, fall-run samples from the Trask River Hatchery were more closely related to other OC fall-run samples than to Trask River Hatchery spring-run samples (Beacham et al. 2006). A similar pattern was seen in wild fall- and spring- run fish from the Siletz River (Davis et al. 2017). Extensive out-of-basin spring (and fall) Chinook salmon hatchery releases in the Trask River may be an explanation for this pattern. Similarly, although relatively few spring-run Chinook salmon hatchery releases have occurred in the Siletz River, that basin did receive > 2 million Columbia River hatchery Chinook salmon releases between 1934 and 1952 (Appendix D in Myers et al. 1998). Additional sampling and genetic analysis of natural-origin fish across the range of return timing in multiple OC and SONCC rivers would help improve our understanding of the genetic relationships among OC and SONCC Chinook salmon populations. However, nothing in the available data indicates that spring-run Chinook salmon spawning in rivers on the Oregon Coast, as a group, form a distinct lineage separate from OC fall-run Chinook salmon.

One recent paper, Davis et al. (2017), describes a detailed study of genetic variation among spring- and fall-run Chinook salmon in the Siletz River using largely neutral markers. This study reported evidence for two genetically differentiated groups within the Siletz River, corresponding

to the spring- and fall-run samples, but the level of differentiation was very low. This does indicate, however, that in this watershed these two run timing groups express some assortative mating. See below for discussion of additional analyses of these samples at the GREB1L region loci.

Patterns of variation at the GREB1L region

In addition to studies that have examined patterns of genetic diversity at 'neutral' loci not associated with run timing, there are five recent studies that have examined run-time-associated variants in the GREB1L region in OC or SONCC Chinook samples (Prince *et al.* 2017; Anderson & Garza 2018; Thompson *et al.* 2019a; O'Malley *et al.* 2020a; O'Malley *et al.* 2020b). These studies have found that heterozygotes between spring-run and fall-run GREB1L-region variants are common, indicating that interbreeding between fish homozygous for the "spring" and "fall" run variants is commonly occurring. This pattern has been most extensively studied in the Rogue River (SONCC; (Thompson *et al.* 2019a; O'Malley *et al.* 2020a; O'Malley *et al.* 2020b)), where researchers have obtained relatively large sample sizes of fish based on carcass surveys and surveys of captured live fish both conducted throughout the run. For the OC, the only river that has been sampled using the most highly associated GREB1L markers is the Siletz River (Anderson and Garza 2018, Thompson et al. 2020). That study also found substantial proportions of heterozygotes, particularly among fish that were phenotyped as spring-run (29%; Table 2). A similarly high proportion of GREB1L-region heterozygotes have been found in other coastal Chinook ESUs (UKTR, Anderson and Garza 2018; Washington Coast, (Thompson *et al.* 2019b)).

The GREB1L region is highly associated with the run-timing phenotype in multiple populations of coastal Chinook salmon (i.e., coastal spring-run Chinook salmon are homozygous for the early alleles, fall-run Chinook salmon are homozygous for the "late" alleles – Anderson and Garza 2018, Thompson et al. 2019a, O'Malley et al. 2020). The finding of substantial proportions of heterozygotes, often in or close to Hardy-Weinberg proportions, therefore provides strong evidence of contemporary interbreeding between alternative homozygotes at the GREB1L region. This, in turn, strongly implies that mating among spring- and fall-run (and likely intermediate timed) fish is common in multiple watersheds (reviewed by Ford et al. 2020). Analysis of recombination events (Anderson and Garza 2018) also indicates that at least in the Upper Klamath River, such interbreeding must have also occurred historically at some level, although the rate of interbreeding was not determined and could be lower than is seen now. An expert workshop has recently reviewed this issue (including largely the same studies reviewed here) and concluded:

> The extent to which observed contemporary levels of interbreeding between individuals with early and late run timing would be typical under historical environmental conditions is unknown. [emphasis in the original] The dynamic nature of the Pacific Northwest environment and geology makes it reasonable to conclude that the direction and amount of interbreeding between early and late runs has been variable over many timescales. However, there is clear documentation that anthropogenic activities have increased opportunities for interbreeding between ecotypes, at least in some locations. For

example, high rates of interbreeding between spring and fall-run fish in the Upper Rogue River appear to be due to changes in water temperature and flow associated with an upstream dam that has allowed fall-run fish to access what was historically spring-run habitat (Thompson et al. 2019a). Workshop participants also cited numerous habitat changes in the Klamath and Chehalis Rivers that likely have increased interbreeding between spring and fall-run Chinook salmon, including modifications to natural low-flow barriers to allow fall-run fish greater access to upstream habitats and/or blockage of upstream habitat (Wendler and Deschamps 1955; Hiss et al. 1985; Olsen and Dix 1991), both of which would be expected to increase relative degree of overlap and thus opportunities for interbreeding between runs. Analysis of recombination events in the Klamath River whole-genome sequencing data indicated that some level of interbreeding between the run types was occurring prior to 200 years ago, but the level of historical interbreeding or the degree to which it has increased has not been quantified (Anderson presentation). However, the type of habitat that creates flow-dependent partial migration barriers is naturally dynamic, so it is reasonable to conclude that the nature and extent of interbreeding has also been variable over space and time. (Ford et al. 2020)

Conclusions regarding the reproductive isolation criterion

In both the OC and the SONCC ESUs, there is strong evidence from GREB1L-region markers that contemporary interbreeding between spring-and fall-run Chinook salmon within a watershed is common, at least for the two watersheds that have been studied to date (Rogue River, Siletz River). These data do not clearly indicate whether these current levels of interbreeding occurred historically. However, patterns of random genomic variation (indicative of population history) do not suggest that spring-run Chinook salmon in either the OC or SONCC ESUs are, as a group, a distinct unit that does not interbreed with fall-run Chinook spawning in OC and SONCC rivers. There is some indication that spring-run Chinook salmon in the Umpqua River may have somewhat reduced gene flow from other OC fall- and spring-run Chinook salmon populations, but past hatchery practices may have also influenced this result. As a whole, however, the available data indicate that the spring-run portions of the OC and SONCC ESUs are not substantially reproductively isolated from the fall-run populations in the ESUs. Additional genetic sampling of fish throughout the period of migration in multiple populations, especially in the OC ESU, would be very helpful for further evaluating this question.

Evolutionary legacy criterion

Both of the petitions noted that the spring/early run timing trait is an important component of diversity within the Chinook salmon species. In particular, the trait allows Chinook salmon to access upstream habitats that are inaccessible to later returning fish in some years. Run time diversity as a whole is also expected to increase viability by broadening the portfolio of traits within a species or an ESU, which leads to increased resilience to environmental variation (Quinn et al. 2016). Recent reviews of ESU/DPS configurations of Chinook salmon (Anderson et al. 2018) and steelhead (Pearse et al. 2019) support this point, as does a recent expert

workshop report (Ford et al. 2020) and the original coastwide status review of Chinook salmon (Myers et al. 1998). Recovery plans for Chinook salmon ESUs that contain populations with both spring- and fall-run fish also emphasize the importance of recovering populations with both life-history strategies (Shared Strategy Development Committee 2007; Dornbush 2013; Pearse et al. 2019).

While recognizing the importance of run-timing variation to species and ESU viability, Myers et al. (1998) concluded that patterns of genetic variation and patterns of variation at other life-history traits indicated that coastal spring- and fall-run Chinook salmon shared the same recent evolutionary history. Coastal ESUs were identified based on concordant patterns of genetic, life-history, and geographic variation, with run-timing variation considered to be an important element of diversity within ESUs. Subsequent reports of Upper Klamath Trinity River Chinook salmon and Northern California steelhead have reached the same conclusion (Williams et al. 2013, Anderson et al. 2018, Pearse et al. 2019). Recent genetic studies have greatly increased our knowledge of the genetic basis of run-timing variation, but these studies do not change or invalidate the previous conclusion that spring- and fall-run Chinook salmon in the currently defined OC and SONCC ESUs share a recent evolutionary legacy and they are, on the whole, more genetically similar to each other than to populations in other ESUs. The two run types display similar characteristics at other life-history traits, and are genetically similar to each other due to a combination of recent common ancestry and ongoing interbreeding. Identifying a spring-run-only ESU carved out of either the OC or SONCC ESUs would therefore be inconsistent with the NMFS ESU policy, both because of high levels of interbreeding between spring- and fall-run fish in these ESUs and because spring-run fish as a group in these ESUs do not form a distinct evolutionary lineage within the species.

Conclusion

We conclude that available genetic data published in the 22 years since the 1998 coastwide status review support, on the whole, the current ESU configuration for both the OC and SONCC ESUs, and do not support the identification of spring-run-only ESUs within either the OC or the SONCC ESUs. The spring-run phenotype and the spring-run variant within the GREB1L chromosomal region are clearly an important part of the diversity within the Chinook salmon species, but the available data indicate that spring-run Chinook salmon in the OC and SONCC ESUs regularly interbreed with and share a recent evolutionary history throughout the vast majority of their genome with fall-run Chinook salmon in the same rivers. We therefore conclude that spring-run Chinook salmon in the OC or SONCC ESUs do not meet the criteria for being separate ESUs under the NMFS ESU policy.

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