Genetic Evaluation of Fall Chinook Salmon Carcasses Collected During Annual Spawning Ground Surveys of the White Salmon River, WA from 2013 – 2021

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Abstract

Hybridization among threatened tule and non-native upriver bright (URB) fall-run Chinook salmon populations has been observed in both the White Salmon River and broodstocks at the Little White Salmon (LWS), Willard (WI), and Spring Creek (SC) National Fish Hatcheries (NFH). Previous genetic studies identified a need to evaluate the impact of these hatchery operations on the native White Salmon River tule population by evaluating whether hatchery-origin tule *x* URB hybrids are straying to the White Salmon River at an elevated rate and thereby increasing hybridization rates on spawning grounds. We also desired to quantify the number of hybrids returning to spawning grounds and investigate concordance of genotypic sample classifications with phenotypic carcass identifications. To accomplish these goals, we evaluated 967 field-identified tule (n = 622) and URB (n = 345) carcass samples collected in the White Salmon River from 2013 through 2021. Carcasses were genotyped using a 344-locus GTseq panel. Analyses revealed a greater proportion of hybrids among natural-origin spawners (30%) compared to hatchery-origin spawners (11%). The overall annual proportion of hybrid spawners was 31.1%, with a greater annual mean proportion of hybrids found among field-identified tule carcasses (38.1%) relative to URBs (15.7%). A large proportion of tule carcasses were tule backcrosses (27%), which were typically recovered from spawning grounds in late October. Just three tule carcasses were hatchery-origin, but parentage-based tagging analysis combined with hatchery-marking data indicated that 30.1% of URB carcasses originated from fish spawned at LWS NFH, rather than the 11.8% inferred from physical hatchery markings. However, the proportion of hybrids among LWS NFH strays (8.2%) matched prior estimates for LWS NFH broodstock (8.4%). Overall, a greater proportion of hybrid spawners in the White Salmon River are wild-origin rather than hatchery-origin. Most of these returning hybrids display run timing intermediate to the main tule and URB spawning runs. This overlap in run timing with non-hybridized fish is expected to result in continued production of wild-origin hybrids.

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Introduction

Human-assisted translocation of animal populations has led to several instances of hybridization among formerly allopatric populations (Seehausen et al. 2008). The resulting hybridization is concerning for threatened and endangered species conservation due to its potentially negative outcomes (Allendorf et al. 2001). These outcomes pose multiple threats to populations, including wasted reproductive effort (Wolf et al. 2001), outbreeding depression (Lynch 1991), and breakdown of discrete population barriers through repeated hybridization and backcrossing (Harrison 1993). However, these outcomes are often unpredictable (Ellstrand et al. 2010), and can be influenced by dynamics of the environments in which the hybridization occurs (Mandeville et al. 2022).

Hybridization among salmon populations remains an active area of study due to impacts from environmental modifications (e.g., dams), construction of hatcheries, and translocation of populations from their historical ranges (Fisher 1994; Araujo et al. 2021; Devlin et al. 2022). Intraspecific hybridization has been studied in Chinook salmon *Oncorhynchus tshawytscha*; focusing on once allopatric populations with divergent life history characteristics (Waples et al. 2004) that have been forced into contact by habitat alterations which restrict access to historical spawning habitat. The White Salmon River, a tributary of the Columbia River in southern Washington, is a place where intraspecific hybridization has occurred between historically allopatric fall-run Chinook salmon. This hybridization became a concern when upriver bright (URB) fall Chinook salmon production began at the Little White Salmon (LWS) National Fish Hatchery (NFH) in 1982, and again when production goals for URB increased from 2 million juveniles to 4.5 million in 2009 (NMFS 2017). These production numbers remained high from 2012 to 2021, with 4.4 million URB juveniles being released annually from LWS NFH (Silver et al. 2022).

The URB fall Chinook salmon reared and released from the LWS and Willard (WI) NFHs are straying into the White Salmon River where they interbreed and compete with the native ESA-listed tule fall Chinook. A variety of environmental and anthropogenic factors have been proposed to explain the incidence of hatchery-origin strays entering the White Salmon River, though the exact causes remain undetermined (Silver et al. 2022). Interactions between hatchery-origin URB strays and native tule fall Chinook are believed to reduce productivity of the native tule population (e.g., through hybridization and redd superimposition) (NMFS 2017).

The potential impacts of hybridization between URB strays and the White Salmon River tule population have been a particular concern (NMFS 2017). A study by Smith and Engle (2011) found that 4.3% to 15% of the fall Chinook juvenile production (n = 1,546 samples) in the White Salmon River between 2006-2008 were F1 hybrids (i.e., native tule × URB). A more recent analysis of 372 juvenile samples collected from 2017 – 2019 suggested that the frequency of F1 hybrids has increased over time, comprising 17% to 32% of recent samples; though more sampling is needed to understand the relative importance of year-to-year variation in hybridization rates (Smith et al. 2021). This analysis also identified backcross hybrids (i.e., individuals with one hybrid parent) in the White Salmon River, indicating survival and contribution of F1 hybrids to the spawning population (Smith et al. 2021). Furthermore, analysis of hybridization rates at nearby hatcheries revealed that hybrids comprised an average 8.4% (range: 7.3% to 9.5%) of the broodstock at LWS NFH between 2015 – 2018, with hybrids predominantly composed of backcross individuals (i.e., F1 hybrid × URB) (Smith et al. 2021). These results raise additional questions concerning the impact of URB straying on the tule population (e.g., risk

of genetic introgression) and the potential causes contributing to straying including the impact of hybrids used as hatchery broodstock.

Superimposition of hatchery–origin URB redds on native tule redds is also thought to occur in the White Salmon River due to the later spawning timing of URBs compared with tules (Hardiman and Allen 2015). Redd superimposition may result in tule egg displacement and reduce the egg-to-fry survival rate leading to a further loss in productivity of the tule population (McNeil 1964; Fukushima et al. 1998). Due to difficulties in directly assessing the impact of redd superimposition, the abundance of hatchery-origin URB spawners is used as a surrogate to measure for impacts on the tule population (NMFS 2017). Annual spawning ground surveys conducted by the Washington Department of Fish and Wildlife (WDFW) are used to estimate the native tule and URB fall Chinook salmon spawning populations, as well as provide estimates of the origin of URB strays. These estimates are critical to evaluating the potential risks of hatchery-origin URB strays on the native tule population.

To understand the impacts of hybridization between hatchery-origin URB strays and the ESA-listed White Salmon River tule fall Chinook population, we analyzed genetic samples obtained from adult carcasses collected by the WDFW during annual spawning ground surveys of the White Salmon River from 2013 – 2021. These samples were evaluated using modern genetic techniques to address the following three objectives. First, we used genotypic data to quantify the proportions of hybrids among field-identified tule and URB carcass samples. Second, we assessed the level of concordance in population assignment of carcasses to tule and URB fall Chinook salmon populations based on genetic evidence and phenotypic characteristics. Finally, we used a parentage-based tagging approach (Steele et al. 2019) to determine the percent of carcasses that were misclassified as wild-origin spawners due to the non-detection of visible hatchery marks or tags, and evaluate the potential impact of hybrids used as broodstock at LWS and WI NFHs by quantifying the proportion of hatchery-origin strays assigned to hybrid classes.

Methods

Fall and Spring-run Chinook salmon carcass surveys were conducted in the White Salmon River from 2013 – 2021. Tissue samples were collected each year and preserved in 95% ethanol or dried on Whatman paper for a total of 1,233 samples (Table 1). Genomic DNA was extracted using DNeasy Blood and Tissue Kits (Qiagen Inc.; Valencia, CA) according to the manufacturer's protocol. Samples were processed using the Genotyping-in-Thousands by Sequencing (GT-Seq) method (Campbell et al. 2015) by amplifying a 344 locus primer pool (Otsh_344; Shawn Narum, CRITFC, unpublished data). Quality assessment/quality control (QA/QC) was performed to determine laboratory error by building a new library from the extracted DNA of 10% of the samples. Resulting libraries were analyzed using MiSeq Reagent v3 Kits on an Illumina MiSeq. Sequence data were demultiplexed and genotyped using the GTseq pipeline (Campbell et al. 2015).

GTseq loci exhibiting excessive missing data (>30%) were removed from the dataset. A filter was then applied to remove carcasses with >15% missing genotypes. Carcasses lacking necessary field data (i.e., field identification as a tule, URB, or spring-run Chinook salmon) were also discarded. Data filtering led to retention of 340 GTseq loci and 998 carcass samples. All filtering steps were performed with custom Python scripts (https://github.com/stevemussmann/GTseqTools) unless otherwise noted.

Genotype data were first analyzed to identify and discard individuals with spring-run Chinook salmon ancestry. The admixture model with uncorrelated allele frequencies was applied in Structure v2.3.4 (Pritchard et al. 2000; Falush et al. 2003). We assumed presence of three populations ($K = 3$), corresponding to tule, URB, and spring-run Chinook salmon, but no *a priori* assignment data were provided for individual samples to the program. Twenty-four independent replicates were performed, each consisting of 100,000 burn-in Markov chain Monte Carlo (MCMC) generations followed by 500,000 generations of data collection. Results were summarized in CLUMPAK (Kopelman et al. 2015) to check for potential multimodality of population assignments. Individuals with spring-run ancestry ($Q > 0.2$) were discarded from the dataset.

The program NewHybrids (Anderson and Thompson 2002) was then utilized to compare genetic classifications of tule and URB carcasses to their field identifications, and to identify hybrid ancestry of returning fish. The program evaluated six possible ancestry categories: 1) tule, 2) URB, 3) first generation (F1) hybrid of tule and URB, 4) second generation (F2) hybrid (i.e., offspring of two F1 hybrids), 5) tule backcross (offspring resulting from an F1 *x* tule cross), and 6) URB backcross (offspring from an F1 *x* URB cross). NewHybrids was executed using 50,000 MCMC generations of burn-in, followed by 100,000 generations of data collection. The Jeffreys prior for allele frequencies and mixing proportion was applied, and the 'z' option was employed to specify reference genotypes for non-hybrid individuals. Samples were selected as reference data based upon time of collection, field identification, and population ancestry assignment in Structure. For example, tule reference individuals were chosen by selecting samples from early returning fish (i.e., collected prior to October 21) that had been field-identified as tule and had genetic ancestry assigning almost exclusively to the tule population based upon Structure results ($Q > 0.99$). Similar criteria were applied to select URB representatives, except samples were chosen from the latest returning fish (i.e., collected after November 19). All samples were chosen from carcasses collected over a 4-year time span (2018-2021). Ultimately, 28 samples were selected as reference data for the tule population and 18 samples for the URB population.

Individuals were assigned to hybrid categories based upon Bayesian posterior probability (BPP) values from NewHybrids. A minimum threshold of BPP > 0.50 was applied when identifying hybrid categories. This threshold was determined from prior simulation work conducted for this study system and GTseq panel (Smith et al. 2021).

The program SNPPIT (Anderson 2012) was utilized to assign carcass samples to the parentage-based tagging (PBT) baseline dataset for salmon spawned at Spring Creek (spawning years 2015 – 2018; n = 20,527) and Little White Salmon (spawning years 2013 – 2018; n = 34,116) NFHs. Data were downloaded from https://www.fishgen.net/ on August 21, 2020. Genetic loci common to both the PBT dataset and our GTseq panel were retained and filtered to remove excessive missing data as previously described. Ultimately, 264 loci were retained for PBT analyses.

Field data associated with carcass samples were compared to genetic data to check for congruence among the two data sources. First, tule and URB field-identifications were compared with genetic assignments from NewHybrids. This was conducted only for non-hybrid individuals. Secondly, the phenotypic sex of carcasses was compared with genetic determinations made from sequencing of the Ots_SEXY3-1 marker included in the GTseq panel. Phenotypic and genotypic sex determinations were also evaluated in the PBT dataset to provide a baseline of congruence rates to which data from the carcass samples could be compared.

Comparisons of genetic and field data were also conducted to determine if unmarked hatchery fish were returning to the White Salmon River, and the proportion of hatchery fish with hybrid ancestry. For this purpose, parentage assignments from PBT analysis were contrasted with relevant field data indicating hatchery origin. Fish with either a coded wire tag (CWT) present, or those lacking an adipose fin, were presumed to be of hatchery origin. Unmarked fish were presumed to be of wild origin. All fish that were assigned to hatchery parents were then cross-referenced with hybrid ancestry results to identify potential hatchery contribution to hybrid groups.

Results

Structure

CLUMPAK indicated high consistency among independent Structure replicates, with 22/24 replicates converging upon the same ancestry proportions. Thirty individuals with a high proportion of spring-run ancestry (Q > 0.2) were identified. These samples were discarded from the dataset, leaving 968 samples for further analysis (Table 1).

NewHybrids

NewHybrids assigned ancestry classes for most carcasses with high confidence (mean BPP = 0.97). Just 92 samples (9.5%) were assigned with BPP < 0.9, and only one sample was assigned with BPP < 0.5. The latter sample was excluded from all further analyses. The proportion of all carcasses belonging to any of the four hybrid classes (Figure 1) ranged from a low of 18.1% in 2018 to a high of 45.7% in 2015 (mean = 31.1% per year). Tule backcross was the largest overall hybrid class, representing just 3.6% of all carcasses in 2018 but 34.6% in 2015 (mean = 18.1% per year). A lower proportion of URB backcrosses was detected, ranging from 0% in 2015 to 12% in 2018 (mean = 5.9% per year). However, intentional URB carcass sampling was not conducted by field crews from 2014 to 2017. First generation (F1) hybrids were relatively rare in all years, ranging from 0% in 2017 to 13% in 2016. Very few 2016 fish were genotyped (n = 23), meaning the 13% F1 proportion represents just three fish.

Hybrid individuals were commonly detected among field-identified tule carcasses (Figure 2). The proportion of field-identified tule carcasses belonging to any of the four hybrid classes ranged from a low of 20.8% in 2019 to a high of 66.7% in 2020 (mean = 38.1% per year). Most hybrids were classified as tule backcrosses, which accounted for just 16.4% of tule carcasses in 2013 but 45.8% in 2020 (mean = 27% per year). First generation (F1) hybrids were rare in all years. No F1 hybrids were detected among tule carcasses from 2017-2019. The greatest proportion of F1 hybrids was observed in 2016 (13%).

Few hybrids were observed among field-identified URB carcasses relative to tule carcasses (Figure 3). The cumulative proportion of all detected hybrid classes among URBs ranged from 14.3% in 2018 to 22.7% in 2013 (mean = 15.7% per year). Backcrossed individuals represented the greatest proportion of hybrids among URB carcasses, ranging from 11.5% in 2019 to 14.9% in 2013. First generation hybrids were again rare. None were detected in 2020-2021, but 2.3% of URB carcasses collected in 2019 or earlier were F1 hybrids (mean = 1.2% per year).

The compilation of carcass collection dates across all study years revealed temporal trends in ancestry class abundance on spawning grounds from September through December. Hybrid individuals were present on the spawning grounds through the duration of both tule and URB fall runs but were most common during late October and early November. Notably, hybrids accounted for most carcasses

collected during the final week of October (63.4%; Figure 4). Tule backcrosses represented a plurality of carcasses (35.6%) collected during this time.

SNPPIT

Field data indicated presence of 25 hatchery-origin fish in our dataset, but hatchery parents were genetically assigned to 59 of 305 carcasses (19.3%) collected from 2018 through 2021 (tule: Table 2; URB: Table 3). The field-identified and genetically-identified hatchery-origin groups overlapped for just 20 samples, meaning 20% of known hatchery-origin individuals (n = 5) could not be assigned to hatchery parents via PBT analysis, and 39 unmarked fish were assigned with high probability (Pr > 0.99) to hatchery parents.

Relatively few hybrids were observed among the hatchery-origin carcasses (Table 4). These fish were almost exclusively field-identified as URB carcasses (61 of 64 fish; 95.3%) and genetically-identified as non-hybrid URB originating from LWS NFH (n = 56; 87.5%). Two field-identified tule carcasses were genetically assigned to hatchery parents, but only one of these fish was genetically determined to be a non-hybridized tule originating from Spring Creek NFH. Six hybrids originated from LWS NFH; of which five were URB backcrosses and one was an F1 hybrid.

The proportion of hatchery-origin hybrids detected among URB carcasses was much smaller than the proportion of wild-origin carcasses classified as hybrids of any type (Table 5). From 2018 to 2021, the proportion of hatchery-origin URB hybrids (0% to 10.53% per year) was consistently lower than the proportion of wild-origin URB hybrids (16.67% to 29.03% per year).

Field ID vs. Genetic ID

Field-identifications of carcasses to Chinook salmon populations mostly agreed with genetic classifications (Table 6). Annually, 5.7% of field-identified tule carcasses were genetically identified as URB carcasses. Most disagreements between field and genetic data (23/42; 54.8%) occurred when a subset of URB fish returned prior to October 1 (Figure 5). The highest rate of disagreement occurred in 2013 when 24 field-identified tule carcasses were genetically classified as URB (17.1%). Twenty of these 24 disagreements (83.3%) from 2013 were collected during September. In contrast, few genetic classifications of URB disagreed with field results. Just 1.0% of field-identified URB carcasses were classified as tule, with disagreements observed only in 2019 (n = 1) and 2021 (n = 2).

Phenotypic sex-identification of carcasses tended to agree with genotypic sex (Table 7). Most carcasses phenotypically identified as female (95.1%) had an XX genotype, while 3.4% were XY. Congruence for carcasses identified as male phenotype was lower, with 73.9% having an XY genotype and 14.9% being XX genotype. Much higher congruence was observed among samples from the PBT baseline, in which >99% congruence was observed for both male and female fish.

Discussion

Our results reveal several important trends of concern for the tule population in the White Salmon River. Notably, we observed proportions of hybrids among wild-origin carcasses exceeding those previously observed among juvenile life stages and in hatchery broodstock (Smith and Engle 2011; Smith et al. 2021). Hatchery strays, typically URB fish from LWS NFH, accounted for a greater proportion of carcasses collected from 2018-2021 than anticipated from adipose fin clips and CWT recoveries. Additionally, the proportion of hybrids among hatchery strays was lower than observed for wild-origin

fish. Here, we compare our results to those from earlier evaluations of this study system and consider the implications of these results for the threatened tule population in the White Salmon River.

Hybridization

Hybrid proportions observed among carcasses from wild-spawning salmon provide a different perspective on hybridization in the White Salmon River compared to previous studies which evaluated out-migrating juveniles or returning fish selected for spawning in hatcheries. The proportion of hybrids detected among carcasses exceeds the proportions detected among hatchery broodstocks from 2015 to 2018. Smith et al. (2021) detected few hybrids (typically tule backcrosses) among tule broodstock at Spring Creek NFH, comprising 1.18% to 1.46% of the tule broodstock per year. During these same years, the hybrid proportions among field-identified tule carcasses ranged from 35% to 45.7% annually. Tule backcross was also the most prevalent hybrid class among field-identified tule carcasses (23.1% to 34.6% per year).

Similar trends were observed for URB; however, drawing direct comparisons with the hybrid proportions calculated by Smith et al. (2021) is challenging because URB carcasses were not sampled from 2014 to 2017. However, trends at LWS NFH again showed that most hybrids among the URB broodstock were URB backcrosses, and these represented a stable proportion of the broodstock annually from 2015 through 2018 (6.73% to 7.89%). The URB carcass hybrid data overlap with this evaluation only for 2018, in which 14.3% of field-identified URB carcasses were hybrids (12.9% = URB backcrosses). Annual hybrid proportions were variable among URB carcasses (16.1% to 22.0%) for the following three years (2019 to 2021), but a stable proportion of URB backcrosses was observed (11.5% to 12.9%). These proportions indicate that although the existence of hatchery-spawned hybrids is concerning, the majority of hybrid spawners in the White Salmon River are of natural origin. We thus conclude that URBs spawning in the White Salmon River are a larger contributor to the hybrid issue than are culture practices at the adjacent hatcheries. We predict that hybridization will continue to pose a threat to the tule population for as long as large numbers of hatchery URBs continue to escape to the White Salmon River.

Comparison of our results to hybrid proportions observed in previous studies for juvenile fish is challenging due to differences in genetic sampling procedures for tule and URB carcasses that occurred over the duration of our study. Most importantly, annual hybrid proportions calculated for 2014 – 2017 reflect only field-identified tule carcasses, and thus could overestimate the actual proportion of returning hybrids that spawned in the White Salmon River due to differences in proportions of hybrids among these two populations (Figures 2 and 3). Additionally, the timing of collection for juveniles (e.g., April) during 2016 – 2018 may have captured more URBs which migrate out of the river later in the year than tules (Smith et al. 2021). With these caveats in mind, we found that the proportion of hybrids among carcasses in any year (18.1% to 45.7%; mean = 31.1%) exceeded estimates for hybrids among juvenile salmon evaluated from 2006-2008 (Maximum proportion = 15.0%, Smith and Engle 2011). Additionally, carcass samples collected in 2016 – 2018 represent parental generations for the cohorts of juveniles sampled by Smith et al. (2021) during 2017 – 2019. We detected greater hybrid proportions among carcasses than juveniles in 2016/2017 and 2017/2018, but these carcass data represent years with no URB collections. For 2018/2019, hybrids represented a lower overall proportion of carcasses (18.1%) than juveniles (28.7%). Due to the differences among these datasets, we are unable to identify clear temporal patterns or relate the proportion of juvenile hybrids with proportions of adult hybrids.

Selection

Outcomes of hybridization are notoriously difficult to predict because disruption of coadapted gene complexes can differentially impact fitness in different generations of hybrids (Wu and Ting 2004; McClelland and Naish 2007). Furthermore, genomic studies indicate that selection commonly acts upon Chinook salmon populations to yield localized adaptation and a diversity of life history traits that include differences in run timing by ecotype or population (Hecht et al. 2015; Narum et al. 2018; Willis et al. 2021), and hatchery practices can influence the genetic makeup of nearby native populations through introgressive hybridization (Hess et al. 2011). Therefore, it is reasonable to assume that both the native tule and introduced URB populations have adapted to the environmental conditions of their native spawning grounds, and different hybrid classes will exhibit different levels of reproductive fitness.

Variability in the relative abundance of different hybrid classes reveals concerning trends among White Salmon River populations. A greater proportion of backcrossed individuals exists relative to F1 and F2 hybrids. This trend was variable among years, but approximately 4.9 backcrossed individuals were observed for every F1 or F2 hybrid. This indicates that disruptive selection, which favors phenotypic extremes relative to intermediate forms (Mather 1953), is acting to maintain some separation of the tule and URB populations. Selection is hypothesized to act against intermediate life history traits in salmonids; but this is typically applied to those with drastically divergent life history traits or those with rigid years of return (Gharrett et al. 1999; Wang et al. 2004). However, tule and URB life histories are similar; primarily differing by time spent in freshwater prior to spawning, time of spawning, and time of out-migration. These similarities possibly contribute to the successful reproduction of hybrids observed in the White Salmon River.

Another important trend is that hybrid carcasses were found throughout most of the spawning season, overlapping the spawning period for both tule and URB runs (Figure 4). F1 and F2 hybrids show intermediacy of run timing, with F2 fish returning over a longer span of time than F1 individuals. Although F1 and F2 hybrids were collected at relatively low frequency, they represent a regular presence on the spawning grounds and provide a potential bridge for introgression of foreign alleles into either population as observed for other salmonid species (Muhlfeld et al. 2009).

Tule backcrosses also show intermediacy of run-timing but comprise a large proportion of the returning fish that arrive on the spawning grounds at the end of the tule spawning period. During approximately the last week of October, they comprise a plurality of returning Chinook salmon (Figure 4A). This timing is concerning due to potential effects on the non-hybridized tule population, some of which overlap with suggested ecological impacts of URB spawning upon the tule population (e.g., superimposition of URB redds on tule redds; Silver et al. 2022). Spatial and temporal overlap of tule and URB spawning typically occurs during the end of October (Dammerman et al. 2022) when the two populations utilize similar habitats (although URBs also utilize upstream habitats relative to tules; Wilson et al. 2020). Therefore, consideration should also be given to impacts of hybrid tule backcross redds superimposed on non-hybrid tules. Based upon their genetic composition, we infer the backcrossed tules to share habitat preferences aligning more closely with non-hybrid tules. Consequently, although hybridization impact has previously been considered in the context of lost population productivity (Silver et al. 2022), we should also consider whether these hybrid individuals pose a yet unquantified threat to the non-hybridized population through other ecological impacts such as redd superimposition.

Hatchery-Origin Strays

Homing behavior of anadromous salmonids is facilitated through olfactory imprinting of juvenile fish on unique odor signatures present at their natal waters (Hasler and Scholz 1983; Dittman and Quinn 1996), hormones and pheromones released by conspecifics (Courtenay et al. 1997, 2001), and an innate genetic component (McIsaac and Quinn 1988; Keefer and Caudill 2014). However, imprinting of these homing queues are disrupted for hatchery-origin salmon, which will stray to non-natal streams at greater rates than wild-origin fish (Ford et al. 2015). Straying is further influenced by hybridization among populations and life history characteristics, with higher incidence of straying observed among hybrid stocks and Chinook salmon populations that out-migrate during their first year of life (Candy and Beacham 2000; Westley et al. 2013). These characteristics describe known conditions of the fish spawned at hatcheries near the White Salmon River (Smith et al. 2021), indicating that straying of hatchery stocks should be expected. Therefore, two issues must be considered: the rate at which hatchery-origin fish stray to the White Salmon River, and the question of whether this straying is driven by tule *x* URB hybrids released from nearby hatcheries.

Unfortunately, our ability to draw conclusions about hatchery strays from Spring Creek NFH is limited since few strays were detected from this hatchery. However, our results show that fish from LWS NFH are straying to the White Salmon River at a greater rate than indicated by hatchery-marked carcasses. Approximately 30% of URB carcasses sampled from 2018-2021 originated from LWS NFH; most of which lacked physical hatchery markings. The number of unmarked fish was greater than expected, with 65% of hatchery-origin carcasses detected exclusively via PBT analysis. The reason for this discrepancy is uncertain, but is consistent with other PBT studies and most likely stems from tag loss (e.g., Hargrove et al. 2021). Adipose regeneration is rare if the fin is completely removed at its base, but incomplete fin removal can yield regrowth (Thompson and Blankenship 1997). Estimated CWT loss rates are low for Chinook salmon (e.g., frequently < 2%; Blankenship 1990; Vander Haegen et al. 2005). However, other studies indicate variable loss rates for different species (Kolari and Hirvonen 2006; Beacham et al. 2019). Tag loss estimates are also influenced by the amount of time that passes between tagging and loss assessment (Kolari and Hirvonen 2006).

It is important that hatchery-origin carcass counts from spawning ground surveys are accurate because models for inferring hatchery stray rates depend upon counts of adipose-clipped carcasses (Wilson et al. 2020). In summary, the total number of returning fish for each of the tule and URB runs is estimated by a trapezoidal 'area under the curve' approach, and proportions of hatchery and wild-origin fish are estimated from the proportion of recovered hatchery-marked carcasses combined with corrections for the proportion of individuals left untagged by mass marking protocols (Parsons and Skalski 2010; Wilson et al. 2020). These models, in the absence of PBT data, estimated that 64.5% of URB spawners in the White Salmon River were of hatchery origin from 2018 to 2021 (WDFW SCoRE website accessed 3/29/2023). These population estimates are more than double the proportion of hatchery-origin fish detected among URB carcasses using PBT data but could be driven higher if expanded knowledge of hatchery returns derived from PBT data are incorporated.

We also note that PBT methods could still provide a slight undercount of hatchery-origin fish for our dataset. Five fish of known hatchery origin were left without parental assignments, possibly resulting from incomplete baseline data. The PBT baseline for Spring Creek NFH tule broodstock begins in 2015. Given the average return age of Chinook salmon at 3-4 years (Healey 1991), it is possible that

parents of some tule carcasses collected in 2018 were not genotyped, thereby reducing our ability to genetically identify hatchery-origin tule in our dataset. The PBT baseline for URB broodstock from LWS NFH begins in 2013, meaning that age of returning fish relative to the those included in the baseline is an unlikely factor for assignment failure. Rather, the inability to assign four hatchery-origin URBs to their parents probably resulted from a small number of genotyping errors or missing parents (Steele et al. 2013). Parental assignment failure also occurs if just one of an individual's parents is absent from the PBT baseline because SNPPIT's algorithm does not reconstruct genotypes for unsampled parents (Anderson 2012).

Annual proportions of hybrids detected among LWS NFH strays indicate that unintentional production of hybrids likely contributes less to contemporary hybridization than does wild reproduction. Similar proportions of hybrids were observed among LWS NFH broodstock (8.4%; Smith et al. 2021) and carcasses from LWS NFH strays (8.2%; Table 5). This is somewhat contradictory to the results of Candy and Beacham (2000) who reported an approximately threefold greater proportion of strays among hybrids. Rather, we observed proportions of wild-origin hybrids 2.8 times greater than hatchery-origin hybrids (Table 5). This is concerning because the return of wild-spawned hybrids will not be directly remedied through changes in hatchery practices, thus limiting potential management options if managers decide this issue must be addressed. Furthermore, the intermediate run timing of hybrids could create a cycle that leads to increased hybridization among White Salmon River populations (Figure 4).

Concordance of field and genetic identifications

Comparison of field and genetic determinations of ancestry revealed that putatively misidentified fish belong to two temporally-defined groups. The first contained genetic URBs that arrived during September to early October but were field-identified as tule. Most of these fish (n = 21 of 25) originated from sample year 2013, which was the first year of expanded spawning ground surveys conducted in the White Salmon River by WDFW. Assumptions of return timing for these fish could have factored into this discrepancy since URB typically spawn later in the fall. The second group of discordantly identified fish arrived in mid-October to early November. This group was mostly composed of genetic URBs field-identified as tules, but three genetic tules field-identified as URBs were also among this group.

Comparisons of field and genetic identifications of fish indicate the number of tule carcasses observed in the White Salmon River might be slightly overestimated in most years. Tules are putatively misidentified at a rate of 5.7% per year compared to 1.0% for URBs but this difference is somewhat moderated when sample year 2013 is excluded from calculations. If we consider 2013 as an outlier, then 4.2% of tules per year were putatively misidentified compared to 1.2% of URBs. Population estimates relying upon accurate counts of carcasses could be impacted by these misidentifications, but the magnitude of impact is uncertain given that misidentifications are frequently 1-2 fish per year. Fewer tule carcasses are sampled in most years relative to URB carcasses. Therefore, removing 1-2 carcasses from tule counts will have a slightly greater impact for tule estimates than adding these fish to counts of URB carcasses. Such impacts could be most visible when estimating proportions of wild and hatchery-origin fish (Wilson et al. 2020).

The patterns of putative misidentification run counter to those noted for live fish used for hatchery spawning. Most discrepancies occurred for carcasses that were genetically URB but called as

tule during field collection. In contrast, 0.09% to 0.46% of broodstock at LWS NFH from 2015 to 2018 were genetically identified as tule while 0% to 0.02% of broodstock per year at Spring Creek NFH were URB (Smith et al. 2021). This indicates that variables confounding field identification of living and dead fish are likely different, as evidenced by relative differences recorded from hatchery and field data. We infer these variables could include state of decomposition or weather conditions, but identification of these confounding variables would require further study.

Comparisons of phenotypic sex data to sex genotypes derived from the GTseq panel indicated high discordance in carcass samples, particularly for male genotypes. The sources of error leading to this discordance are unclear. Previous studies using the Ots_SEXY3-1 genetic marker demonstrated 100% concordance of genotype and phenotype for Snake River Chinook salmon populations (Steele et al. 2012). Sex marker accuracy can vary among Chinook salmon populations (Von Bargen et al. 2015), but this is an unlikely explanation for the observed discrepancies because >99% concordance of genotypes and phenotypes was observed in the PBT baseline dataset (Table 7). Additionally, no differences in sex marker accuracy were apparent when carcass samples were grouped and reanalyzed according to run or hybrid class (data not presented). This suggests a potential for lower accuracy in sex genotyping that we hypothesize could be correlated with carcass condition, and indicates a need to better explore sources of error that can influence genetic sex marker accuracy in carcass samples (Venditti et al. 2022).

Conclusion

Genetic analysis of tule and URB carcasses has elucidated several important trends with implications for population monitoring and management of fall-run Chinook salmon populations of the White Salmon River. We found that hatchery URBs are straying to the White Salmon River at a greater rate than previously detected, as evidenced by parental assignment of untagged individuals to hatchery-spawned parents. The improved quantification of untagged hatchery-origin URBs will aid in refining estimates of hatchery stray rates. We also found that field-identifications of non-hybridized fish were largely in agreement with genetic classifications, meaning misidentification of non-hybridized tules and URBs likely has minimal impact on population size estimation. Rather, hybridization among wild-origin fish is more common than previously expected and is occurring at a greater rate than observed among hatchery fish. Most carcasses recovered during the overlap between tule and URB runs in late October were hybrids, meaning the hybridization among wild fish has likely resulted in a greater overlap of run timing between the tule and URB populations. Backcrossed individuals were detected in both populations, and were especially prevalent in the tule population, meaning that wild-origin hybrids are returning to the White Salmon River and successfully reproducing with both the tule and URB populations. These factors, combined with the intermediate run-timing of hybrids will continue to yield wild-origin hybrids in the White Salmon River if current trends are sustained.

Acknowledgements

Data Management

Fin clips and extracted DNA have been permanently archived at AFTC's facility and the associated biodata stored on internal servers. Raw genotypes have been saved on AFTC databases and will be made available to other researchers and the public upon request.

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Tables

Table 1. The number of tule, URB, and Spring-run Chinook salmon carcass samples collected per year (left) and retained for analysis (right) following completion of all data filtering steps. Carcasses with >15% missing genotypes were removed from analysis. No URB samples were collected from 2015 through 2017.

Year	Collected			Analyzed		
	Tule	URB	Spring	Tule	URB	Spring
2013	174	174	2	140	141	0
2014	253	1	16	190	1	0
2015	173	0	13	127	0	0
2016	35	0	7	23	0	0
2017	44	0	1	40	0	0
2018	13	76	6	13	70	0
2019	26	60	0	24	52	0
2020	28	32	0	24	31	0
2021	43	56	0	41	50	0
Total	**789**	**399**	**45**	**622**	**345**	**0**

Table 2. Assignment of fall-run tule Chinook salmon carcasses to hatchery parents. Parentage Based Tagging (PBT) genotype data were available for fish spawned at Spring Creek NFH beginning in 2015. Carcasses sampled from 2018 to 2021 were considered for this analysis, as these represent potential return years for the PBT data. (A) shows the counts of samples belonging to each of several categories. The 'Genotyped' column represents the total number of tule samples genotyped per year. 'Assigned' indicates samples that were assigned to hatchery parents via PBT analysis. 'Marked' indicates the number of samples with a clipped adipose fin or coded wire tag present. 'Unassigned' represents marked samples that could not be associated with hatchery parents. 'Unmarked' carcasses are those in the 'Assigned' group that lack hatchery markings. (B) shows the percent of samples for each group in (A), as well as the percent of all individuals determined to be of hatchery origin by any method (%Hatchery).

(A)

Year	Genotyped	Assigned	Marked	Unassigned	Unmarked
2018	13	1	1	1	1
2019	24	1	0	0	1
2020	24	0	0	0	0
2021	41	0	0	0	0
Total	102	2	1	1	2

(B)

Year	%Hatchery	%Assigned	%Marked	%Unassigned	%Unmarked
2018	15.38%	7.69%	7.69%	7.69%	7.69%
2019	4.17%	4.17%	0.00%	0.00%	4.17%
2020	0.00%	0.00%	0.00%	0.00%	0.00%
2021	0.00%	0.00%	0.00%	0.00%	0.00%
Overall	2.94%	1.96%	0.98%	0.98%	1.96%

Table 3. Assignment of fall-run URB Chinook salmon carcasses to hatchery parents. Parentage Based Tagging (PBT) genotype data were available for fish spawned at Little White Salmon NFH beginning in 2013. Carcasses sampled from 2018 to 2021 were considered for this analysis, as these represent potential return years for the PBT data. (A) shows the counts of samples belonging to each of several categories. The 'Genotyped' column represents the total number of URB samples genotyped per year. 'Assigned' indicates samples that were assigned to hatchery parents via PBT analysis. 'Marked' indicates the number of samples with a clipped adipose fin or coded wire tag present. 'Unassigned' represents marked samples that could not be associated with hatchery parents. 'Unmarked' carcasses are those in the 'Assigned' group that lack hatchery markings. (B) shows the percent of samples for each group in (A), as well as the percent of all individuals determined to be of hatchery origin by any method (%Hatchery).

(A)

Year	Genotyped	Assigned	Marked	Unassigned	Unmarked
2018	70	15	8	1	8
2019	52	16	3	3	16
2020	31	7	1	0	6
2021	50	19	12	0	7
Total	203	57	24	4	37

(B)

Year	%Hatchery	%Assigned	%Marked	%Unassigned	%Unmarked
2018	22.86%	21.43%	11.43%	1.43%	11.43%
2019	36.54%	30.77%	5.77%	5.77%	30.77%
2020	22.58%	22.58%	3.23%	0.00%	19.35%
2021	38.00%	38.00%	24.00%	0.00%	14.00%
Overall	30.05%	28.08%	11.82%	1.97%	18.23%

Table 4. Hatchery origin carcasses detected from 2018 through 2021. Rows indicate field-identifications of Chinook salmon fall runs, whereas columns indicate their genetic classifications as determined in NewHybrids. F1 = first generation hybrid; F2 = second generation hybrid; Tule Bx = tule backcross; URB Bx = URB backcross.

	Tule	URB	F1	F2	Tule Bx	URB Bx	Total
Tule	1	-	-	-	1	1	3
URB	-	56	1	-	-	4	61
Total	1	56	1	0	1	5	64

Table 5. Proportions of hybrids among wild and hatchery-origin carcasses collected from 2018 through 2021 for tule and URB fall-run Chinook salmon. 'Hatchery Origin' includes all hatchery-origin fish determined by any method. 'Wild Origin' includes all untagged fish. The 'Samples' columns indicate the number of carcasses determined to be either wild or hatchery-origin. 'Hybrids' indicates the number of samples genetically determined to belong to any hybrid category by the program NewHybrids. The '%Hybrid' column shows the proportion of hybrids among wild and hatchery-origin groups per year. No hatchery-origin tule carcasses were documented in 2020 or 2021.

Year	Run	Wild Origin				Hatchery Origin		
		Samples	Hybrids	%Hybrid		Samples	Hybrids	%Hybrid
2018	**Tule**	11	3	27.27%		2	2	100.00%
	URB	54	9	16.67%		16	1	6.25%
2019	**Tule**	23	5	21.74%		1	0	0.00%
	URB	33	8	24.24%		19	2	10.53%
2020	**Tule**	24	16	66.67%		-	-	-
	URB	24	5	20.83%		7	0	0.00%
2021	**Tule**	41	18	43.90%		-	-	-
	URB	31	9	29.03%		19	2	10.53%
Total	**Tule**	99	42	42.42%		3	2	66.67%
	URB	142	31	21.83%		61	5	8.20%

Table 6. The number of fall-run Chinook salmon carcasses per year for which field and genetic assignments disagreed. Columns under the "Field ID" heading indicate the number of field-identified tule and URB carcasses that were successfully genotyped per year. Columns under the "Misidentified" heading represent the number of carcasses from each field-identified sample group that were genetically classified as belonging to the other sample group (e.g., the "Disagree Tule" column shows the number of field-identified tule that were genetically-identified as URB). The "% Disagree" columns show the proportion of samples collected in each year for which field and genetic identifications disagreed. Samples assigned to hybrid categories were not considered. No field-identified URB carcasses were collected in 2015-2017.

Year	Field ID Tule	Field ID URB	Disagree Tule	Disagree URB	% Disagree Tule	% Disagree URB
2013	140	141	24	0	17.1%	0.0%
2014	190	1	10	0	5.3%	0.0%
2015	127	-	2	-	1.6%	-
2016	23	-	1	-	4.3%	-
2017	40	-	1	-	2.5%	-
2018	13	70	1	0	7.7%	0.0%
2019	24	52	1	1	4.2%	1.9%
2020	24	31	2	0	8.3%	0.0%
2021	41	50	0	2	0.0%	4.0%

Table 7. Sex-identification concordance for genetic samples of (A) fall-run Chinook salmon carcasses collected from the White Salmon River, and (B) hatchery-spawned fish from Little White Salmon and Spring Creek National Fish Hatcheries. Columns represent phenotypic sex (i.e., Female and Male), whereas rows indicate genotypic sex (XX and XY). 'Unknown' indicates that either the sex phenotype was not recorded at time of sample collection (columns) or that genotyping efforts failed (rows). Intersections of rows and columns show concordance (e.g., 639 female phenotype carcasses were XX genotype) or discordance (e.g., 23 female phenotype carcasses were XY genotype).

(A)

	Female	Male	Unknown
XX	639	44	0
XY	23	218	0
Unknown	10	33	0

(B)

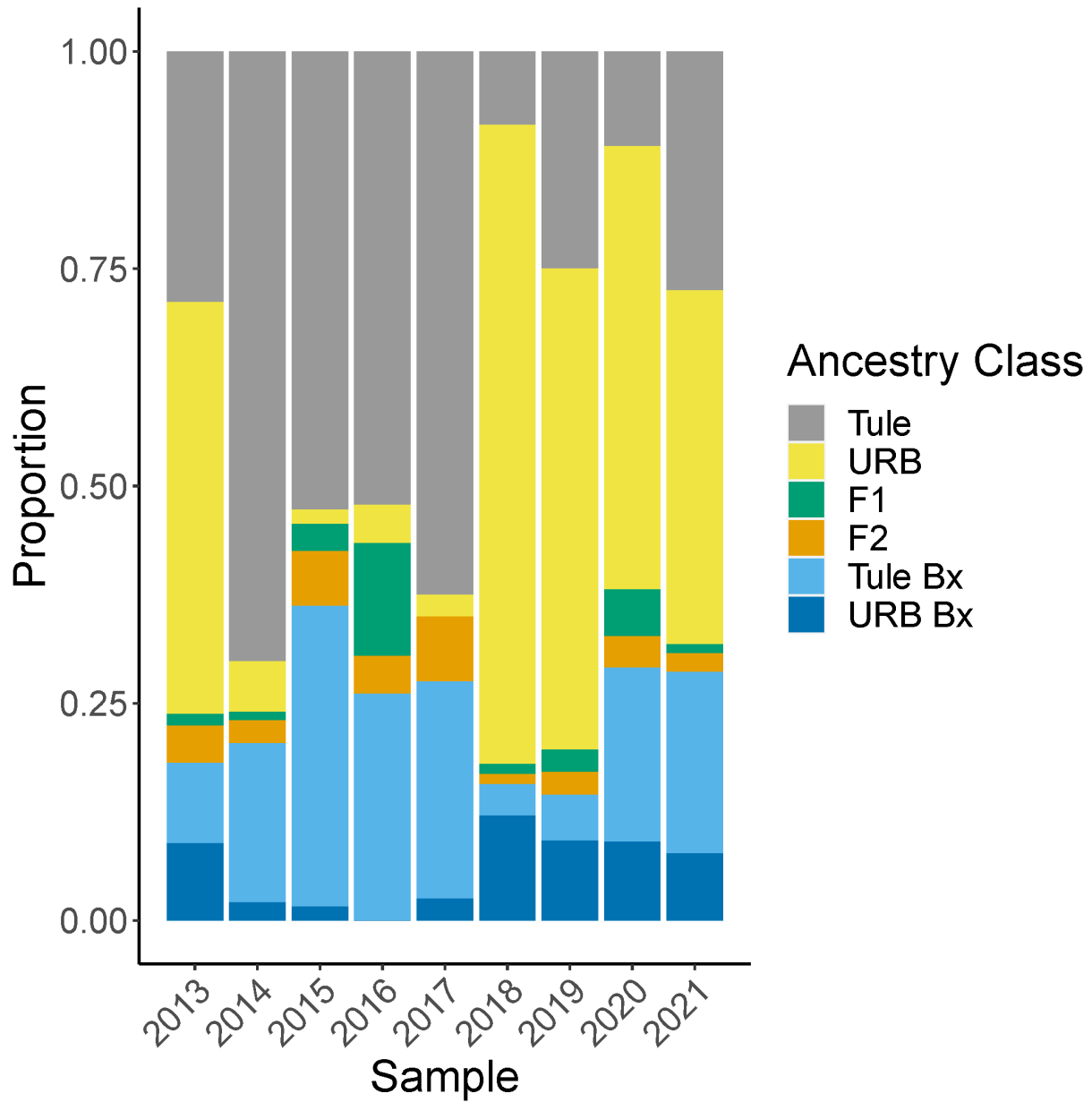|	Female	Male	Unknown
XX	29,145	134	123
XY	132	24,984	8
Unknown	55	61	0

Figure 1. Genetic ancestry classifications in NewHybrids of 967 Chinook salmon carcasses collected from the White Salmon River from 2013 to 2021. The colored segments of each bar represent the proportion of carcasses assigned to each genetic ancestry class in each sample year.
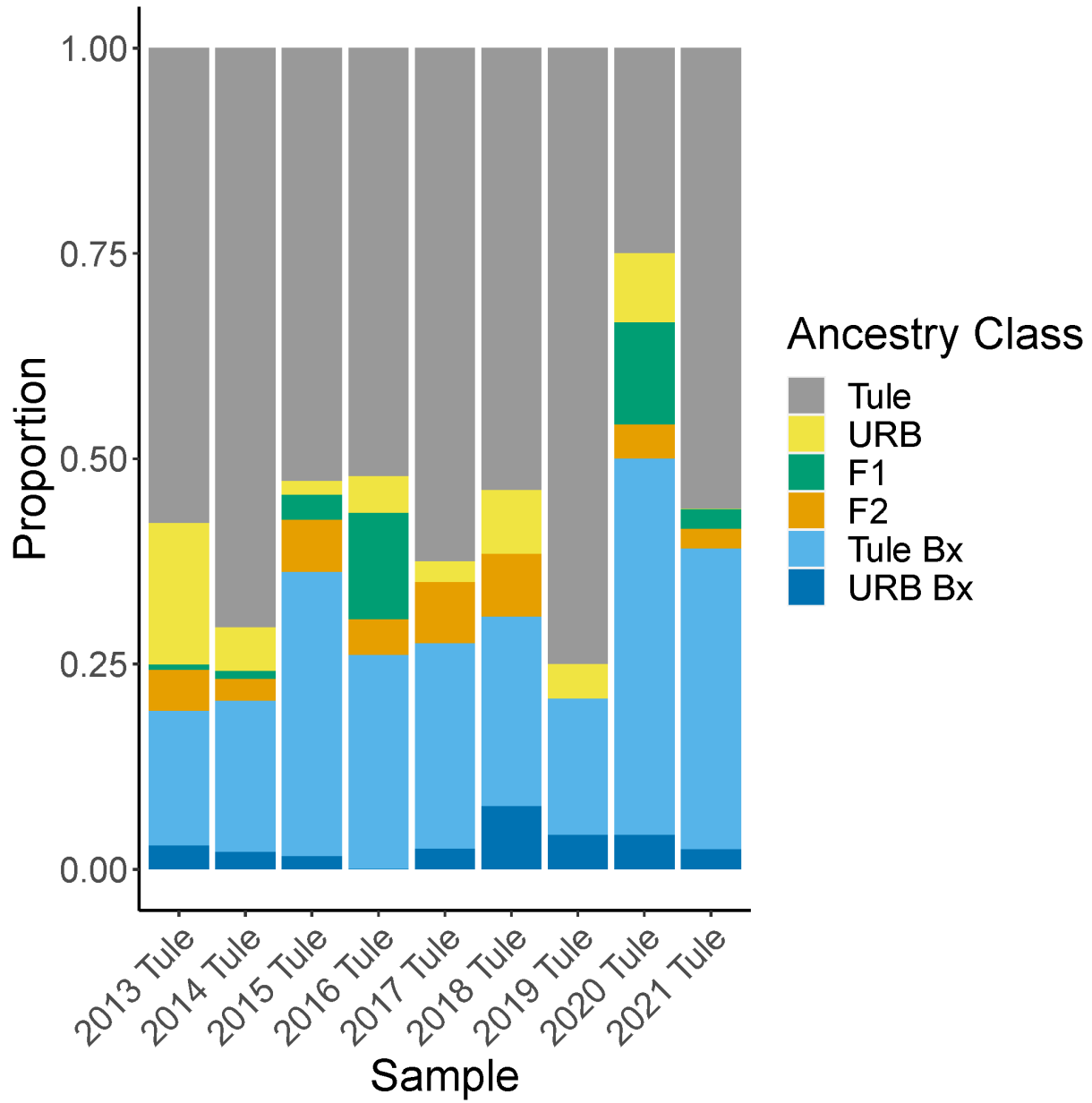
Figure 2. Genetic ancestry classifications in NewHybrids of 622 field-identified tule carcasses collected 2013 to 2021. The colored segments of each bar represent the proportion of tule carcasses assigned to each genetic ancestry class in each sample year.
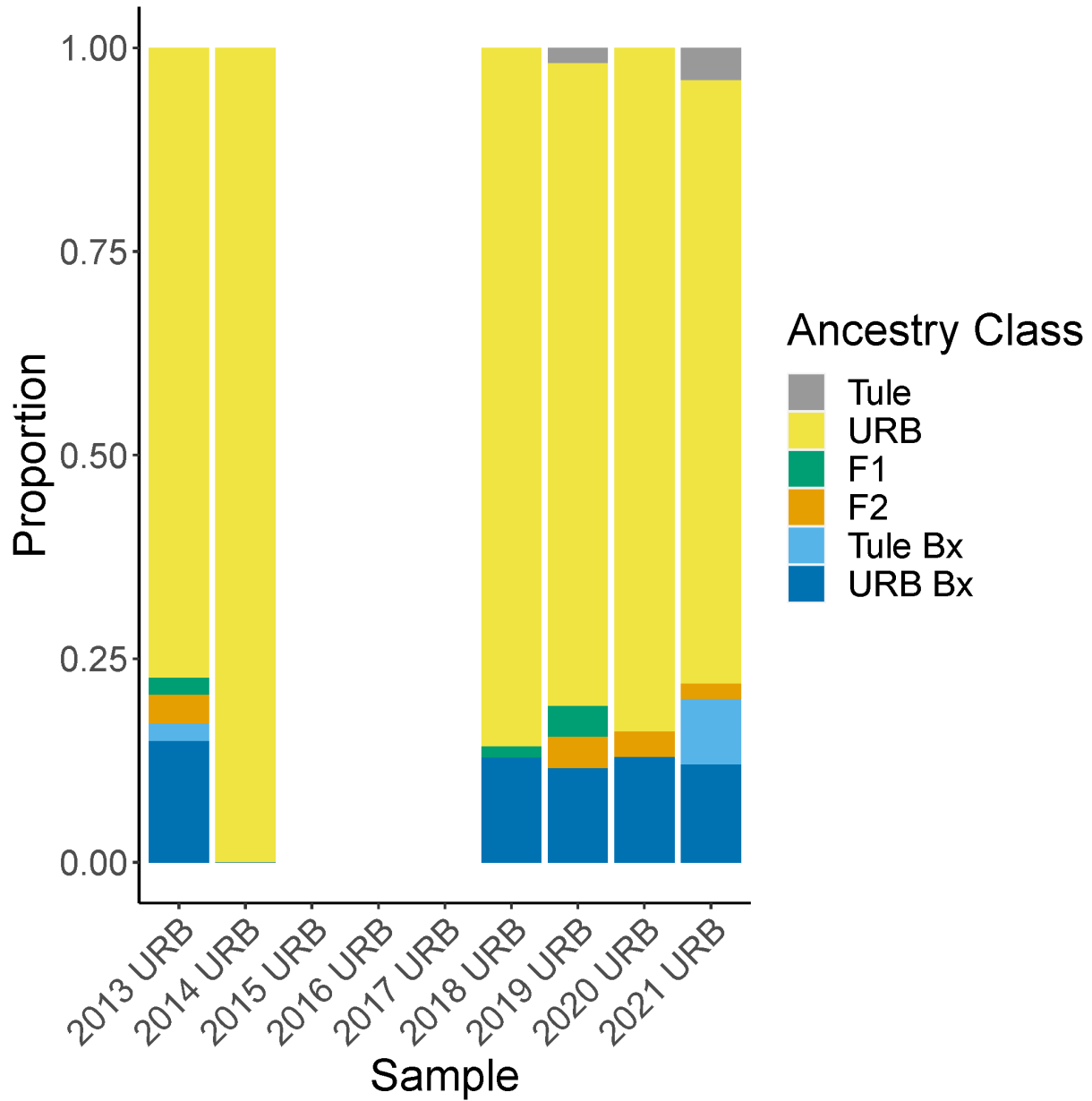
Figure 3. Genetic ancestry classifications in NewHybrids of 345 field-identified URB carcasses collected 2013 to 2021. The colored segments of each bar represent the proportion of URB carcasses assigned to each genetic ancestry class in each sample year. A single URB individual was collected in 2014, and no URB collections were made from 2015-2017.
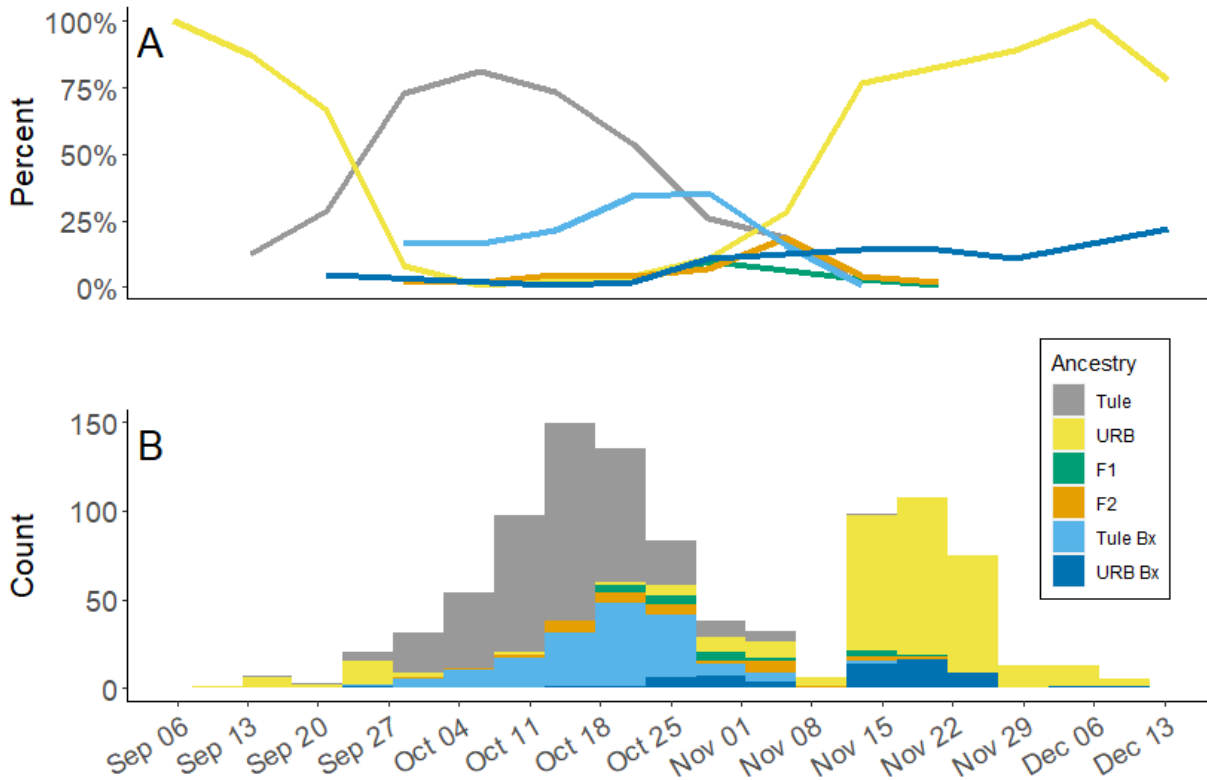
Figure 4. The proportion (A) and count (B) of samples belonging to each ancestry class arranged by sampling date. The x-axis represents the duration of carcass sampling for fall-run Chinook salmon collected from 2013 to 2021. Bins represent 1-week intervals. Colors represent the six ancestry classes to which samples were assigned based upon genotype data. Carcass collection dates may not correspond directly to spawning dates due to individual variability in post-spawn lifespan as well as time lapse between death and carcass collection.
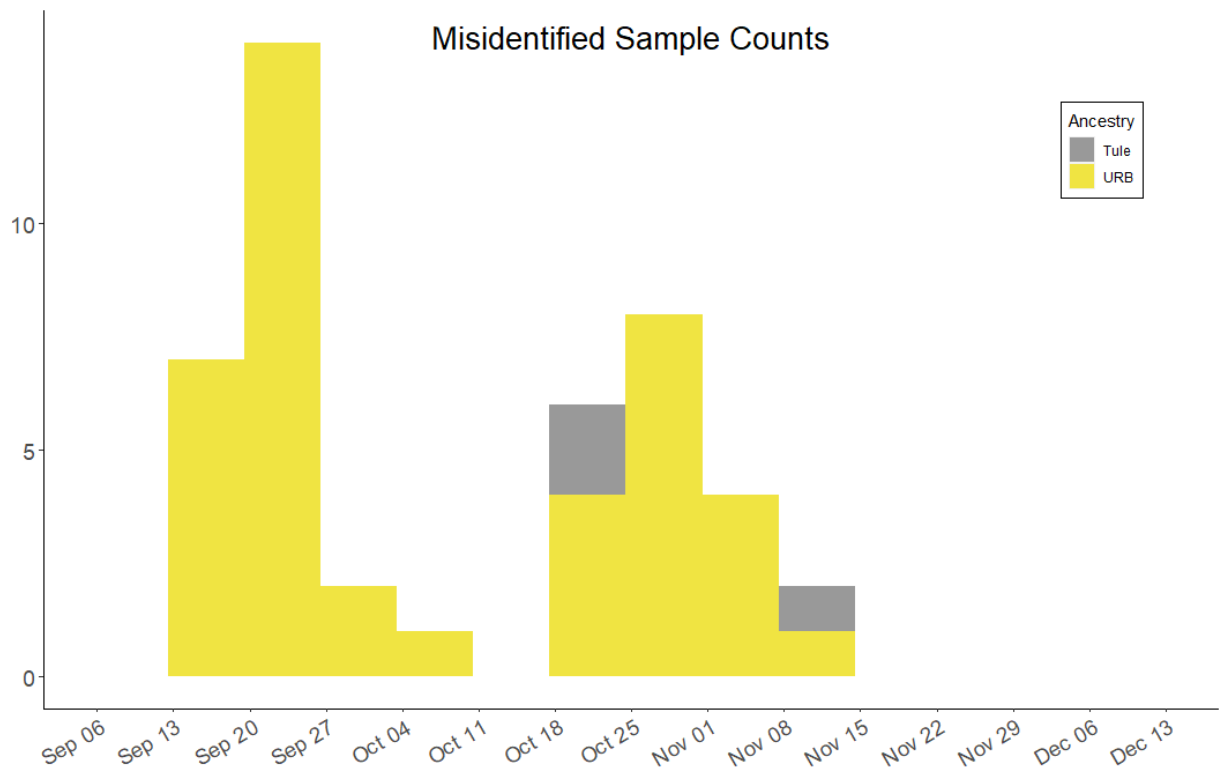
Figure 5. The counts of putatively misidentified samples arranged by sampling date. The x-axis represents the duration of carcass sampling for fall-run Chinook salmon collected from 2013 to 2021. Bins represent 1-week intervals. The y-axis shows counts of putatively misidentified carcasses within each bin. Color signifies the genetic ancestry of each misidentified carcass.

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