INFORMING CONSERVATION ACTIONS: GENETIC APPROACHES TO CHINOOK SALMON MANAGEMENT IN THE SACRAMENTO RIVER OF CALIFORNIA

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Integrative Biology – Doctor of Philosophy
Ecology, Evolutionary Biology, and Behavior – Dual Major

2023

ABSTRACT

Biocomplexity provides many benefits to ecosystems, chief among them allowing for species persistence and resilience. Genetic diversity is the fundamental building block that allows for intraspecific diversity. Unfortunately, anthropogenic changes to the environment have led to a sharp decrease in the abundance of many species, therefore reducing genetic diversity within and among populations. This increases the need for ways to monitor this complexity, and genetic methods are a very promising tool. In this dissertation, I explore biocomplexity through the lens of life history diversity in Chinook salmon (*Oncorhynchus tshawytscha*), a culturally, ecologically, and economically important species. The Sacramento River of California is the only place throughout the entire range where fish return at four different times during the year to spawn (Fall, Late Fall, Spring, and Winter), providing them with important adaptive variation to ensure population persistence and resilience. This phenotype, known as their run type or run timing, can be identified using genetics and is important for monitoring, as the run types are morphologically indistinguishable at most life stages and two of the run types are federally listed as threatened and endangered (Spring and Winter, respectively). My research explores broadly how we can use genetic methods to assess and monitor biocomplexity of imperiled species in highly altered environments using genetic tools and methods.

In Chapter 1, "Genetic Assessment of Floodplain Habitat Use by Juvenile Chinook salmon", I explore how biocomplexity in juvenile Chinook salmon in a managed floodplain in California can buffer the effects of climate change. To do this, I genetically identified juvenile Chinook salmon samples from surveys in the Sacramento River and Yolo Bypass to run type using an 80 loci informative Fluidigm panel. I first analyzed how accurate current management methods are at identifying run-type as compared to genetic methods, finding that current methods are often inaccurate. I further found that drought conditions had negative impacts on imperiled populations of juvenile Chinook salmon. Despite this, I found that even during periods of drought, the Yolo Bypass juvenile Chinook salmon attained larger sizes than the adjacent Sacramento River, suggesting that managed floodplain is critical for maintaining diversity in this system.

In Chapter 2, "Remnant salmon life history diversity rediscovered in a highly compressed habitat", I explore how genetic tools can be utilized to understand run timing in the Yuba River of California. I did this by assigning individuals to early or late migrating groups, based on

informative genetic markers from the GREB1L region of the Chinook genome and compared that to date of entry in the system. I found that despite large amounts of anthropogenic alteration, the Yuba River supports life history diversity of Fall and Spring run types, and this diversity is correlated with the genetic markers in the GREB1L region of the Chinook genome. This study highlights the incredible resilience of Chinook salmon populations in the Yuba River, as well as validates exciting new genomic regions that can be used for monitoring populations in the Sacramento River.

In Chapter 3, "Genetic divergence of recently introduced populations of Chinook salmon (*Oncorhynchus tshawytscha*) in New Zealand", I explore how New Zealand populations of Chinook salmon compare genetically to each other, as well as how they have diverged from their source, Chinook salmon from the Sacramento River of California. This research is the first time New Zealand Chinook salmon have been compared to all four run types in the Sacramento River and is a critical first step in understanding those relationships. To do this, I analyzed genomic data obtained from genotyping by sequencing and restriction site associated DNA sequencing data from Chinook salmon sampled in rivers in New Zealand and all major tributaries and run types from the Sacramento River. I found that there is genetic structure between the different rivers in New Zealand, and that although New Zealand fish have diverged from the Sacramento River fish, they appear more genetically similar to contemporary Fall and Spring run populations than to contemporary Winter run. This research highlights the importance of genomic tools to understand genetic relationships and could inform restoration efforts such as genetic rescue.

This body of work highlights the importance of using genetic tools for management of imperiled species, especially identifying and monitoring biocomplexity. It also addresses how anthropogenic activities can impact species and systems, which will be important in informing how to mitigate potential impacts on imperiled species.

To all those who wander and are lost: this is the beginning of the journey, not the end. One day you will find the light that was taken from you again.

ACKNOWLEDGMENTS

The PhD is an immense undertaking with many challenges that cannot be completed without the help of others. I am incredibly thankful for all those who have helped and mentored me on this journey. At the top of this list is my advisor, Dr. Mariah Meek, for answering the email of a wandering professional in need of a doctoral home, and for all the guidance and tutelage after that. Mariah built a wonderful community that is a testament to her skill and compassion. My academic home was truly a home thanks to her. I would next like to thank my committee for their mentorship. Kim Scribner, Sarah Fitzpatrick, and Nadya Mamoozadeh provided invaluable feedback to make this dissertation the best it could be, all while suffering through numerous proposal and chapter drafts. I would also like to thank the endless support of my lab mates, who provided commiseration and solace through this difficult journey. In particular, I thank Miranda Wade for taking this journey with me, providing many opportunities for books, coffee, and analysis to help me through these last 6 years. I also want to acknowledge Ben Kline, who brought light and laughter to our lab, and was always there with a baked good and hug when the journey was difficult, pushing me through the last stage of my dissertation when it was darkest. I also thank my other lab members, past and present, for their support including Charlene Tarsa, Sierra Kaszubinski, Shannon O'Leary, Tasha Thompson, Hannah Rothkopf, Erin Collins, Alexandra Zhang, Emily Bardwell, Arianna Troia, and Scott Jackson.

This dissertation would not be possible without the support and resources provided to me of many people and institutions. I received financial support from fellowships and grants, including Ecology, Evolution, and Behavior summer fellowships, travel fellowships through the Council of Graduate Students, the MSU Graduate School, and the American Fisheries Society Genetics Section, and the MSU Dissertation Completion Fellowship, just to name a few. I would also like to acknowledge our many authors and collaborators. Special thanks to Ted Sommer, Louise Conrad, Alisha Goodbla, Brian Schreier, and Pascale Goertler for your time and expertise on the Yolo chapter. Special thanks to Malte Willmes, Rachel Johnston, Flora Cordoleani, Anna Sturrock, and Natalie Stauffer-Olsen for your help writing the Yuba Chapter. Thank you to Andrea Schreier and Emily Funk for use of your genomic resources, and particularly Andrea Schreier for all your help with friendly review and genetic analysis. Thank you to Daniel Gomez-Uchida and Rodrigo Marin Nahuelpi at the Universidad de Concepción for your genetic resources on the New Zealand samples. Lastly, thank you to Melanie Cheung and especially the

Winnemem Wintu tribe for your precious resources and for helping the New Zealand chapter become a reality.

I also give thanks to the many other friends that helped me along this journey. To all the IBIO and EEB girlies, this community at MSU wouldn't have been possible or even nice without you. In particular, I thank Emily Liljestrand for indulging me in rants, providing me snacks, and calling me when times got tough. I also thank those the friends I had before graduate school. Eleni Shenk for being the best friend a girl could have, Adriana Hertel-Wulff for our solace together as scientists at a young age, and Bryce Williams and Ryan Price for your friendship and laughter over the years.

Getting into graduate school and pursuing my dreams would not be possible without the help of countless mentors throughout my educational career. Thanks to the many biology teachers who encouraged my love of all natural things, but in particular, Herond Hoyt, who pushed me to be the best biologist I could be. My thanks go out to Carl Safina, who gave a young high school student career advice and then later provided mentorship and letters of recommendation to get me here. To Wayne Potts and Jimmy Ruff, who first gave me my first research experience and opened the door to graduate school, one doorknob I would not have even seen. Special thanks to Jimmy who taught me invaluable genetics skills and mentored me for hours to interview for PhD programs. To Mark Elvin, who reminded me again of my value when the degree ground my confidence down.

And lastly, I am thankful to my family. Grey, Drew, but especially Mom and Dad for your support throughout my entire life to helping me pursue crazy dream. To my parents for supporting me through endless standardized tests, trips to destinations to nurture the budding biologist, helping me move more than once without a sun visor, and supporting me through the process of applying to graduate school not once or twice but five times. I will be forever grateful. And most thanks to my small family, Fluff and Zac, who have perhaps suffered most on my behalf in this doctoral journey, having encountered a very different woman six years ago when I started this journey. Thank you Fluff for the near constant snuggles and diligent supervision when writing this dissertation. And most of all Zac. I couldn't imagine a better companion on this dissertation journey, and you have been there since the start. I am grateful for all the tears you wiped away when I truly believed I couldn't do it. This dissertation is here because of you.

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CHAPTER 1: GENETIC ASSESSMENT OF FLOODPLAIN HABITAT USE BY JUVENILE CHINOOK SALMON

Chapter 1: This chapter has been submitted for publication to San Franscisco Estuary and Watershed Sciences and is currently under peer-review.

Other contributing authors: J. Louise Conrad, Alisha Goodbla, Ted Sommer, Mariah Meek

ABSTRACT

Climate change is having widespread negative effects on freshwater environments, including an increasing frequency and severity of droughts. Drought conditions present unique challenges for the federally listed Central Valley Chinook Salmon (*Oncorhynchus tshawytscha*), which use the already limited floodplain in the Central Valley as rearing habitat. In this study, we examined how differing hydrologic conditions influence the run composition of juvenile Chinook Salmon in the floodplain (Yolo Bypass) versus the mainstem of the Sacramento River. Juvenile Chinook Salmon from the Yolo Bypass and areas along the Sacramento River were identified to the genetically distinct runs (fall, late fall, winter, and spring) from 2013-2019. We found overwhelmingly that Length at Date methods are misclassifying fish, particularly late fall and spring run fish, and winter-run fish in the bypass. Using this genetic run-timing, we found that the abundances of endangered runs (spring and winter) are reduced during low flow periods in both the bypass and Sacramento River. Even during drought conditions, juvenile Chinook Salmon rearing in the Yolo Bypass attained significantly larger sizes than those in the Sacramento River. When comparing fish growth across time, during wet years fish in the bypass start smaller and get significantly larger over the course of the year as compared to drought years, while during both wet and dry years fish in the Sacramento River largely attain the same size. This suggests that floodplain habitat is critical to maintaining diversity in juvenile Chinook Salmon.

KEYWORDS

Chinook Salmon, genetics, monitoring, drought, floodplain, life history diversity, Yolo Bypass, Sacramento River

INTRODUCTION

Climate change presents a distinct threat to freshwater systems, as these systems often have a lack of connectivity between habitats making it often impossible for species to migrate to more favorable environments. The rise in temperature in freshwater basins is likely to lead to

changes in habitat quality and quantity, and conditions are predicted to worsen (Woodward et al. 2010; Ficklin et al. 2014). Overall changing water conditions (such as increase in temperature or habitat fragmentation) have already greatly reduced some freshwater population sizes, likely altering the overall amount of biodiversity and biocomplexity in these systems (Ficke et al. 2007; Brucet et al. 2012). Maintaining genetic and phenotypic diversity is necessary for population resiliency in the face of these fluctuations, and loss of biocomplexity further reduces any given population's ability to respond to climatic change and drought (Crozier et al. 2008).

The Central Valley of California is predicted to become one of the most water scarce areas in the world due to climate change and increasing water use (Famiglietti et al. 2011). Recently, the Central Valley experienced one of the longest and most severe droughts in California history, spanning the years 2012-2016 (Xiao et al. 2017). During this time, water in the largest watershed in California, the Sacramento River watershed, was at an all-time low, with the worst period of drought occurring in 2015. This led to vastly reduced connectivity between Sacramento River and its adjacent floodplain habitats, which have already been negatively impacted by extensive development and channelization (James and Singer 2008). Reduced access to floodplain habitat is particularly troubling because seasonally flooded habitats in the Sacramento River are critical for native freshwater species, providing important spawning, rearing, and feeding opportunities (Sommer et al. 2001a; van Dyke and Wasson 2005). For example, the Yolo Bypass, one of the few remaining large scale seasonal floodplain habitats in the upper San Francisco estuary, provides habitat for 45 different animal species and flood protection for the lower Sacramento Valley (Salcido 2012).

One species that utilizes the bypass is Chinook Salmon (*Oncorhynchus tshawytscha*), which includes two federally listed ESUs (Sommer et al. 2001a). Many juvenile Chinook within the Sacramento River basin use the bypass as feeding and rearing habitat as they make outward migrations to the Pacific Ocean. For fish migrating from the Sacramento Valley, the primary alternative route is the mainstem of the Sacramento River, which is extremely channelized and has high water velocities (Sommer et al. 2001c). This mainstem habitat is often suboptimal for Chinook Salmon rearing and is correlated with high mortality (Michel et al. 2015). In contrast, off-channel habitats often provide more favorable conditions in the form of increased food resources and shelter from predators (Jeffres et al. 2008; Limm and Marchetti 2009).

Within the bypass, more favorable habitat conditions are correlated with an increase in the overall abundance and size of juvenile Chinook (Katz et al. 2013; Hellmair et al. 2018). Furthermore, evidence suggests that the bypass facilitates increased biocomplexity in the form of variation in juvenile size at out-migration, which can have significant impacts on ocean survival (MacArthur 1955; Schindler et al. 2010; Woodson et al. 2013; Goertler et al. 2018). Evidence from other off-channel habitats in the Central Valley suggest that areas like the bypass can provide a "shifting habitat mosaic" which leads to differing growth rates during differing hydrological conditions (Cordoleani et al. 2022). This diversity of habitats across space and time is important for maintaining biocomplexity, leading to an overall portfolio effect (Greene et al. 2009). This can lead to some life history traits performing better under different conditions, providing population buffering and overall stability of the species (Sturrock et al. 2015).

In addition to the portfolio effect provided by variation in size, the Central Valley is the only location within the Chinook Salmon range that has four distinct Chinook Salmon spawning life-histories (runs), named for the time they return from the ocean to freshwater rivers to spawn (Meek et al. 2014). It is widely accepted that this life history diversity provides important biocomplexity necessary to mitigate adverse effects of changing conditions in the environment on any one population (Hilborn et al. 2003; Carlson and Satterthwaite 2011). However, the spring and winter-run are experiencing population declines in excess of 90%, and the U.S. Fish and Wildlife Service currently lists them as threatened and endangered, respectively, under the US Endangered Species Act (National Marine Fisheries Service 2014). The loss of either or both the spring and winter-run would represent an extreme loss of the biocomplexity of the region. Intensifying drought conditions in the Central Valley have led to extremely low water conditions that may reduce its ability to provide adequate habitat for all runs.

Currently, we do not know to what extent the bypass supports juvenile Chinook of the different runs in terms of abundance or residence time. Many of the natural resource agencies working in the Central Valley have used non-genetic methods to identify juveniles to run type, mainly using a Length at Date (LAD) model (Harvey 2011). These criteria were introduced in the 1970s and incorporate fork length and date of capture to determine a classification. Although this method is expedient for use in the field, there is evidence the classifications are highly inaccurate (Harvey et al. 2014).

The purpose of this study was to examine the differences (if any) in run biocomplexity between the two habitat types—floodplain of the bypass (YBY) and mainstem river habitat of the Lower Sacramento River (LSR) by addressing the following questions: 1) Do genetic methods and the LAD model show similar patterns of run compositiacross the YBY and adjacent LSR? And 2) Do genetically determined run and size distributions differ between the YBY and LSR? By answering these questions, we will be able to better understand how Chinook Salmon (and available habitat) can be better managed to promote run biocomplexity. On a more regional level, this study provides insights into the degree to which different run identification methods (LAD vs. genetic) are usable in different habitats (e.g., floodplain vs. channel).

METHODS

Study Site

The bypass is a managed floodplain that provides flood control for the city of Sacramento. The 24,000-ha region is one of the only remaining floodplain habitats within the Sacramento River basin. Habitat in the bypass includes grasslands, managed wildlife areas, agriculture, tidal wetlands and channels and perennial ponds (Sommer et al. 2001b; Sommer et al. 2005). Water enters the bypass from a few sources, creating access points for juvenile Chinook Salmon (Fig 1.1). Downstream migrating Chinook Salmon can most easily enter the bypass when the Sacramento River overtops the Fremont Weir, located at the northern part of the bypass (Sommer et al. 2001a). When water overtops the weir, water fans out across the bypass, creating suitable Chinook rearing habitat (Katz et al. 2017; Takata et al. 2017). During dry periods when the Sacramento River does not spill over the Fremont Weir, there are still substantial tidal river flows in Yolo Bypass from its base near Rio Vista, allowing young salmon to access the region (Goertler et al. 2018). During these periods, there are additional flow inputs from smaller westside tributaries (e.g. Putah and Cache Creeks) that enter a perennial channel called the “Toe Drain.” Consequently, juvenile salmon can access the region in both flood and non-flood years, but connectivity between Yolo Bypass and Sacramento River is greatest in wet years (Sommer et al. 2005). In contrast, the adjacent Sacramento River channel, is a deep and fast-flowing river system with water reaching depths of >5 m and flows as high as ~311 m^3/s with little vegetation (Sommer et al. 2001c). This channel is always available for juvenile Chinook Salmon, but provides almost no opportunities for rearing, feeding, and protection from predators (May and Brown 2002; Brown and Bauer 2010).

Sampling

Morphometric data, DNA samples, and environmental water conditions were obtained from monitoring projects operating within the Sacramento River from both the California Department of Water Resources (CDWR), which operates the bypass Fish Monitoring Program (Pien and Kwan 2022), and the United States Fish and Wildlife Service, which operates the Delta Juvenile Fish Monitoring Program (Mahardja et al. 2019).

In the bypass (YBY), sampling occurred during winter and spring by two main methods, a rotary screw trap and beach seines, a program operated by CDWR that started in 1998. The rotary screw trap sits at the base of the toe drain of the bypass and is approximately 2.6 meters in diameter. It was operated and fished 5 to 7 days a week depending on water conditions. Beach seines measuring 7.62 by 1.22 m were towed parallel to the shoreline in 17 spots along the bypass with 10 spots along the toe drain, 3 perennial ponds, and 4 high flow sites. These sampling events occurred once every other week as water conditions allowed (Sommer et al. 2001c; Goertler et al. 2018; Schreier et al. 2018).

Samples from the Lower Sacramento River (LSR) were collected by USFWS using three different methods. In the tidal Sacramento River at Sherwood Harbor, a Kodiak trawl was operated from October to March and towed between two boats (Brandes and Mclain 2001; del Rosario et al. 2013). During the months of April and September, a midwater trawl was used and towed with one boat. Sampling by both trawls was at the surface and usually consisted of 10 tows per day, 3 days per week. The second method was beach seines, conducted at two sites downriver from the Fremont Weir entrance to the bypass and adjacent to sampling seines in the bypass (Fig 1.1).

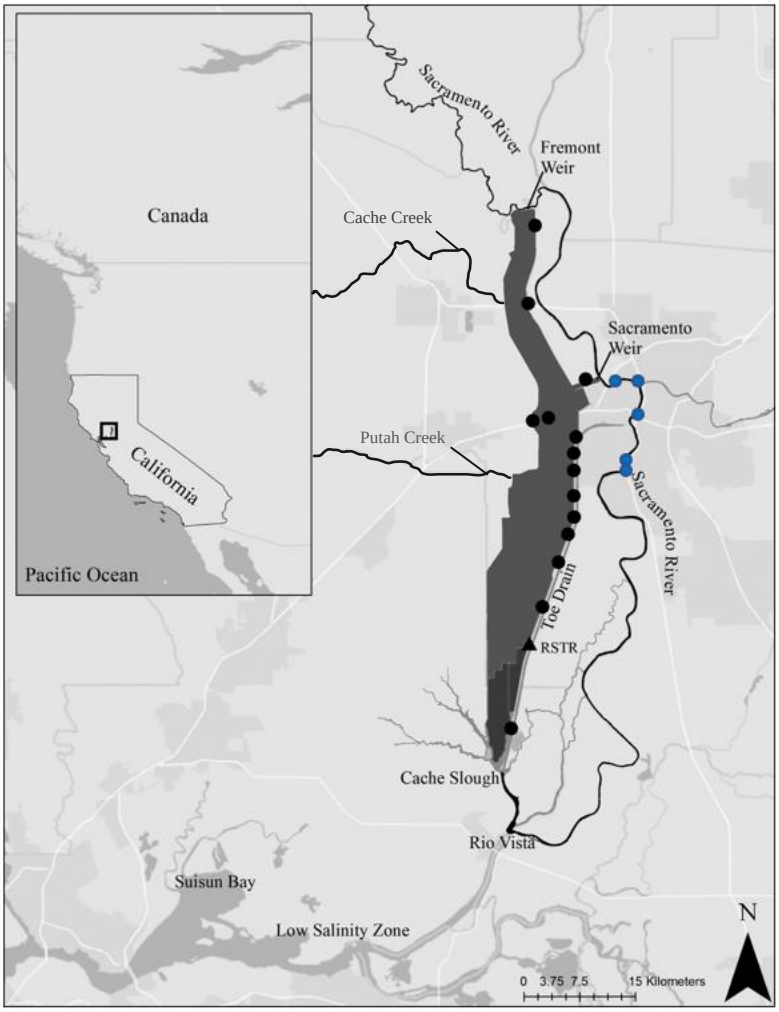

Figure 1.1: Map of the sampling region. Black symbols indicate Chinook juvenile sampling locations within the bypass, collected by the DWR. Blue symbols indicate sampling locations collected by the USFWS. Adapted from Goertler et al. 2018a.

Juveniles in both regions were then measured for fork length (mm) and assigned to run, across the years of 2013-2019 (Table 1.1). The primary method currently used by many management agencies to assign individuals to run in both systems is “Length at Date” (LAD) method (Fisher 1992). This model uses fork length and date of capture to assign individuals to run. The bypass uses the “Delta” version of the model and the “River model” is employed for identification in the mainstem Sacramento River. The primary difference between these models is based on different algorithms for length-at-date calculation (Fisher 1992; Harvey et al. 2014).

Table 1.1: Summary of fish sampled per location and year reported here form all sampling sites mentioned in Figure 1. LSR= Lower Sacramento River and YBY = Yolo Bypass.

Sample Numbers		
Year	**YBY n**	**LSR n**
2013	60	139
2014	211	165
2015	23	67
2016	199	289
2017	983	632
2018	42	249
2019	453	517

Because we were interested in the ecology of the wild populations in the Central Valley, we excluded all known hatchery fish by excluding fish that lacked an adipose fin. Throughout the Sacramento River System, hatcheries clip the adipose fin of Chinook Salmon juveniles of all runs to signify hatchery origin. Only 25% of fall-run fish raised in hatcheries have their adipose fin clipped, therefore it is possible that some fall-run hatchery origin fish are included in our dataset. However, during the period of our study, hatchery fish were released in the river only during 2013. After that, conditions in the river were so dry that hatchery fish were transported directly to the Delta (a site below our study area) to increase survival, making us confident that no (or very few) hatchery origin fall-run fish were included in our analyses for the other years (Sturrock et al. 2019).

Genotyping and run assignment

We collected fin clips for genetic analyses from 10 randomly sampled fish per LAD run classification per sampling site per day. Tissue samples were placed in 95% Ethanol and transported back to the lab. We extracted DNA from fins using the DNeasy® Blood and Tissue extraction kit (Qiagen, Valencia, CA). Samples were then genotyped using a Fluidigm Single Nucleotide Polymorphism (SNP) assay of 80 run-type informative markers following the protocol of Meek et al. 2016. This assay was developed using adult Chinook populations of known run throughout the Central Valley. We then assigned samples to run using ONCOR and

the baseline described in the previous study (Kalinowski et al. 2008; Meek et al. 2016). We assigned a genetic run to samples with 80% or greater probability of assignment to a particular run, while those below that threshold were assigned as "unknown." Samples that were "unknown" by genetic methods were not included in the analysis.

Statistical analyses

To assess the accuracy of LAD identification, we assigned all samples a value of 0 or 1, 1 indicating a match between LAD and genetic run assignment, and 0 indicating mismatch between LAD and genetic run assignment. We then separated samples by run (fall, late fall, spring, and winter) and assessed for mean accuracy employing bootstrap methods using the 'boot' function in the R program boot. In this code, means from a random sample of the assigned values were calculated 1000 times.

To evaluate if there were differences in proportion of run among individual years in the bypass vs the Sacramento Mainstem, we ran a chi-squared test of independence in R using the "chisq.test" function. We then used the R package "corrplot" to evaluate the residuals in each year and run to determine which values were contributing the most to the overall statistic. Each year is classified to a hydrologic classification based on the Sacramento Valley water year Hydrologic Classification Indices (Whitney 2007; Chronological Reconstructed Sacramento and San Joaquin Valley water year Hydrologic Classification Indices). Within this system there are 5 different classifications: Critical (C), Dry (D), Below Normal (BN), Above Normal (AN), and Wet (W). This metric is determined by taking into account the levels of unimpaired runoff and the previous year's index (Davis et al. 2000).

Next, we analyzed size differences in the bypass vs the Lower Sacramento River Fork Length and Date of fall-run fish by putting these data into a linear regression and using the "glm" function in R. We separated the data by year and location (YBY vs LSR), assuming a normal distribution. We then evaluated these models for statistical differences between years by using a two-sided t-test to compare the difference between the relative slopes. We compared each slope within one year individually and by each location. In both these analyses, we only used fall-run fish to reduce the chance that differences in size were due to life history characteristics present in other runs. Additionally, fall-run was the only population with large enough sample sizes to provide meaningful and statistically sound comparisons.

To further evaluate differences between size among juveniles in different hydrological regimes, we compared all mean fork lengths of fall-run fish by water year. We evaluated these means by two statistical methods. To compare between the Yolo Bypass and the Sacramento River, we completed a t-test between each location in each water year. To compare all water years, we ran all samples within a specific location through an ANOVA. To compare what years were contributing the ANOVA statistic, we did further analysis by running a post-hoc Tukey test.

RESULTS

Concordance between LAD and genetic methods for inferring run type varied by run type and habitat. We found higher concordance between LAD and genetic run assignment in fall-run in both habitats and winter-run in the Lower Sacramento River (Fig. 1.2). Concordance between assignment methods was very low for spring and late fall-runs in both habitats and winter-run in the bypass. During all years of sampling, we classified no juvenile Chinook as late fall by the LAD method in the bypass. In the Sacramento River, we classified a very small number as late fall. However, our genetic assignments show in both systems, there was a non-negligible amount of genetically late fall fish.

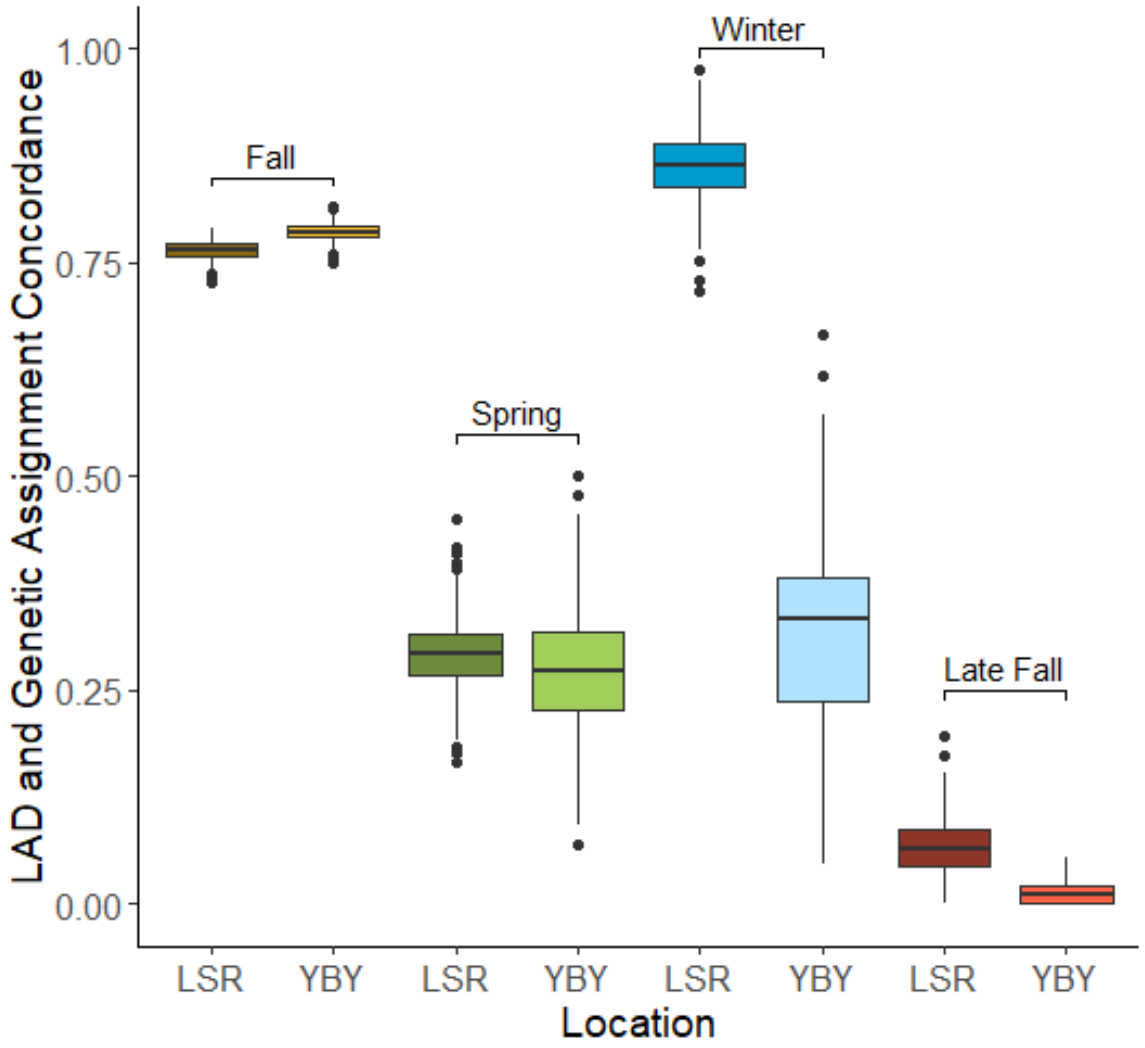

Figure 1.2: Results from a bootstrap analysis of Length at Date Classification Mismatches, organized by genetic run classification and location. A value closer to 1.0 indicates higher concordance between genetic and length at date classification. LSR= Lower Sacramento River and YBY = Yolo Bypass.

Table 1.2: Summary of fish sampled per location and run, and results from the bootstrap analysis depicted in Figure 2 comparing concordance between genetic and LAD classification methods. A value closer to 1.0 in the bootstrap mean column indicates higher concordance between genetic and length at date classification. LSR= Lower Sacramento River and YBY = Yolo Bypass. Sample numbers of fish classified to run in each system (LSR vs YBY) and each method of classification are also reported.

	Sample numbers			
Run	**LAD n**	**Genetic n**	**Boot mean**	**95% CI**
Fall				
YBY	1827	1890	0.76	±0.02
LSR	1573	1811	0.76	±0.02
Late Fall				
YBY	0	96	0	
LSR	16	97	0	
Spring				
YBY	453	58	0.2	±0.1
LSR	476	120	0.2	±0.1
Winter				
YBY	36	29	0.31	±0.2
LSR	178	81	0.85	±0.04
Unknown	0	530	-	-

To ascertain which misclassifications were contributing the most to the lack of concordance between genetic and LAD in these statistics, we compared both methods of classification across all years by plotting fork length versus date of capture (Fig. 1.3). Strikingly, we found that the majority of spring-run misclassifications were genetically fall-run individuals. Most of the genetic fall-run fish above a certain size are reflected in the LAD classifications in the spring graph, leading to spring-run juveniles to be massively overestimated. Alternatively, many genetic late fall-run fish were classified as fall, leading to those juveniles to be largely underestimated.

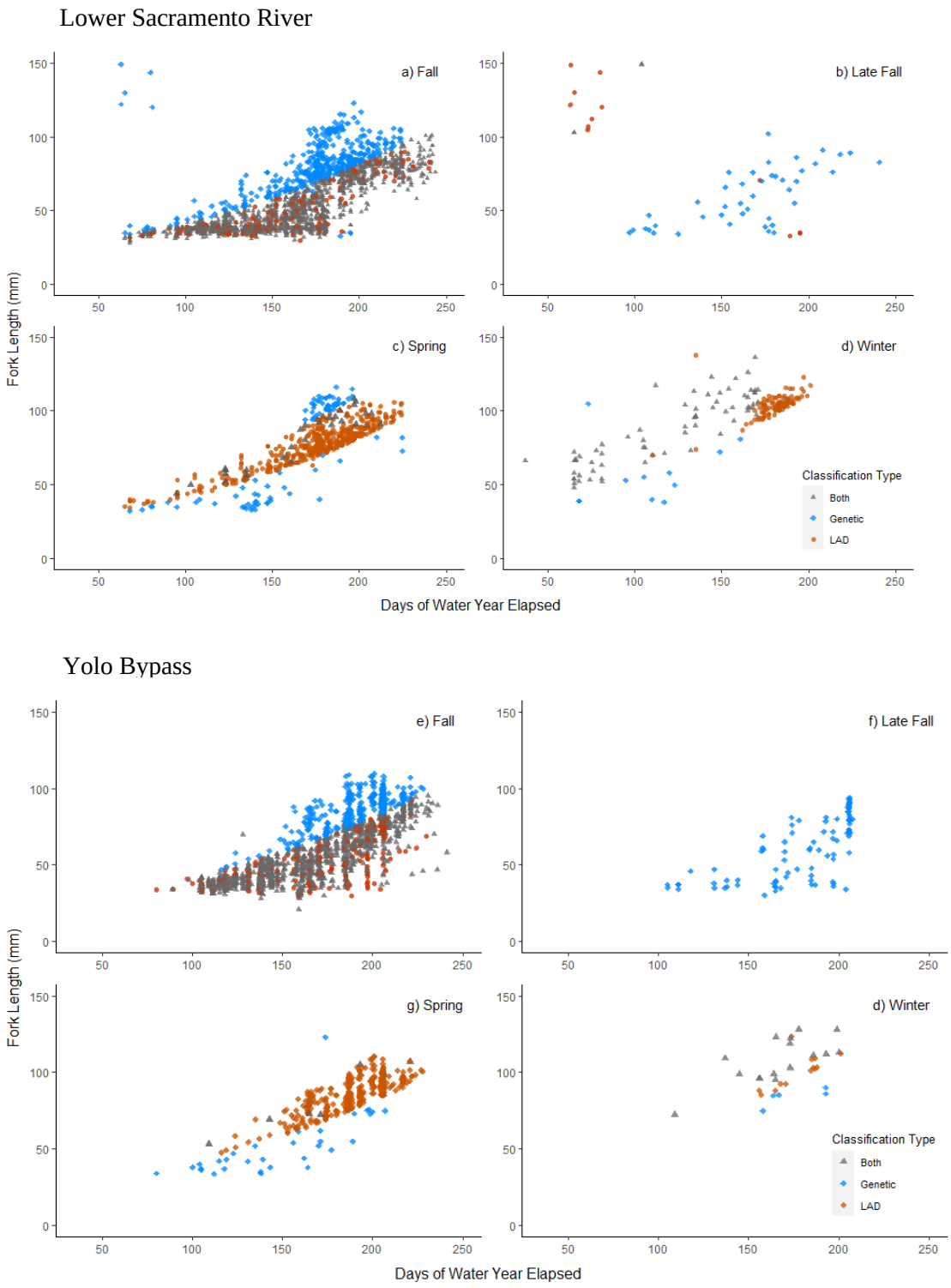

Figure 1.3: Comparison of the distribution of run-timing based on classification method across the years 2013-2019. In each plot, the blue and grey colored dots represent the genetic identification, while the orange points show the LAD misidentified individuals. In order, starting with the sites on the Lower Sacramento River we show the a) fall, b) spring, c) late fall, and winter-runs; followed by the e) fall, f) spring, g) late fall, and h) winter-runs in the bypass.

We found a significant difference in run proportion between years in both the bypass and Lower Sacramento River sites (Yolo Bypass: X-squared = 128.58, df = 18, p-value < 2.2e-16, Sacramento River: X-squared = 103.86, df = 18, p-value = 4.316e-14). When we calculated the residuals, it was clear that some proportions were contributing more to the chi-square statistic than others (Figure 4). In the years 2013 and 2014, our results showed more spring and winter-run fish in the bypass than expected when compared the proportion of other runs as well as proportions of winter and spring across the years, contributing positively to the chi square statistic. These results were similar to those in the Lower Sacramento River, where only winter-run proportion was higher than expected in 2013 and spring was similarly higher in 2014. Particularly in the later years of the drought (2015-2016), there was a dearth of ESA listed runs (spring and winter) when compared to fall-run. Interestingly, we saw an increase in late fall proportions in the years 2018 in the Sacramento River and 2019 in the bypass (Figure 5).

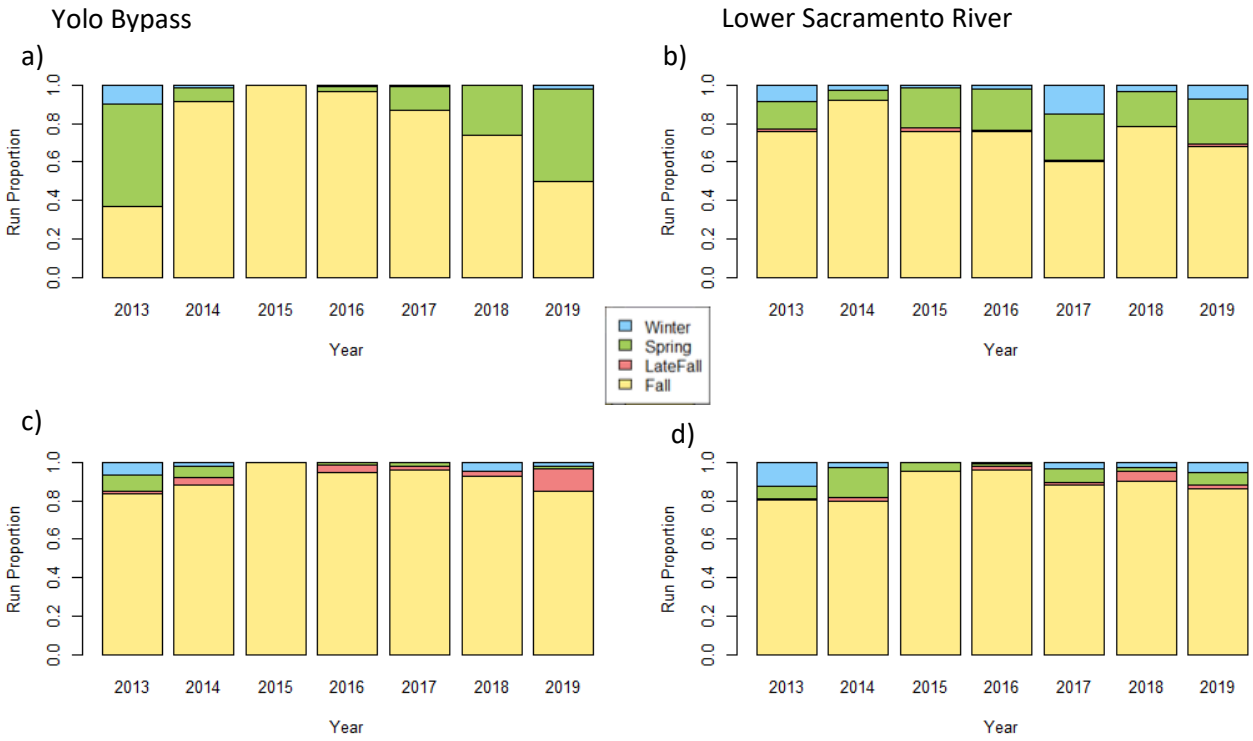

Figure 1.4: Length at Date and Genetic run proportions within the bypass and Sacramento River. Length at Date proportion of each run within year in the (a) Yolo Bypass and (b) the Lower Sacramento mainstem. Genetic proportion of each run within year in the (c) Yolo Bypass and (d) the Lower Sacramento mainstem. Residuals from a chi-square test for independence in the (e) Yolo Bypass and (f) Lower Sacramento mainstem indicating significance in that particular cell is compared to all other cells. Larger dots indicate a higher contribution to the chi-square statistic, while blue dots indicate a positive contribution and red dots indicate a negative contribution.

Figure 1.4 (cont'd)

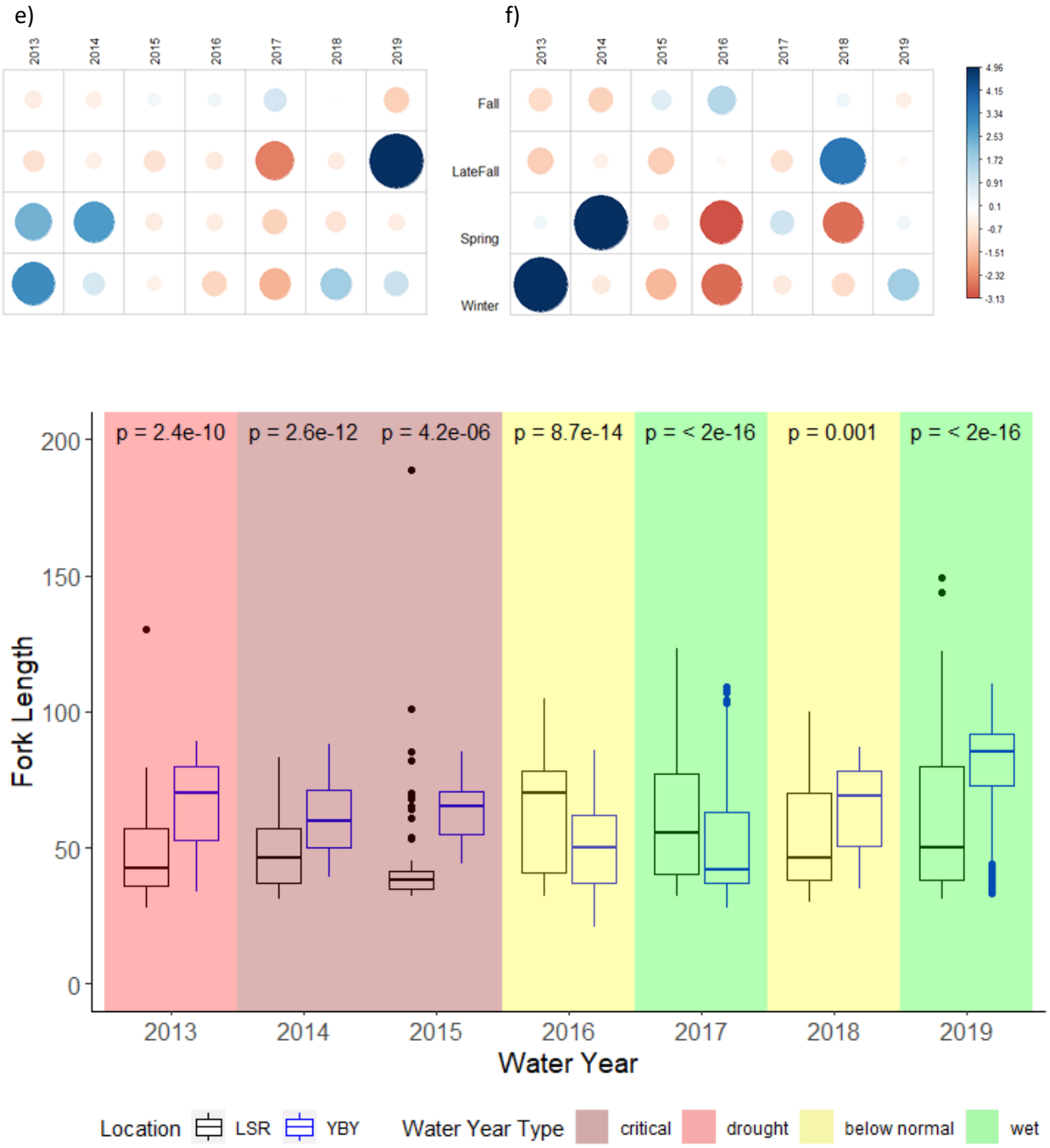

Figure 1.5: Mean fork length (mm) of genetically assigned fall-run juvenile Chinook in the Lower Sacramento River (LSR) vs the bypass (YBY), organized by water year. Comparisons for all years were statistically significant, as indicated by the p-values.

Table 1.3: Results from the posthoc Tukey test, comparing Fork Length of fall-run juveniles within each year in the a) Yolo Bypass and the b) Lower Sacramento River. Each Tukey group represents a significantly different mean fork length as it contributes to the overall significant difference in the ANOVA. Groups with the same letter are similar to each other, while groups with different letters are significantly different from each other.

a)

Fall-run – Yolo Bypass			
Water year	Mean Fork Length	Tukey group	Water year Type
2013	67.92857	b	Drought
2014	59.62445	c	Critical
2015	63.8333	bc	Critical
2016	51.78302	d	Below Normal
2017	51.33795	d	Wet
2018	62.82222	bc	Below Normal
2019	77.94915	a	Wet

b)

Fall-run – Lower Sacramento River			
Water year	Mean Fork Length	Tukey group	Water year Type
2013	49.19048	bc	Drought
2014	49.60440	bc	Critical
2015	46.02941	c	Critical
2016	63.64217	a	Below Normal
2017	65.16715	a	Wet
2018	54.96350	b	Below Normal
2019	62.6407	a	Wet

We found different fork lengths in fall-run between habitat types across all years, with the bypass having significantly larger fall-run fish in every year except 2016 and 2017, where the means were significantly smaller (Table 1.3). We also found a significant difference among years within habitat type (Table 1.4). We found that in both habitats in 2019, both habitats had a

larger mean fork length as compared with other years. In the Lower Sacramento River in years classified as Wet or Below Normal, we found fish attained greater size. This same pattern is not reflected in the bypass, where the smallest mean fork lengths were in the years of 2016 and 2017.

To further explore the effect habitat has on the size distribution of juvenile fall-run Chinook in the bypass, we evaluated the fork lengths of fish over time in the bypass as compared to the Lower Sacramento River. When we used the linear regression based on year and location, the slope of fall-run fish fork lengths over time from the bypass were significantly different from that in the Sacramento mainstem in the years 2016, 2017, and 2019 (Table 4, Fig. 1.6), with sizes increasing more over time in the bypass. We found that the slopes of fork lengths over time were not significantly different between habitat types for 2013-2015, the three Drought and Critical years (Table 1.4, Table S1.1, Figure S1.1-7).

Table 1.4: Changes in fork length over time between habitat types as input in a linear regression. Here we show the results from comparison of slopes of both sampling regions as input in a glm model. The coefficient indicates the level of interaction between the location and sample date, where the bypass is the point of reference. An asterisk indicates a significantly larger slope between locations.

Fall-run			
Water year	Coefficient	P value	Water year Type
2013	0.1072	0.0892	Drought
2014	0.0125	0.787	Critical
2015	0.0871	0.634	Critical
2016	0.1644	0.0012*	Below Normal
2017	0.0452	0.0188*	Wet
2018	0.1082	0.496	Below Normal
2019	0.2982	2.48^{-11} *	Wet

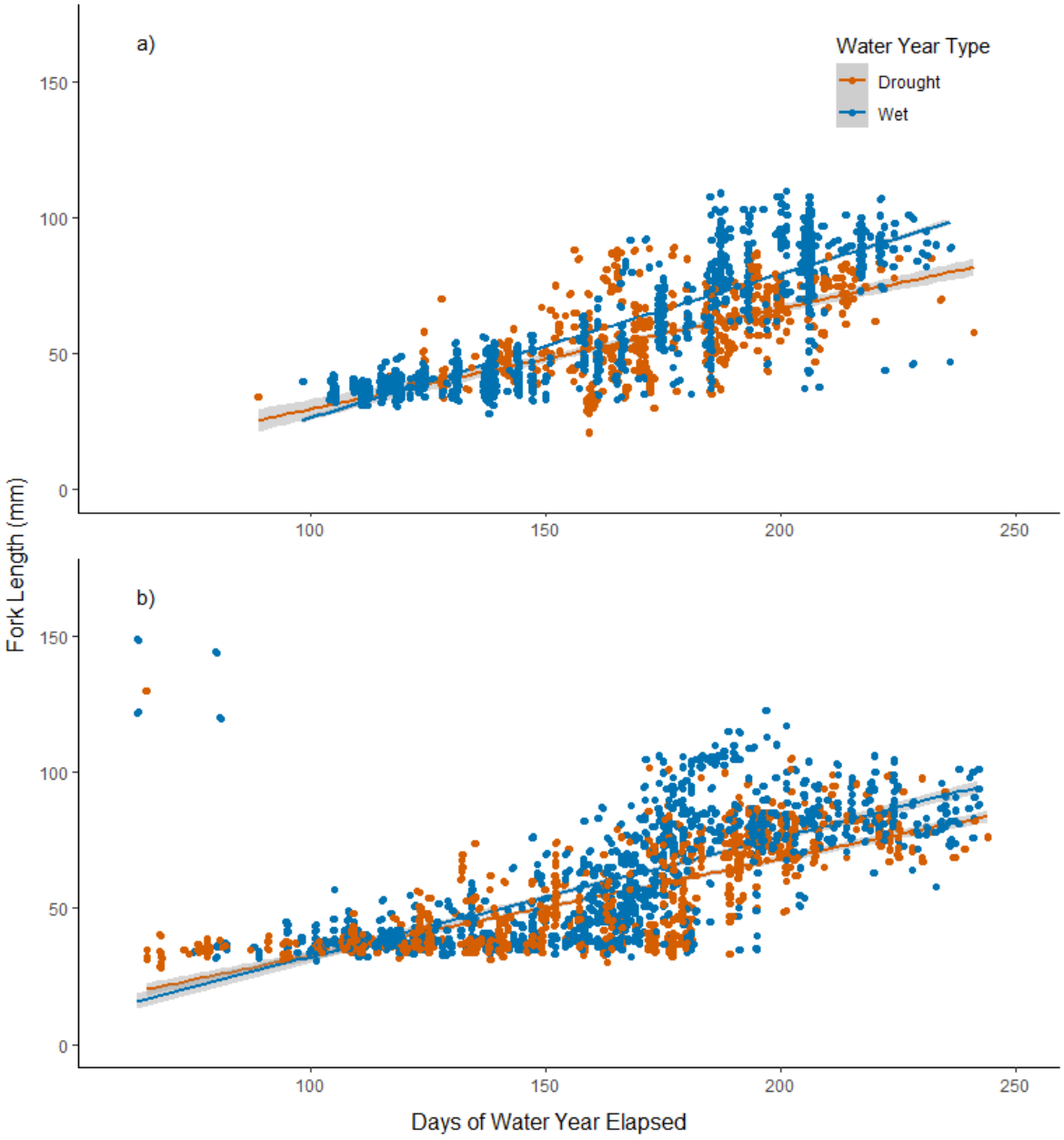

Figure 1.6: Changes in size of fall-run fish over time in all years classified as Wet (blue) and Drought (orange)with 95% confidence intervals (gray). Here we show the comparison between wet and dry years in the bypass (a) versus wet and dry years in the Lower Sacramento River (b). In both locations you can see there is a significant difference between wet and dry years, with the bypass experiencing a larger change in slope across time.

DISCUSSION

Habitat diversity is essential for supporting diverse juvenile salmon populations, particularly as climate change creates large fluctuations in environmental conditions (Beechie et al. 2013; Herbold et al. 2018). The diversity of migration timings, natal homing strategies, and outmigration tactics provides population buffering under differing environmental conditions, such as differing water levels and temperatures (Hilborn et al. 2003; Greene et al. 2009; Schindler et al. 2010). It is therefore imperative to monitor and manage life history diversity accurately and how that diversity is impacted by varying habitat conditions. Our study found that genetics methods would be most effective, and that floodplain habitat in the Central Valley is vital for supporting diverse Chinook Salmon populations. In particular, the diverse habitat provided by the bypass supports all runs as well as a diversity in fish size.

LAD versus Genetic Methods

Because monitoring life history diversity requires accurate classification of this diversity, we first compared classification methods in the system. LAD methods are still widely being used as the main method of classification, so we sought to understand how that compared with genetic methods. We found a large mismatch between the length at date and genetic run assignments, indicating a lack of accuracy in the LAD model. Our work shows that overwhelmingly, length at date metrics are overestimating the occurrence of spring-run and underestimating fall, late fall, and winter-run (Fig. 1.3). This is an important issue because it means that the LAD approach does not present an accurate picture of how many threatened and endangered juvenile fish are in the system. For example, this has contributed to a knowledge gap around the abundance of threatened spring run in the system (Nelson et al. 2022). For endangered winter-run, the export of water by state and federal pumping operations is directly tied to how many fish are in the system (NOAA NMFS 2009; Harvey et al. 2014). These management decisions rely heavily on quantifying the exact number of winter-run juvenile fish in the system to mitigate negative impacts. Therefore, accurate classification methods are vital to enabling the protection of the listed run and sustainable use of the water resource (Brown et al. 2009; Brown and Bauer 2010; Stewardship Council Delta Science Program 2019).

Chinook Salmon Life History Diversity

Our data indicate that all four runs and a diversity of sizes are present in the bypass. The is likely because the bypass provides several benefits to juvenile Chinook, including increased food resources and protection from predators (Jeffres et al. 2008; Limm and Marchetti 2009). Unfortunately, during low water periods, especially years that experience drought conditions, our work shows the proportion of spring, late fall, and winter-run decreased over time, suggesting that life history diversity is compromised when the bypass is more difficult to access (Fig. 1.4). Spring and winter-run did not begin to increase proportionally until 2019, when flooding occurred. Proportions of runs other than fall were similarly negatively affected in the Lower Sacramento River. These data indicate that maintaining higher flows help support life history diversity in juvenile Chinook Salmon, regardless of habitat. This is consistent with studies showing that spring-run phenotypes lose habitat with increased drought periods and have decreased survival rates (Cordoleani et al. 2021; Notch et al.).

Previous work has shown the importance of habitat diversity at all life stages for growth and fitness of many Pacific salmon species (Healey 1991). In the Sacramento River, there is evidence that wetland and managed floodplain habitats provide important opportunities for juvenile salmon growth, and different habitats have different food resources that impact growth (Jeffres et al. 2008; Cordoleani et al. 2022). Size and growth in the early life stages is imperative for success in the marine environment and entering the ocean at a larger size can make the difference between survival or death, so it is important to maintain habitats that provide opportunities for growth (Beamish and Mahnken 2001; Woodson et al. 2013). We found that there is a significant difference in juvenile fall-run Chinook fork length every year between the bypass and Sacramento River, as well as across years in both systems during different hydrological conditions (Fig. 1.5). In particular, fall-run juveniles were significantly bigger in the bypass almost every year. This suggests that the bypass is an important habitat for juvenile Chinook that allows growth and refuge, even during drought conditions. Because the floodplain conditions have shown to be important for growth, particularly periods of wet conditions or inundation, managing the bypass with an aim of promoting this life history and run diversity will be imperative for the persistence of these populations.

Although fish tended to be larger in Yolo Bypass than the Sacramento River although there are two notable exceptions. For example, fish were relatively smaller in two divergent

water year types, 2015 (extremely dry) and 2017 (extremely wet). This is likely because fish size is an imperfect metric of growth since the target habitats are open to immigration and emigration. This is because fish size in either Yolo Bypass or Sacramento River is a complex function of not only regional growth, but also influx of new individuals from upstream areas. Fish tagging or otolith measurements would have been a better tool to better characterize growth patterns but was not within the scope of this study. There is ample evidence from tagging methods that growth is consistently faster in Yolo Bypass (Sommer et al. 2001c; Katz et al. 2017; Takata et al. 2017)

In addition, our research indicates during wet conditions the bypass supports smaller fish earlier in the season while sustaining higher growth rates than the adjacent Sacramento River later in the season. This suggests that the bypass proffers benefits for size diversity during wet conditions (Table 1.4 and Fig. 1.6). The greatest fold difference slope of fork length over time between the two locations was during 2019, which had many weir-overtopping events (51 days) as compared to years that had less (in comparison 2014 had 0 days and 2015 had 3 days) (California Department of Water Resources). When all data points are combined from wet and dry years, there is still a significant pattern of increased growth in the bypass over time (Fig. 1.6). This aligns with other research that has shown increased rearing opportunities in the bypass leads to increased variation in size and growth rates in juvenile Chinook Salmon (Goertler et al. 2016; Goertler et al. 2018)

MANAGEMENT IMPLICATIONS

The Central Valley is predicted to be one of the areas most impacted by drought in the world (Famiglietti 2014). As the climate warms and intensifies, this is predicted to cause longer, more intense, and frequent droughts in the Central Valley, and more intense flood years (Gershunov et al. 2013; Trenberth et al. 2013; Swain et al. 2016). During more intense drought periods, young juvenile Chinook Salmon may have reduced access to off-channel habitat. Our work shows the diversity of habitats is essential to preserving a diversity of run-types and size distributions in juvenile Chinook Salmon. Consequently, reduced habitat variation could lead to a loss diversity of juvenile Chinook Salmon, which could weaken the portfolio effect and long-term stability and resilience. For this reason, managing connectivity between the bypass and Sacramento River represents a potentially valuable tool to sustain Chinook Salmon populations. For example, a major habitat restoration project is underway that will allow Yolo Bypass to be

inundated at lower flows (USBR and CDWR 2019). Similarly, several large-scale tidal wetlands projects are being constructed in lower Yolo Bypass, which could improve access to the floodplain and habitat quality during dry years and seasons (CDWR 2021; CDWR).

Another important finding from our study is that the LAD method is relatively inaccurate at identifying the full range of salmon runs. For example, the LAD models overestimate spring-run fish because large fall-run fish in the system are being misclassified as spring-run. This issue is already relatively well-recognized (Harvey et al. 2014; Brandes et al. 2021; Nelson et al. 2022), so genetics is increasingly being added as a monitoring and management tool in the Sacramento watershed and downstream estuary. Hence, we strongly support expanded use of genetic methods to monitor and manage Chinook Salmon in the system more accurately.

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APPENDIX 1A: SUPPLEMENTAL TABLE AND FIGURES

Table S1.1: Results from comparison of slopes of both sampling regions as input in a glm model. The coefficient indicates the level of interaction between the location and sample date, where the Yolo Bypass is the point of reference. An asterisk indicates a significantly larger slope between locations.

	Fall Run		
Water Year	Coefficient	P value	Water Year Type
2013	0.1072	0.0892	Drought
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2015	0.0871	0.634	Critical
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2018	0.1082	0.496	Below Normal
2019	0.2982	2.48^{-11} *	Wet

Figure S1.1: Graph comparing fork lengths of fall run fish between the Yolo Bypass (blue) and Lower Sacramento River mainstem (red) with a linear model generated for the year 2013 with 95% confidence intervals (gray). Significance is indicated by a start symbol.

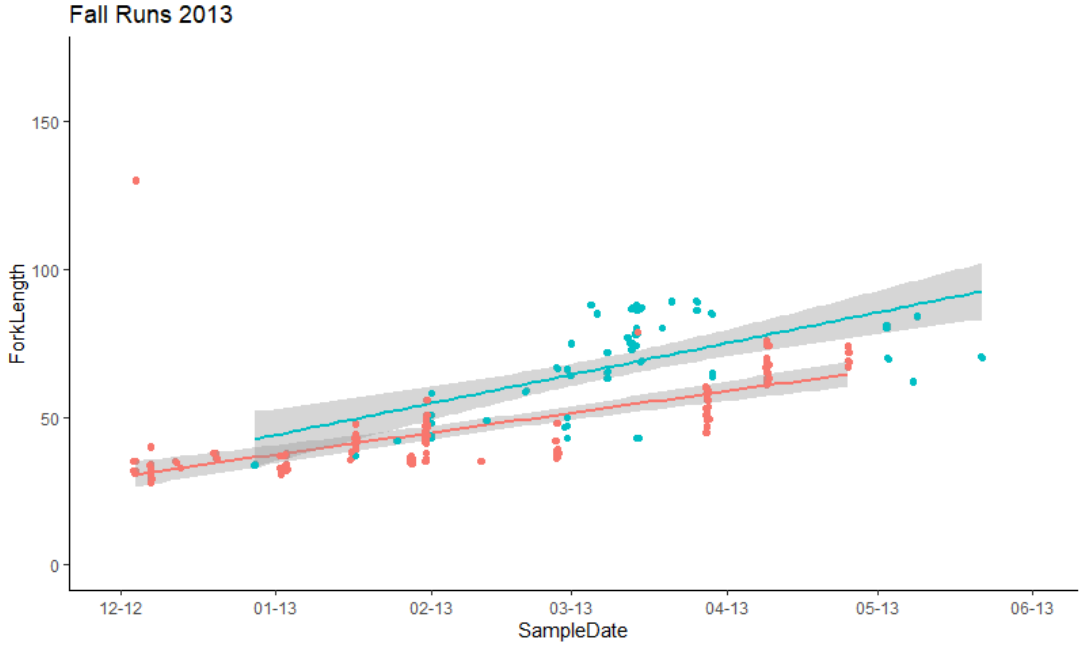


Figure S1.2: Graph comparing fork lengths of fall run fish between the Yolo Bypass (blue) and Lower Sacramento River mainstem (red) with a linear model generated for the year 2014 with 95% confidence intervals (gray). Significance is indicated by a start symbol.

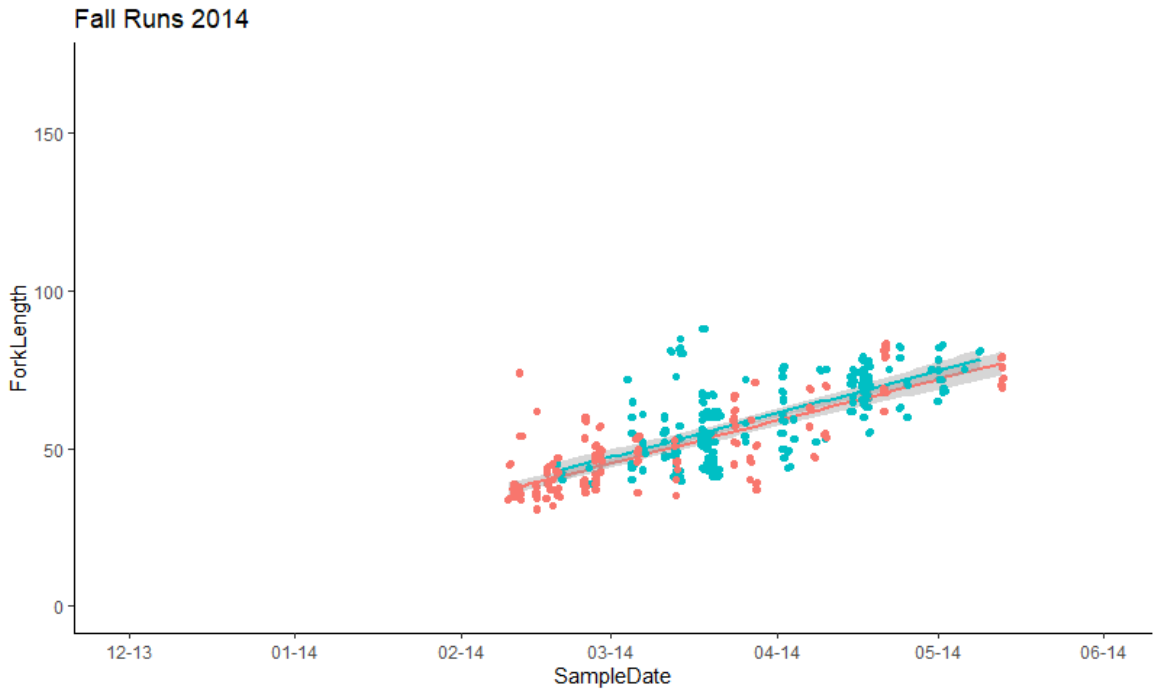


Figure S1.3: Graph comparing fork lengths of fall run fish between the Yolo Bypass (blue) and Lower Sacramento River mainstem (red) with a linear model generated for the year 2015 with 95% confidence intervals (gray). Significance is indicated by a star symbol.

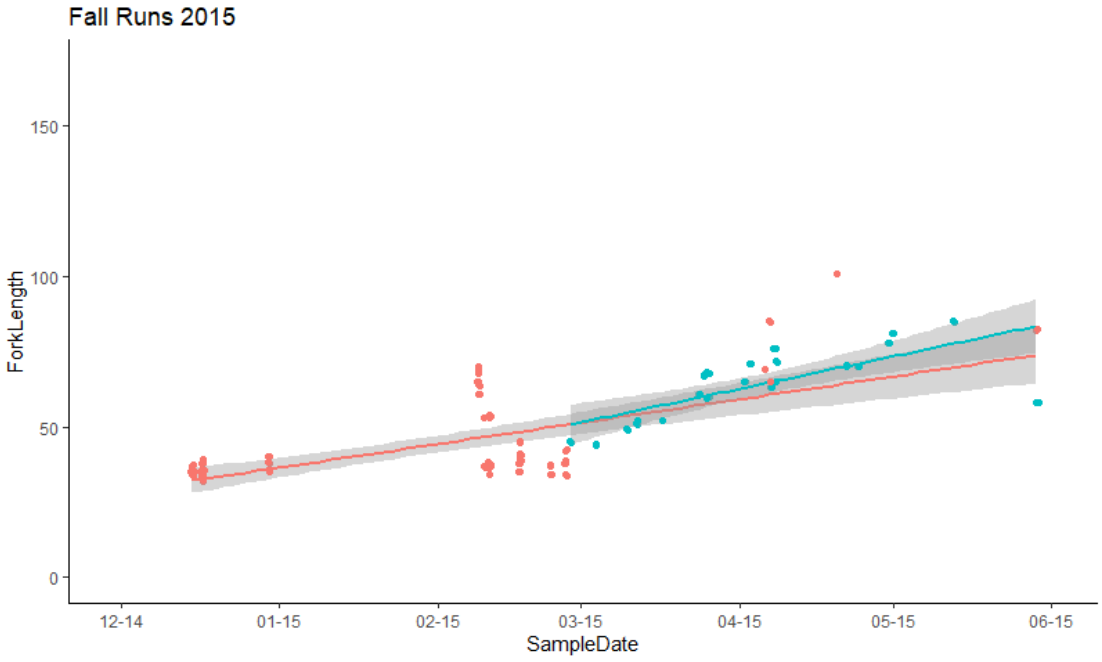


Figure S1.4: Graph comparing fork lengths of fall run fish between the Yolo Bypass (blue) and Lower Sacramento River mainstem (red) with a linear model generated for the year 2016 with 95% confidence intervals (gray). Significance is indicated by a star symbol.

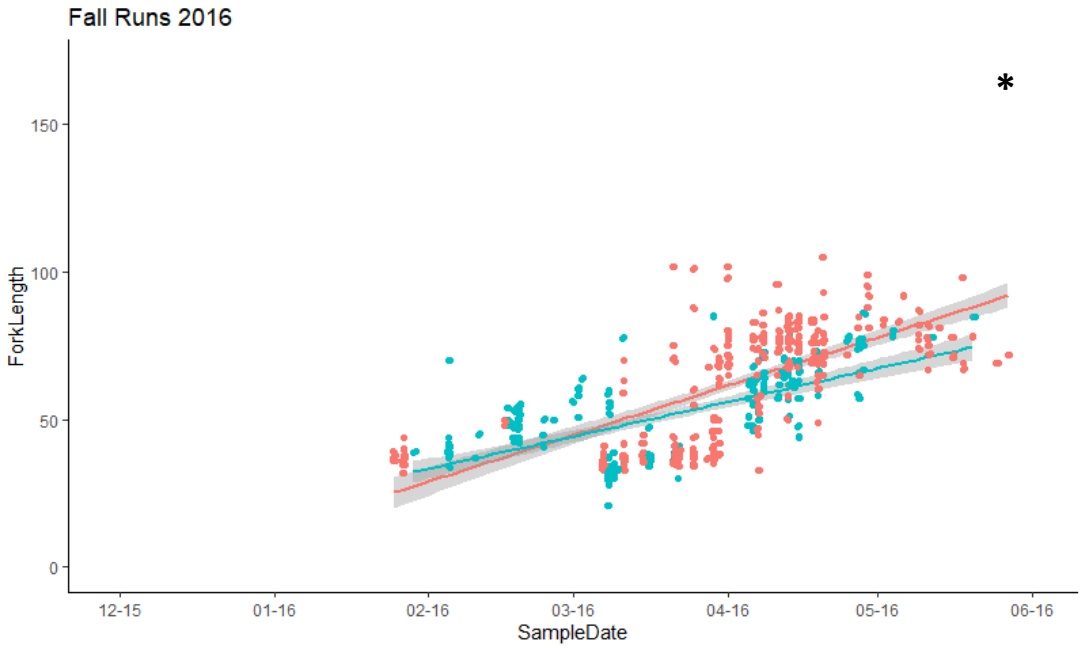


Figure S1.5: Graph comparing fork lengths of fall run fish between the Yolo Bypass (blue) and Lower Sacramento River mainstem (red) with a linear model generated for the year 2017 with 95% confidence intervals (gray). Significance is indicated by a start symbol.

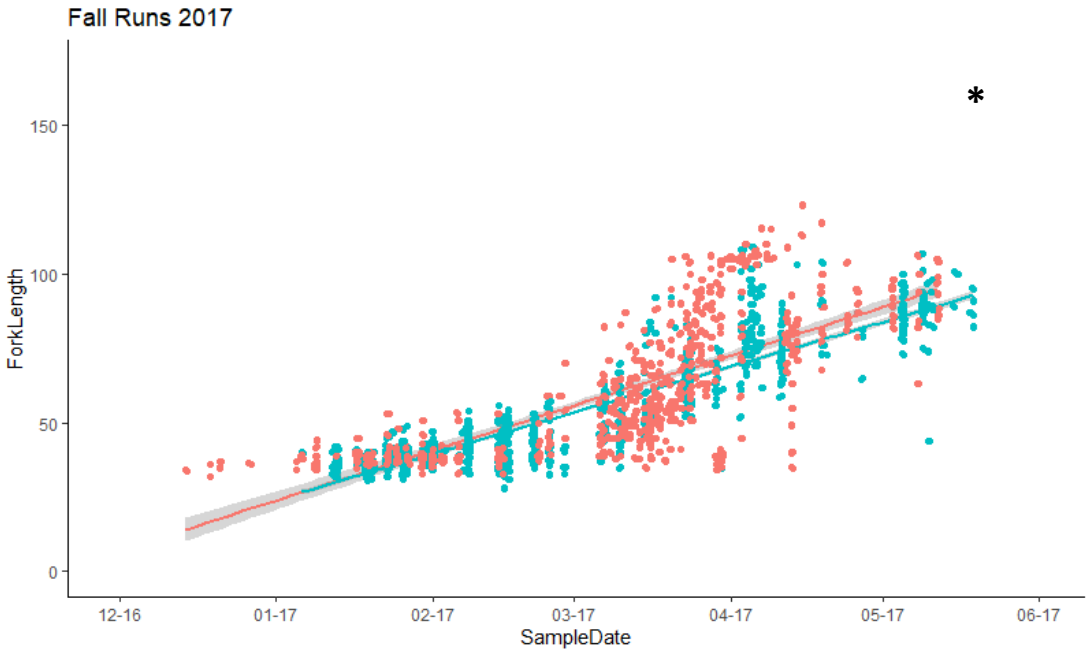


Figure S1.6: Graph comparing fork lengths of fall run fish between the Yolo Bypass (blue) and Lower Sacramento River mainstem (red) with a linear model generated for the year 2018 with 95% confidence intervals (gray). Significance is indicated by a start symbol.

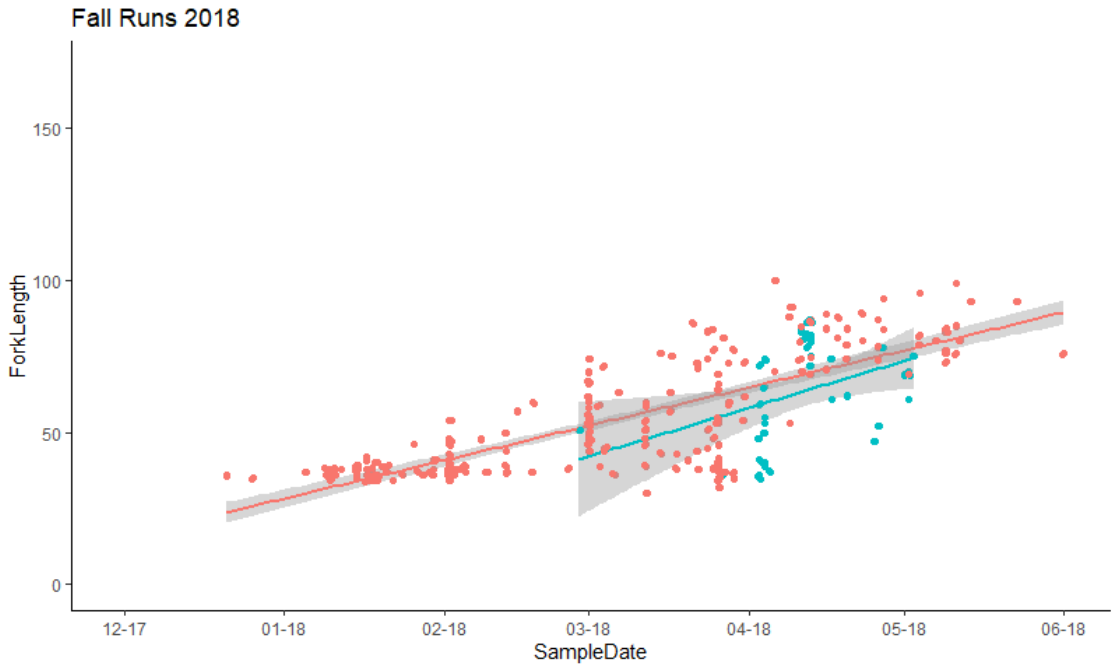
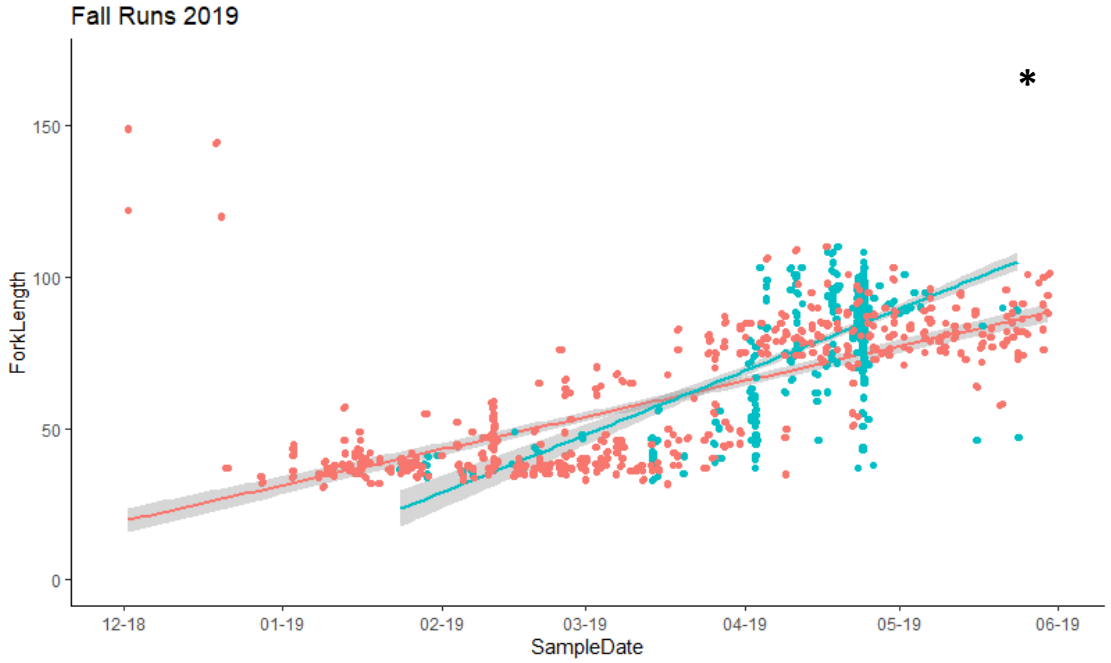


Figure S1.7: Graph comparing fork lengths of fall run fish between the Yolo Bypass (blue) and Lower Sacramento River mainstem (red) with a linear model generated for the year 2019 with 95% confidence intervals (gray). Significance is indicated by a start symbol.



CHAPTER 2: REMNANT SALMON LIFE HISTORY DIVERSITY REDISCOVERED IN A HIGHLY COMPRESSED HABITAT

Chapter 2: This chapter has been submitted for publication to Evolutionary Applications and is currently under peer-review.

Other contributing authors: Anna Sturrock, Malte Willmes, Tasha Thompson, Rachel Johnson, Flora Cordoleani, Natalie Stauffer-Olsen, George Whitman, Mariah Meek

ABSTRACT

Chinook salmon (*Oncorhynchus tshawytscha*) display remarkable life history diversity underpinning their ability to adapt to environmental change. Maintaining life history diversity is vital to the resilience and stability of Chinook metapopulations, particularly under rapidly changing climates. However, the conditions that promote life history diversity are rapidly disappearing, as anthropogenic forces promote homogenization of habitats and genetic lineages. In this study, we use the highly modified Yuba River in the Central Valley of California to understand if distinct genetic lineages and life history still exist, despite reductions in spawning habitat and hatchery practices that have promoted introgression. There currently is a concerted effort to protect federally listed spring run populations, given that few wild populations still exist. Despite this, we lack a comprehensive understanding of the genetic and life history diversity of Chinook salmon present in the Yuba River system. To understand if this diversity still exists, we collected migration timing data from acoustic tagging and carcass surveys and GREB1L genotypes from Chinook salmon in the Yuba River between 2009-2011. Variation in the GREB1L region of the genome is tightly linked with run timing in Chinook salmon but the relationship between this variation and entry on spawning grounds is little explored in the Central Valley. We found that the date Chinook salmon crossed the lowest barrier to spawning habitat (Daguerre Point Dam) was tightly correlated with their GREB1L genotype. Importantly, our study confirms that ESA-listed spring run Chinook salmon are spawning in the Yuba River, promoting a portfolio of life history and genetic diversity, despite spawning in a compressed habitat. This work highlights the need to identify and protect this life history diversity in heavily impacted systems to conserve and promote diverse and healthy Chinook salmon metapopulations. Without this, we run the risk of losing the last vestiges of important variation.

KEYWORDS

Life history diversity, GREB1L, acoustic tagging

INTRODUCTION

Life history diversity is critical for species to respond to environmental variability (Beechie et al., 2006; Moore et al., 2014). This diversity often includes differences in morphology, size, and age at maturity and is often influenced both by environmental and genetic factors (Healey, 1991; Thibaut and Connolly, 2013). In particular, genetic diversity is important as it often harbors the adaptive potential for populations to respond to future or changing conditions (Brooks et al., 2006; Chapin et al., 2000). Additionally, genetic diversity within a species or population can result in the expression of diverse life history strategies that spread survival risk across time and space, stabilizing populations and ecosystem services. This phenomenon is referred to as biocomplexity (Hilborn et al., 2003) and can help buffer the effects of natural and anthropogenic change (Narum et al., 2018). Unfortunately, biocomplexity, and in turn genetic diversity, is being lost at alarming rates due to anthropogenic change, particularly in freshwater ecosystems (Allendorf et al., 2014; Des Roches et al., 2021; Heino et al., 2009; Sih et al., 2000). To protect biocomplexity and promote life history diversity, it is vital to identify, monitor, and protect unique phenotypic and genetic traits present within and among populations.

In general, salmonids in the United States have been losing biocomplexity over the last century due to anthropogenic stressors (Dittman and Quinn, 1996; Finney et al., 2002; Malick and Cox, 2016). For example, Chinook salmon (*Oncorhynchus tshawytscha*) have faced declines in excess of 99% of their original population sizes in their native range due to overfishing, damming, mining, and climate change (Mahnken et al., 1998; National Marine Fisheries Service, 2014). This is particularly troubling because Chinook salmon are a keystone species of high cultural, economic, and ecological value (Bottom et al., 2009; Colombi, 2012; Layman et al., 2006). With large population losses, many Chinook salmon populations have also experienced marked reduction in genetic diversity (Johnson et al., 2018; Thompson et al., 2019; Weeder et al., 2005). These significant losses in genetic diversity have had negative consequences in terms of reduction in phenotypic diversity and adaptive capacity (Carlson and Satterthwaite, 2011; Griffiths et al., 2014). Thus, it is vital that we identify and protect the remaining biocomplexity found in Chinook salmon populations to promote population persistence and resilience in an anthropogenically influenced system.

The California Central Valley (CCV) is the southernmost portion of the native Chinook salmon range and populations are greatly imperiled due to the negative impact of anthropogenic

stressors such as dams, historic mining operations, and extensive urbanization (Herbold et al., 2018; Moyle et al., 2017). Due to its southern location, Chinook salmon populations in the CCV are also highly vulnerable to climate change (Crozier et al., 2019). Despite these threats, the Sacramento River is the only part of the entire species' range that contains four distinct spawning life history timings while all other systems have only two distinct run timings. This makes the Chinook salmon in the CCV a uniquely diverse population complex (Williams, 2006),. These life history phenotypes are referred to as "run-types" and are named after the season by which they migrate upriver to spawn (fall, late fall, spring, and winter). Historical temporal and spatial separation have resulted in limited gene flow among CCV runs within the same river system, leading to these populations becoming genetically distinct (Meek et al., 2020). This genetic variation provides the adaptive capacity necessary to result in phenotypically diverse populations. This biocomplexity in run-types is essential in maintaining Chinook salmon stock abundance across years, facilitating a "portfolio effect" that allows the species to withstand environmental heterogeneity and perturbations (Schindler et al., 2010). Although we know much about the biology of Chinook salmon, much is still unknown about the heritability or genetic basis of life history traits of Central Valley populations (Cordoleani et al., 2020).

Spring run Chinook salmon were once the most abundant run in the CCV, existing in the hundreds of thousands prior to the construction of impassable dams, extensive levees that converted floodplain and marsh habitat to agriculture, and overfishing (Lindley et al., 2004; Yoshiyama et al., 1998). Spring run fish display a unique spawning strategy of migrating into the system early when water temperatures are low from high spring flows and oversummering in cool headwaters before spawning in the fall (Quinn et al., 2016). Unfortunately, dam construction in the CCV, which began in the early 1900s, cut off access to historical spring run spawning habitat for most populations throughout the CCV. This forced spring run to face the double threat of both having to oversummer in much warmer downstream waters while also spawning in the same habitat as fall run, which enter the system after the heat of the summer and spawn immediately in downstream reaches (Healey, 1991). Consequently, spring run numbers have decreased precipitously, with most populations going entirely extinct in the CCV. As a result, they are now listed as threatened under the Endangered Species Act (National Marine Fisheries Service, 2014).

The Yuba River, a tributary of the Feather River within the Sacramento River watershed, once supported an independent spring run population, but like much of the rest of the CCV, due to extensive damming, historic spring run spawning grounds are no longer accessible, making it an excellent system for identifying and understanding if and how various life history forms co-exist in a heavily impacted system (James, 2005). The Yuba River Chinook population is currently managed as one independent fall-run population even though it is unknown how much life history variation within the system exists and is assumed to be largely influenced by strays from the nearby Feather River Hatchery (Lindley et al., 2004). It is unknown if there is an independently spawning spring run population in the Yuba River. If a Yuba River spring run population still exists, it will be critical to manage this watershed appropriately to protect the ESA listed population and, in turn, promote the spring run portfolio.

In recent years, notable progress has been made towards understanding the genetic underpinnings of run timing diversity in Chinook salmon. Research in other systems has shown that variation in return timing of fall and spring run Chinook salmon is tightly correlated with variation in the GREB1L to ROCK1 region of the genome, hence referred to in this paper as GREB1L (Prince et al., 2017; Thompson et al., 2019). Chinook salmon homozygous for the early returning variant exhibit an early run timing distribution in the spring while individuals homozygous for the late returning variant exhibit a later distribution in the fall. Heterozygotes in other systems exhibit an intermediate return timing that overlaps to some extent with homozygotes of both alleles. Although this correlation has been well studied and documented in other river systems (such as the Rogue River, Oregon and Klamath River, California) using well-phenotyped samples from migrating adults, studies in the CCV to date have relied on phenotypic proxies for run timing, such as carcass collection date or entry time into a hatchery (Thompson et al., 2020, 2019). While these studies were sufficient to demonstrate the strong correlation of the GREB1L region with run timing in the CCV, the information from live individuals in the midst of their migration provides much more precise information about the timing distributions of each genotype. More precise timing distributions in the CCV could prove to be an invaluable monitoring tool for the conservation of Chinook salmon populations throughout the Central Valley, given the rarity of spring run. In this study, we seek to both identify how many wild-produced migration phenotypes are present in the Yuba River and to explore the relationship between GREB1L genotypes and return time of Chinook salmon in the CCV. Understanding this

in the highly impacted Yuba River system will be invaluable for not only the management of the Yuba River, but also will be important for understanding how life history diversity is maintained in highly impacted systems and how we should identify, monitor, and protect this life history diversity to promote salmonid recovery.

METHODS

Study Site

The Yuba River is a tributary of the Feather River, which flows into the Sacramento River. The Yuba has 3 main tributaries, the north, middle, and south forks, which were once historic Chinook salmon spawning habitat but are now inaccessible due to dams on the river. The Yuba River has two main dams that serve as barriers to Chinook salmon migration: the Daguerre Point Dam (DPD), which is located at river mile 11 and passable by salmon via two fish ladders on either side, and the Englebright Dam, which is located at river mile 24 and impassable by salmon (Fig. 2.1). In addition to these complications, upstream from the lower Yuba River there is a large hatchery located on the Feather River that produces both spring and fall run that are thought to potentially stray into the Yuba River during spawning migrations. A key management objective in this system is the Yuba River Accord which is an agreement between all agencies in the Lower Yuba River Management Team (RMT) to manage for improved salmon and steelhead habitat. Within the Yuba River Accord Fisheries Agreement, is a stated purpose to evaluate the presence and viability of spring run Chinook salmon in the lower Yuba River (Yuba County Water Agency et al., 2007).

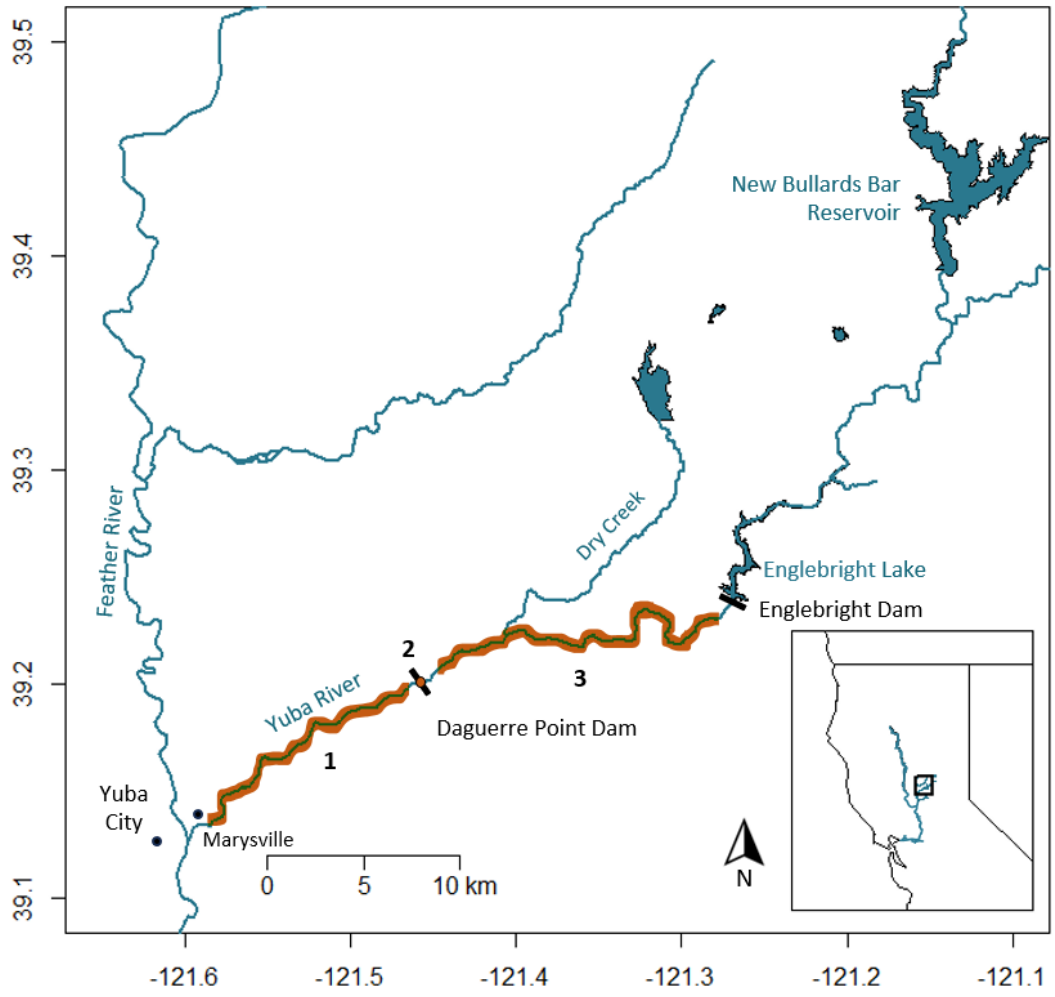


Figure 2.1: Map of the Yuba River system, a tributary of the Feather River. Black bars indicate dams. Orange highlighted areas indicate sampling locations: 1) spawner survey sampling location, 2) acoustic tagging sampling area, and 3) carcass sampling area.

Sample collection

Two sampling efforts, an acoustic telemetry project and a carcass survey, were conducted by the RMT between the years 2009-2011 during their annual spawner surveys to characterize Chinook migration up the Yuba River to the spawning reaches. For the acoustic telemetry project, adult fish were caught via hook-and-line sampling, targeting fish in the lowermost reaches from the confluence of the Yuba and Feather Rivers to DPD from May to October (Sampling Area 1, Fig. 2.1). Fin clips were collected from all captured fish (N=122), but only fish that were determined to be in "good condition" (showing no signs of disease or injury) were also acoustically tagged (N=42, we refer to these as the "acoustic tagging samples" and those that

were just fin clipped but not tagged as “spawner survey samples"). The acoustic tagging samples were tagged with VEMCO V13-1L acoustic transmitters via esophageal/gastric insertion and were detected via two ultrasonic receivers located in the north and south side of the top of the fish ladder to detect fish successfully passing DPD from both sides (Sampling Area 2; PSMFC, 2011; VEMCO, 2010). The most upstream area was sampled via carcass surveys that occurred upstream of the DPD on a weekly basis (Sampling Area 3, Fig. 2.1), starting 10-15 days after the first spawning redds were detected each year. Only fresh carcasses (possessing at least one clear eye and gills that are red or pink) were sampled to avoid sampling fish that had degraded DNA and had already been in the system for a long period of time. In 2009 and 2010, tissue samples were taken from carcasses throughout the river reach between the DPD and Englebright Dam (Sampling Area 3). All fin clips, regardless of survey method, were dried and placed into individual envelopes then sent to the Meek genetics lab at Michigan State University for processing.

Table 2.1: Analyzed genetic samples. Numbers are presented by year and survey type. Note that Acoustic tagging individuals were first surveyed in the spawner survey and then again when they passed DPD, and as such are a portion of the spawner survey individuals.

Sample Year	Survey type	N
2009	Spawner Survey	0
	Acoustic Tagging	0
	Carcass Survey	37
	Total	37
2010	Spawner Survey	92
	Acoustic Tagging	18
	Carcass Survey	35
	Total	127
2011	Spawner Survey	30
	Acoustic Tagging	24
	Carcass Survey	0
	Total	30
	Total	**194**

Run-type Assignment

We first assigned individuals to phenotypic run-timing by the date of their detection in the system. The Yuba River RMT uses two "differentiation days" to classify individuals to either early spring, late spring, or fall run timing category. If an individual fish passes DPD prior to July 15th, they are considered early spring run migrants, while after that but prior to October 1st they are considered late spring run migrants. All fish after October 1st are considered fall run migrants (Poxon and Bratovich, 2020). We used these same metrics to classify individuals to their phenotypic run-timing and compare with their GREB1L genotypes. We used this same method of classification for fish surveyed below DPD.

To genotypically assign run-type, we extracted DNA from fin clips using the DNeasy® Blood and Tissue extraction kit (Qiagen, Valencia, CA). We genotyped fish at the GREB1L locus by selecting five Single Nucleotide Polymorphisms (SNPs) across the GREB1L region of the genome that had been identified as strongly associated with run timing in previous analyses (Koch and Narum, 2020; Prince et al., 2017; Thompson et al., 2020, 2019). SNPs were screened from the input design sequences (Suppl. Table 2.1) by cross-checking against a multi-population dataset utilized by Thompson et al. (2019). We developed those SNPs into Fluidigm SNPtype assays. Individuals were genotyped at the five SNPs using the Fluidigm EP1 platform (Fig. 2.2). From those markers we were able to make assignments to either homozygous early, homozygous late, or heterozygous genotypes. Genotypes were only allowed to have a total of two or fewer missing SNP genotypes otherwise they were deemed ambiguous and reported as "not called." Those samples were not included in the final analyses.

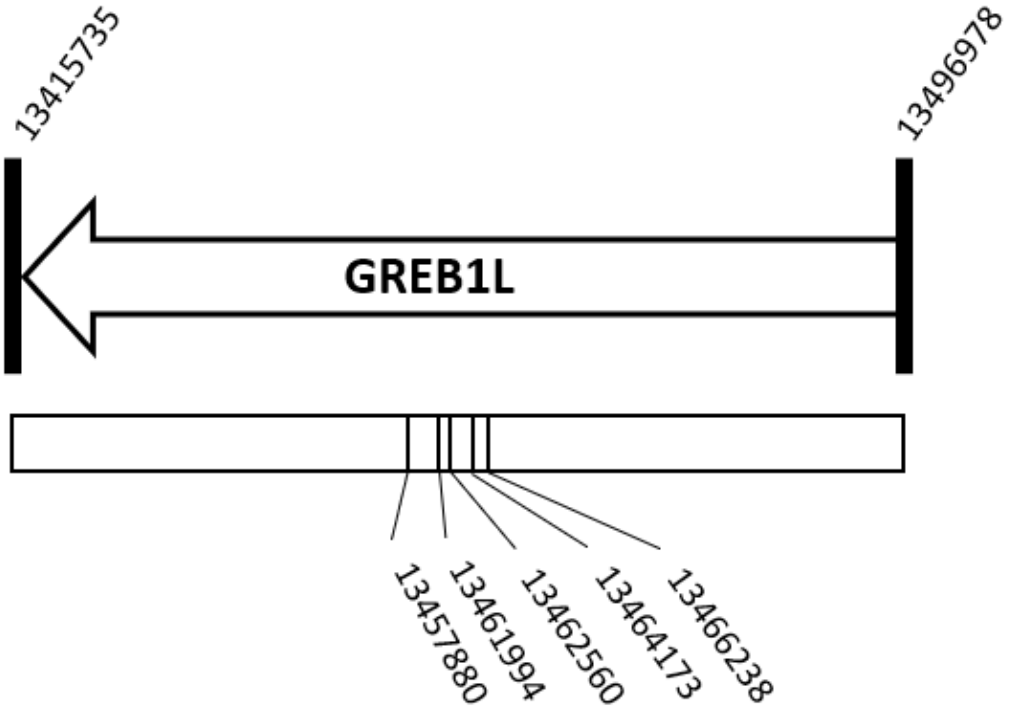


Figure 2.2: Diagram of relative SNP positions in the GREB1L region on chromosome 28 of the Chinook genome, Otsh_v2.0 (GCF_018296145.1) used for genotyping analysis (Christensen et al., 2018).

Statistical analysis

We calculated the mean return date for each run using the day of year converted to Julian date of detection in the system by each of the three methods: spawner surveys, acoustic tagging, and carcass surveys. To test if there was a significant difference in mean detection date for each of the three genotypes within each survey method, we used a Kruskal-Wallis test due to the unequal variance among sampling dates. After determining whether the differences between mean detection dates for the genotypes were significant, we then ran a Dunn test of significance to see which genotype detection dates specifically were significantly different from each other within each method, with a full pairwise comparison: homozygous early vs heterozygous, heterozygous vs homozygous late, and homozygous late vs homozygous early.

RESULTS

Within the Yuba River, genetic assignments show there are genetically spring run (GREB1L homozygous early), fall run (GREB1L homozygous late) and GREB1L heterozygous individuals in the system. In total, we found 125 homozygous early, 25 heterozygous, and 44

homozygous late individuals. When compared with survey data, we found that genetic versus date assigned run type were not in perfect agreement. We found homozygous early individuals in both spring early and spring late migrant phenotypic categories, while homozygous late individuals show up in the fall phenotypic category (Fig. 2.3). Interestingly heterozygous individuals appear below DPD at the same time as homozygous early individuals and were categorized as spring early and spring late based on sample date (Fig. 2.3A), however all heterozygous fish with acoustic tags crossed DPD later in the season. This caused them to be categorized as spring late and fall based on sample date (Fig. 2.3B). We found that this was likely because although homozygous early and heterozygous individuals arrive at the dam at the same time (as early as May 25th, Fig 4A), they crossed the dam at different time periods with homozygous early fish crossing the dam earliest (as early as June 30th). We did not see the heterozygous individuals crossing the dam until later (at the earliest by August 28th, Fig 4B). For the post-spawning carcass surveys, we saw a similar, albeit less protracted pattern, with homozygous early being detected at earlier dates, homozygous late being detected at later dates, and heterozygous individuals being detected at intermediate times (Fig 4C).

Our results clearly show that homozygous early individuals cross the dam earlier while homozygous late individuals cross the dam later in the season, with the mean return date being statistically significantly different ($p = 0.0004$). The same pattern was statistically significant across all sampling methods, with homozygous late mean return dates being later than homozygous early (spawner survey: $p = 0.0067$, carcass survey: $p = 5.58 \text{ e } -11$). Across all sampling methods, heterozygous mean migration dates were not significantly different from homozygous early, despite slight differences in the mean migration date (Table 2.2).

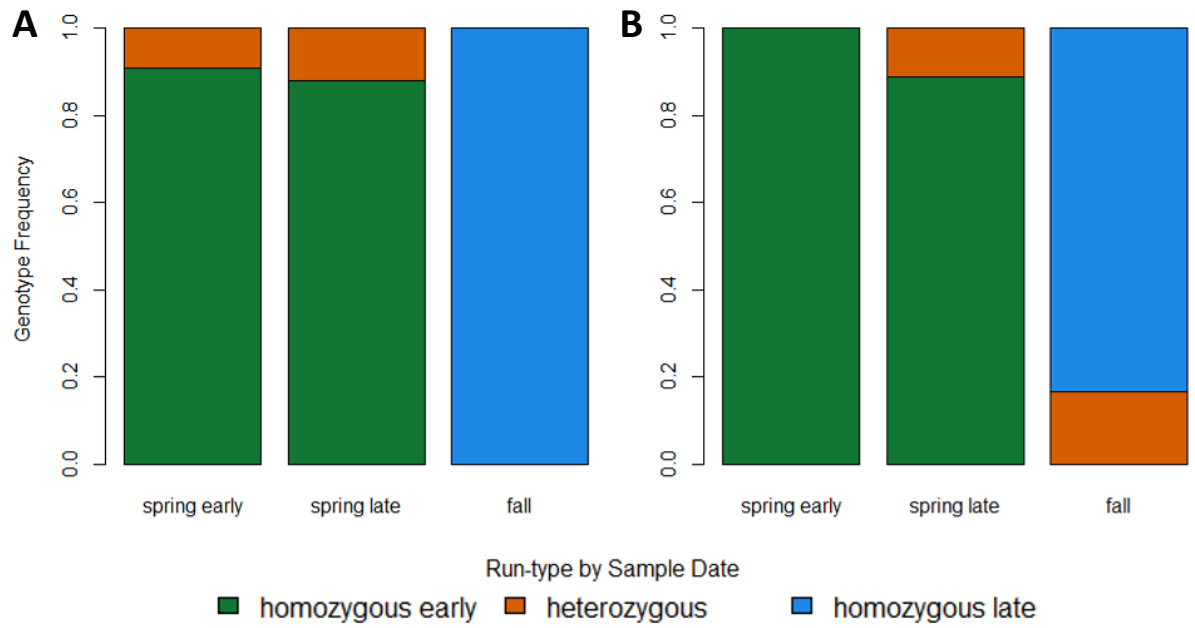


Figure 2.3: Stacked bar graphs of GREB1L genotyped proportions of individuals sorted into phenotypes classified by when they entered the system as Spring early (before July 15th), Spring late (after July 15th but before October 1st) or Fall (after October 1st) using A) Fish surveyed when they first arrived in the system below DPD, B) Fish in A that were acoustically tagged by the date they passed DPD.

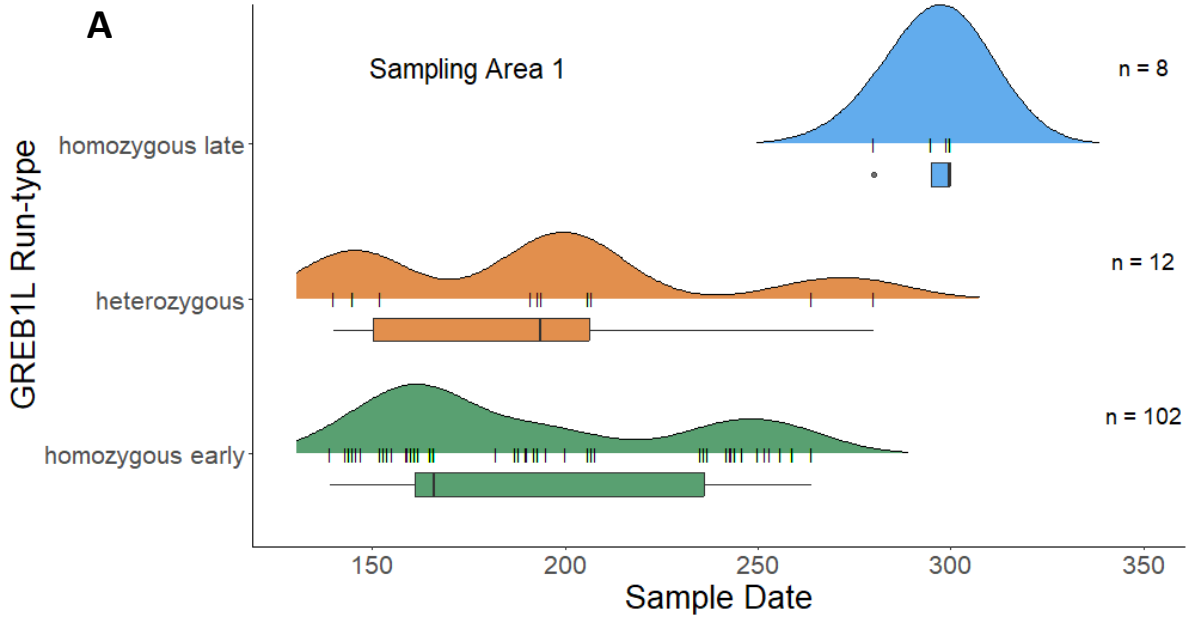
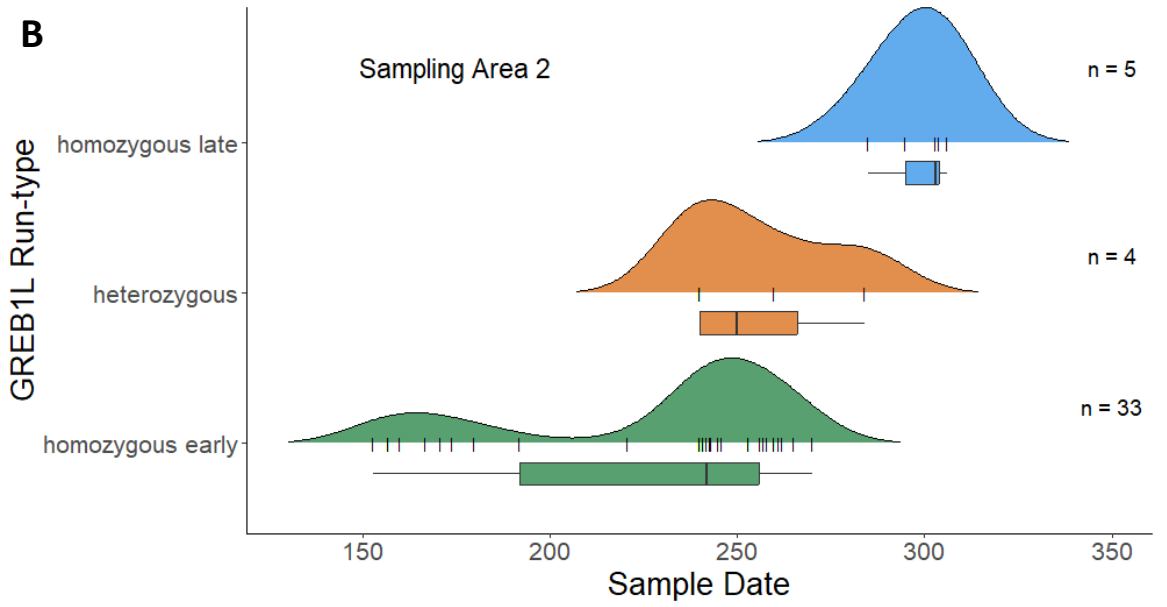


Figure 2.4: Genotypic assignments plotted against date of entry into the Yuba River system colored by GREB1L genotype and median return date using A) Fish surveyed as they entered the Yuba River below DPD, B) acoustically tagged fish in Panel A that passed DPD, and C) Fish detected in carcass surveys, post-spawn. Sample Date is in Julian days, with the equivalent calendar days as follows: 150 = May 30th and day 350 = December 16th.

Figure 2.4 (cont'd)



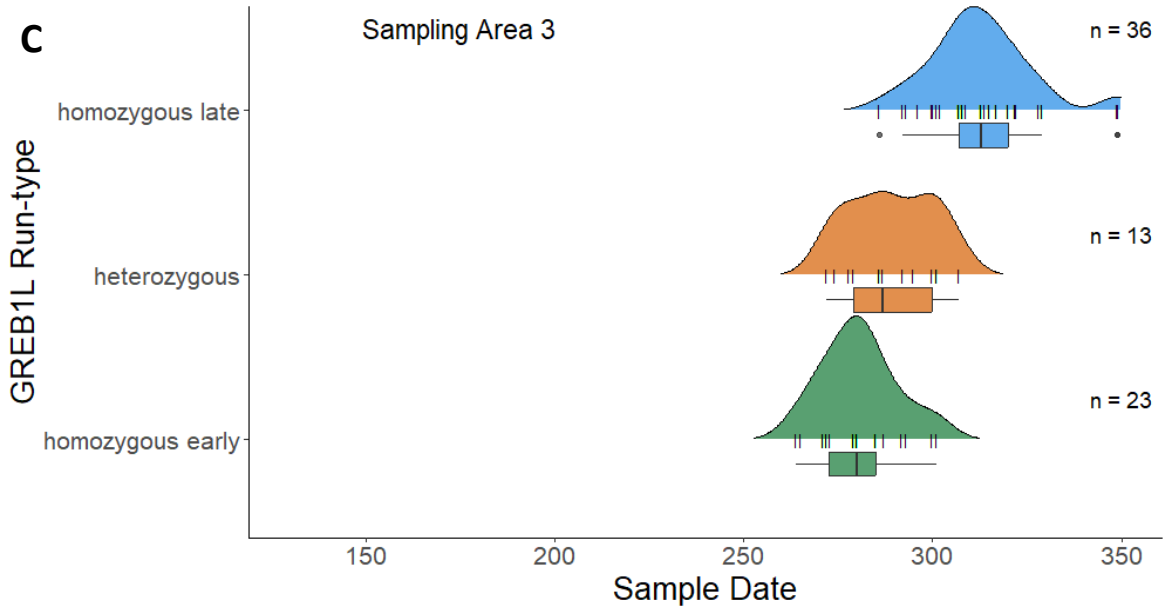


Table 2.2: Statistical results for Kruskal-Wallis comparisons and Dunn test of detection date for each of the three collection methods, comparing within each method for each of the three genotype classifications, where * indicates a significant value.

Survey Type	Genotype	Mean Return Date	Kruskal Wallis χ^2	Kruskal Wallis p	Comparison	Dunn test p
Spawner Survey	Homozygous early	198.41	10.23	0.01*	Homozygous early/ heterozygous	0.30
	Heterozygous	183.13			Heterozygous/ homozygous late	0.002*
	Homozygous late	298.33			Homozygous late/ homozygous early	0.006*
Acoustic Tagging	Homozygous early	227.03	13.45	0.0011*	Homozygous early/ heterozygous	0.777
	Heterozygous	256.00			Heterozygous/ homozygous late	0.067
	Homozygous late	298.60			Homozygous late/ homozygous early	0.0004*
Carcass Survey	Homozygous early	280.57	46.93	6.24e-11*	Homozygous early/ heterozygous	0.2883
	Heterozygous	289.08			Heterozygous/ homozygous late	0.0001*
	Homozygous late	312.75			Homozygous late/ homozygous early	5.58e-11*

DISCUSSION

This study provides direct evidence of spring run Chinook salmon in the Yuba River, and further validation that the GREB1L run timing genotypes are correlated with early or late river sample date. Our data show that individuals entering the system early in the season are genetically homozygous for the early migrating allele or heterozygous, while individuals that enter the system late are homozygous for the late migrating allele. From the acoustic tagging data collected, it appears that heterozygous individuals are passing the dam at a slightly intermediate time point, even though they first appear in the system at the same time as homozygous early running fish. We recognize that our sample numbers for heterozygotes are lower than one would prefer (Fig. 2.4, Suppl. Table 2.2) and additional acoustic tagging would assist in further elucidating the strength of these relationships, however, given the extremely threatened nature of these fish and their very low population sizes, we think the information provided by these samples is incredibly valuable. Additionally, the fact that we didn't find more heterozygotes in this system also points to the maintenance of these distinct life histories and genotypes, despite homogenizing anthropogenic influence. We show there is clearly a pattern of homozygous early genotypes entering the system early through all survey methods. In addition, we see a clear significant difference in spawning time between homozygous early and homozygous late that maintains their temporal segregation in spawning time despite the elimination of spatial separation. Although it is plausible the carcasses were not surveyed until after fish had entered the system, we are certain that surveys were carried out weekly and decomposition rates in this system are fast enough for us to be confident that those samples had not spawned in the system for many additional days beyond when they were sampled.

Our validation of the relationship between GREB1L genotypes and migration phenotypes in the Central Valley is exciting because it means GREB1L can be used to detect, monitor, and quantify the presence of different runs in the Central Valley. The advent of SHERLOCK, which allows especially fast, economical, and field deployable genotyping of the GREB1L locus, makes this possibility even more feasible and has the potential to revolutionize our ability to understand and monitor Chinook salmon life history diversity throughout the Central Valley (Baerwald et al., 2020). In addition, the results found in this study and the combination of tagging and carcass surveys could be used to provide spring run spawner abundance estimates each year, which is critical information for managing this spring run separately from fall run.

Our study also shows that although the dam has eliminated spatial separation between the runs creating some overlap between the presence of spring and fall returning individuals in the system, it does appear that time of entry in the system can also be used as a proxy to determine run-type in the Yuba River. Our research shows that despite anthropogenic influence and very limited to no historical access to spring run spawning habitat due to dam construction, there are still both spring and fall returning populations that are genetically distinct and temporally separated from each other in the Yuba River. This temporal separation is likely only possible due to cold water pools above the DPD and below the Englebright Dam that allow for spring running fish to survive over summer and spawn (Pasternack et al., 2010). It is encouraging that the Yuba River has maintained a spring run population, indicating that important diversity needed to maintain federally listed populations still exists within this altered landscape. Unfortunately, populations have been excluded from large areas of historic oversummering habitat and the remaining habitats are predicted to disappear with a warming climate, leaving only the north fork of the Yuba as potential habitat for spring-running fish (Cordoleani et al., 2021). To ensure the persistence of spring-running fish, it will be necessary to maintain and manage cold water access for these populations.

Discovering that distinct early migrants exist within the Yuba River provides evidence that the system may be able to recover if appropriate conservation efforts and management actions are taken. There is currently an agreement among state, federal, and local officials to reopen large portions of habitat for Yuba River fish. This planned restoration includes the testing and creation of a comprehensive reintroduction plan to reintroduce CCV spring run Chinook salmon into the upper Yuba River habitats as well as habitat restoration design to allow more natural passage around Daguerre Point Dam (California State Government, 2023). This is an important step towards spring run Chinook salmon recovery, however, given impending threats posed by climate change, further actions may be required to ensure that spring run populations recover and persist. Research has shown that intraspecific diversity within spring run Chinook salmon is critical for responding to changing climatic conditions, particularly increases in river and ocean temperatures, helping populations to maintain biocomplexity necessary for resilience and persistence (Cordoleani et al., 2021). More research is needed to fully understand how the amount of migration timing diversity, particularly withing spring run, contributes to an overall portfolio effect, but this will likely be curtailed by lack of available habitat. Because spring run

Chinook salmon rely on cool water to hold over during the summer months, this makes them more susceptible to future threats and continued anthropogenic change such as climate change and water diversion (Meyers et al., 1998; National Research Council, 2004; Quinn et al., 2016). It will therefore be important to ensure that any management actions in the Yuba River promote both the genetic and phenotypic diversity in the system, as well as the hydrological conditions needed to support that diversity.

The Central Valley is a complex and highly altered system with many historical and contemporary threats to life history diversity in fishes (Fisher, 2016; Williams, 2006). However, our work shows that altered ecosystems can still sustain genetic and life history diversity. Life history diversity in salmon has been especially important to maintain species resiliency and persistence, and will continue to be of high importance as we experience more development and more extreme climate regimes (Beechie et al., 2006; Bourret et al., 2016; Pearson et al., 2014). It is often assumed that systems where subpopulations are extirpated or contain introgressed individuals are lacking or have lost life history diversity and biocomplexity. Without a full understanding of variation in genotypes and phenotypes in degraded systems, it is all but impossible to manage them to maintain this diversity. This study highlights the importance of identifying, monitoring, and protecting diversity, even in highly altered environments. In order to ensure the persistence and resilience of the populations in the face of climate change, it will be necessary to protect the little diversity that is left before it is lost forever.

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APPENDIX 2A: GREB1L FLUIDIGM SEQUENCES

Table S2.1: Single Nucleotide Polymorphisms used in Fluidigm type assays from the GREB1L region with their name, genomic positions, original publication, and sequence.

SNPtype Fluidigm Name	Otsh_v1.0_NC_scaffold	Otsh_v1.0_NW_scaffold	Otsh_v2.0	Original publication identifying SNP	Sequence used as input for Fluidigm assay design
GREB1l_pos2194538	NC_037124.1:12273002	chr28_NW_020128528.1|:2194538	NC_056456.1:13457880	Thompson et al. (2020)	GATAAGGGGATAAGGGAGGTCATGCAAATTCCATACCATCCAGGTCAGACAGTGCTAGAACTTTAACCGGAACGCTGC ATGAGTTTAGGGAACATTCTCTTTAGTA[T/C]CAGACTGAACATCCAAATCTTCCTTCACTTCTAGATACACGCTTTAAGG GCCCTCTAGGCAGCTAACTCTGCATCCACAGTAATATAACCCATTCTAGGAGACATTCTTATAACACTGGCCTAGACTAC AAATCACTCTTAACATAACCCTGTAGCTGTGTCCATGATCACAGGGTCACTATCAA
GREB1l_pos2198644	NC_037124.1:12277108	chr28_NW_020128528.1|:2198644	NC_056456.1:13461994	Koch & Narum (2020)	TTTGTCTTCCATTGATATTTGACCTCATGTGGATGTGCCAATGACAACATTATTATTCTCACTCTTAAATCCAACATTAGG GAGACTTAAAACAACCTCAAAAGAGCTACACAATATATTCACGATAACACCATATGTCGYTTGTYTCCTTCACCTGCAA CCTTCTATTCAACAGTCCATTCTTAGAAAAATGACAAGCCYGAGTAAGCCAGTCGGTGAGCCATTCATAACAATCTTAA CATTACTTT[T/A]CAAAAATATTGGATTCGGAATATGGATTCATAACATAATTATGTTATCCTGGATCATTCAAGAGAAAT GAACAGACGGATGAAACATTAAGTCAGAGGATGTTGATCATGACCATATTGTTTAACTGTAATTCTTTCATTTTCATCTT TGCATAGCCAGGCAAGCCGTGTGACTGACTGACTGCCTTAGTCTTCAGTTCATTACAGCAGATCTAGTCAACAGTTGGTT TAATCTGTCCGTATAACTCTTCTCACCTCCT
GREB1l_pos2199210	NC_037124.1:12277674	chr28_NW_020128528.1|:2199210	NC_056456.1:13462560	Koch & Narum (2020)	CTCCACACCACTCATTCATCATACACACATCGCGCATTCTATGCTGAACSTGGCGGTTCGTGTCCATTGCATTATTATACG ACACAGCGTCTGTCTSTCTGWATGGACTCTRTAGGCTCCCGGGGGTAGTCCATTTGAAACAGTTGGAGTAAAGAATGAA AGAAAGAGATGACTTGTKCCCTAAGAGGAGACGAGCATTACAGTTAGTAAACATTACAGTTTCC[T/A]GTCTGAGGTAA ATCAACATATGACCACTCGAAAACTCCCCAAATAAGCTCATTTGGTACAGACCAGCACTAGCAGCAAGTTCAACCTGGG AAGAGGAGTCTCACGGKGTGATTAATCTCCCCCAGCTCCCAGCAGTAGCTCCCTCCCTCCCYGACTTTGACACAGCAGC CACGTTTAAATAGACCCGTTTGAAATGAAGATAATGAGTAAACCCAGCGGTTTCTTTGGCCTCAAAMGAGCCCTGTGTG GGAAAACAAAAGAGCCT
GREB1l_pos2200828	NC_037124.1:12279292	chr28_NW_020128528.1|:2200828	NC_056456.1:13464173	Koch & Narum (2020)	TAAGGGTTGTGGGTGGTGGGGTGGATTAGCCAGTGGGGACTATAAAGGGGAGTGAACTAGGGTTTAAGGCCTGTTGTGA CAGAGGAGCTGGGGAAGGGCTGATGGGGGGGCKGGGGGGAGGCGGACAAAAGGAGCATTTGGGCAGATGAAGAAGTC ATCATCATTAAGCCACTGGAAGTTTACTGTCCAGTTATAAAAGTCATTTCAAAATTAGGRGGTTAGGGGGTGCGTGTGA AAGG[G/A]GAGAAGGGCTCAGAGTGCCTGAGAAGGCCTGGGGGYGGGGCAGATGAGAGCTGTGGCCTGTGGTTGTGAG GGACTCTGTGGGACTGGGGGGCCAATTCATTAGGGGCACAGCCCAGCCTTTGTGTTTGCACCAGGTTGATTGGAGTGCT GACCTTGCCTTGCCTCCCAGCCTTCCCTGCACACTCTGCTCTGGCCCAGTGGAGGATGAGTATAAGGGCAAGGCATTTAA CCTTCAACTAAATCCCAGCCTCAACCACAGCAGACAAAGG
GREB1l_pos2202893	NC_037124.1:12281357	chr28_NW_020128528.1|:2202893	NC_056456.1:13466238	Thompson et al. (2020)	ATTTACCTCCCTGCCCCAGACAATTCTTGAATCACATGGCTGCTGCATTTCATAATGAAAAACAAGGCCA[A/T]ATCAGG AAGTTCAGCCCTCTTTAAATGTGGAAAAMAAAATACAKAGAACATTTTCACTTAGTGTTGTTCTTTTTAAATTTAATTTG AGGCCTGGAGGACAAACTCAATCAATGTGCGGAATTACTGATAATTGACCATGCTCGCTGAGAAGGCCRAATAAAATTG AAGCCCTGAKTGAACCCGCTCTGCATTTTACAACACTGC

APPENDIX 2B: GENOTYPES BY SAMPLING METHOD

Table S2.2: Number of samples in each genotype category organized by each type of survey. Note that samples from individuals in the that were in the acoustic tagging survey were also included in the spawning survey, since that is where they were first detected.

Survey Type	Homozygous early	Heterozygous	Homozygous Late
Spawner Survey	102	12	8
Acoustic Tagging	33	4	5
Carcass Survey	23	13	36
Total	92	21	41

CHAPTER 3: GENETIC DIVERGENCE OF RECENTLY INTRODUCED POPULATIONS OF CHINOOK SALMON IN NEW ZEALAND

INTRODUCTION

Over the past century, anthropogenic activities have resulted in a loss of biodiversity so severe that scientists have classified it as earth's sixth mass extinction event (Tilman 2009; Pyšek and Richardson 2010; Bellard et al. 2012; Intergovernmental Panel on Climate Change 2014; Ceballos et al. 2015). Overfishing, habitat loss and fragmentation, introduced species, and changes in environmental conditions due to climate change have impacted aquatic systems disproportionately (Beddington et al. 2007; Worm et al. 2009; Sadovy de Mitcheson et al. 2013). One of the key challenges facing conservation and management practitioners is how to address this widespread loss of biodiversity. Biodiversity encompasses ecosystem diversity, species diversity, and genetic diversity. In particular, genetic diversity is important as it provides the fundamental building blocks for speciation, as well as harboring the adaptive potential for populations (Chapin et al. 2000; Brooks et al. 2006). Additionally, genetic diversity within a species or population can result in a diversity of phenotypes that stabilizes populations over time, often referred to as biocomplexity (Hilborn et al. 2003). This type of diversity buffers the effects of natural and anthropogenic change (Narum et al. 2018).

Imperiled populations often have reduced genetic variation due to the consequences of large population declines in abundance (Nei et al. 1975; Vrijenhoek 1994). This reduced genetic variation as well as inbreeding due to low population sizes can affect populations' ability to persist (Frankham 2005; Frankham 2015). One way to potentially combat the ill effects of inbreeding and recover these populations is to use genetic rescue, translocating genetically diverse individuals from another region to provide an influx of genetic diversity (Whiteley et al. 2015; Fitzpatrick et al. 2020). Although there are some potential risks to introducing individuals to a population, such as outbreeding depression, increasing empirical evidence also shows that a small amount of gene flow to genetically depauperate populations can result in increased abundance and reproductive success (Frankham 2016; Fitzpatrick et al. 2020).

Chinook salmon is an ecologically, culturally, and economically important species that displays an extraordinary amount of phenotypic diversity that contributes to their overall persistence and resilience (Yoshiyama 1999; Bottom et al. 2009; Raheema et al. 2009; Bourret et al. 2016; Quinn 2018). One life history trait that is particularly important for population

resilience is spawn return timing, with most Chinook populations across the range displaying some variation in what time of year they make their spawning migrations (Moore et al. 2014; Bourret et al. 2016). Chinook salmon in the Central Valley of California (CCV) are unique in that they are the only populations that four genetically distinct run-timing phenotypes (Fall, Late Fall, Spring, and Winter run) co-occur (Williams 2006; Meek et al. 2016). Unfortunately, populations in the CCV have been heavily impacted by human activity (Yoshiyama et al. 1998; National Research Council 2004; IUCN 2017). These activities include overfishing and exclusion from historical spawning grounds, leading many populations to be extirpated and others to be numerically depressed and listed under the Endangered Species Act (Spring and Winter runs) (Williams 2006).

Declines in abundance and distribution of salmon have negatively impacted native peoples of California, including the Winnemem Wintu people, a state recognized tribe of California(Houck 2019). Chinook are spiritually important to the Winnemem Wintu and healthy Chinook populations are of paramount importance(Dallman et al. 2013). The Winnemem are currently engaging in several initiatives to recover and restore their native lands, which largely fall under the more holistic indigenous feminist paradigm of rematriation and can include efforts like Land Back and Water Back (Gray 2022; Leonard et al. 2023; How the Winnemem Wintu won their ancestral land back and help save Chinook Salmon - Vox). Currently, the Winnemem are seeking to rematriate populations of Chinook salmon in the McCloud River, a tributary of the Sacramento River in the CCV. This habitat previously served as spawning grounds for Chinook salmon, but was blocked with the construction of the Shasta Dam, leading to their extirpation above the dam (Houck 2019).

One source of Chinook being considered for the McCloud River rematriation is that of Chinook salmon (*Oncorhynchus tshawystcha*) found in New Zealand (NZ). Between 1901 and 1907, Chinook salmon from the CCV were introduced into the Waitaki River in NZ (McDowall 1994) (Fig. 3.1). It is currently unknown which tributary in the CCV the NZ Chinook originated from because many of the records were lost in a fire (McDowall 1994). From what records do exist, it is clear that the Chinook in NZ originated from a tributary of the Sacramento River, the largest river in the CCV (McDowall 1994). Previous work used microsatellite data to show divergence of the NZ salmon from Battle Creek Fall run in the CCV, one of the hypothesized sources, while another study showed divergence from the Feather River Spring run in the CCV

(Quinn et al. 2001; O'Malley et al. 2007). Unfortunately, these microsatellite data were of low resolution, and to date further comparisons of NZ populations to other populations characterized by different spawning timing from the CCV have not been conducted (Kinnison et al. 2002). Given this context, we sought to understand the genetic structure and diversity of NZ Chinook using other molecular methodology. By comparing all populations in both the CCV and NZ we aimed to explore the genetic diversity and structure of NZ Chinook salmon compared to current day CCV populations.

Since the initial introduction, NZ Chinook have populated several other rivers near the Waitaki River by natural processes such as straying. This has potentially allowed for different populations in each new river or tributary in NZ to adapt and thus generate unique genetic diversity. NZ populations of Chinook also exhibit divergence in phenotypic traits, including freshwater growth rate, reproductive output, and run timing (Quinn et al. 2001). Because adaptation has potentially taken place in the NZ populations, it is possible reservoirs of genetic diversity exist in NZ Chinook that could be used to inform rematriation efforts, for example genetic rescue aimed at decreasing the negative effects of low genetic diversity in CCV populations. Empirical evidence suggests that an influx of genetic diversity from an evolutionarily similar population can increase population growth, making the NZ Chinook an excellent system to explore for CCV Chinook recovery efforts (Whiteley et al. 2015).

Here, we investigate the spatial patterns of genetic diversity in a novel environments for a newly introduced species using high throughput sequencing, showing divergence within and among chinook populations in NZ and the CCV, and address how that can inform a rematriation effort of NZ Chinook salmon to the CCV. The purpose of this research is to assess the spatial patterns of genetic diversity in NZ Chinook salmon, and compare the diversity patterns found within NZ and compared to the CCV. We examine the following questions:

1. Is there population structure within Chinook salmon populations in NZ, and if so;
2. How do NZ Chinook salmon compare in terms of overall allelic richness, heterozygosity, and levels of inbreeding?
3. Using this marker set, do NZ Chinook salmon appear genetically unique compared to CCV populations?

METHODS

Sample Collection and Sequencing

The data analyzed here were obtained from two separate sources that corresponded with CCV vs NZ Chinook salmon. We obtained NZ adult Chinook salmon DNA extracted from fin tissue collected by the Cawthron Institute as part of regular post spawning surveys from three main river catchments between the years 2017-2018 (Fig. 3.1). DNA was extracted from these samples using the high salt method described in Clarke et al, and dried down 20 uL(Clarke et al. 2014). We then rehydrated these samples using a low TE solution and quantified the DNA using a Qubit 3 fluorometer and the High Sensitivity quantitation kit (Thermofisher Scientific). SNP panel design and selection was completed by Danile Gomez-Uchida and Rodrigo Marin Nahuelpi at the Universidad de Concepción. They selected a set of single nucleotide polymorphisms (SNPs) previously identified from restriction site associated DNA RAD sequencing experiments mentioned in previous Chinook studies (Hecht et al. 2015; McKinney et al. 2016; Narum et al. 2017). Briefly, they pulled down the sequence metadata from Hecht et al 2015, Narum et al 2017, and McKinney et al 2016 to get the sequences associated with the polymorphic SNPs, aligned them to the reference genome available at the time (GCA_002872995.1) (Christensen et al. 2018), and used BLASTn in order to get position information for each polymorphic SNP, keeping only one SNP per rad tag. We then used this raw data for our study. The studies the SNPs were obtained from were all performed single digest RAD sequencing on Chinook utilizing the SbfI restriction enzyme using the methods explained in Miller et al 2007 and Baird et al 2008, which allows for direct comparison to the CCV samples used from Meek et al 2019 (Miller et al. 2007; Baird et al. 2008; Meek et al. 2019a). The research these sites were pulled from found these SNPs to be useful for delineating populations, but some were possible sources of adaptive variation. In total, 17,062 were sent for probe development to LGC Biosearch Technologies (hereafter referred to as LGC) to create a SeqSNP panel based on each individual SNP. LGC uses the SNP locations to design high-specificity probes (no off-targets) to create a genotyping panel for targeted genotyping by sequencing using single primer enrichment technology (LGC Group 2023). DNA samples were sent to LGC to be sequenced with this custom panel using the Illumina NextSeq 500 platform, single-end 1 x 75 bp run. In total we obtained genetic information from 89 samples from 3 locations in NZ (Table 3.1).

CCV genetic information was obtained from a previously available dataset as described in Meek et al 2019. Briefly, this was a RADseq paired end dataset with read lengths of 150 bp. This research included individuals from all major runs of Chinook Salmon (Fall, Late Fall, Spring and Winter run) from all major tributaries within the CCV, with sampling spanning the years 2001-2010 (Fig. 3.1) (Meek et al. 2019b). In total, we obtained genetic information from 563 individuals from 11 tributaries in the CCV (Table 3.1).

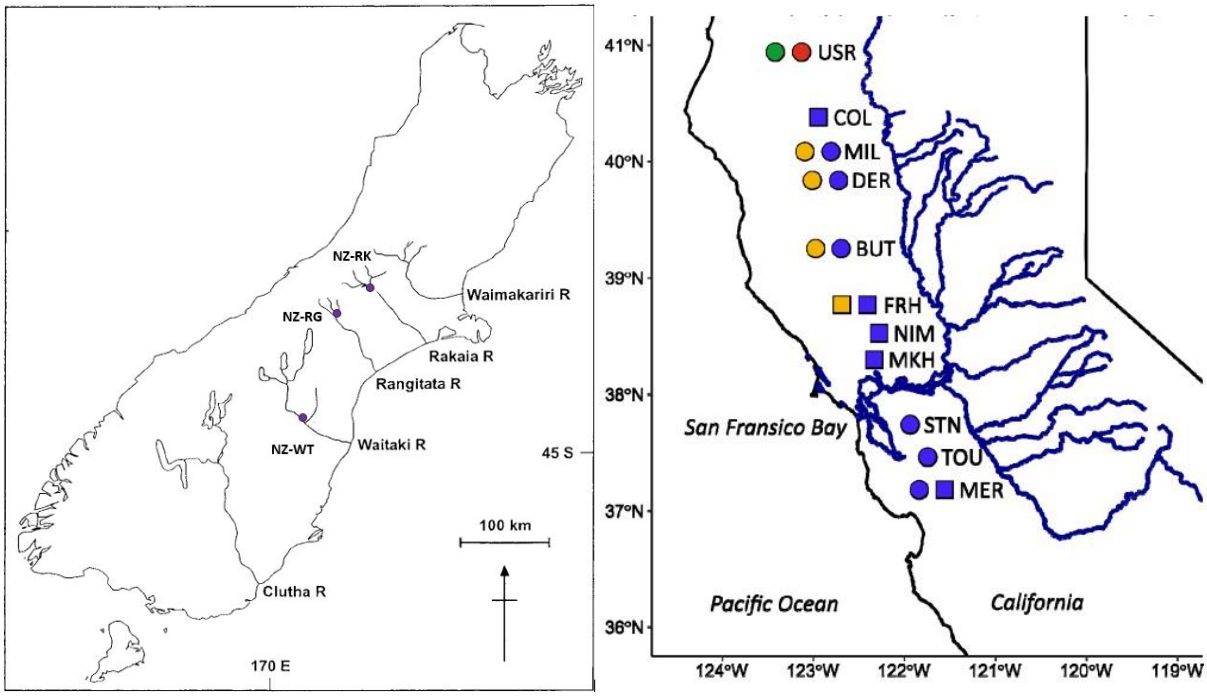

Figure 3.1: Maps of sampling locations. The map on the left is of sampling locations in NZ as adapted from Quinn et al 2001(Quinn et al. 2001) and the right is locations in the CCV as adapted from O'Leary et al 2021(O'Leary et al. 2021). Sampling locations in the major rivers of NZ (Rangitata NZ-RG, Rakaia NZ-RK, and Waitaki NZ-WT) are colored in purple dots. Sampling locations in the Sacramento River are colored by run-timing (Fall = blue, Late Fall = red, Yellow = Spring, and Winter = Green) with squares indicating hatcheries. The tributary abbreviations are as follows: MER = Merced River, TOU = Tuolumne River, STN = Stanislaus River, MKH = Mokelumne River Hatchery, NIM = Nimbus River Hatchery, FRH = Feather River Hatchery, BUT = Butte Creek, DER = Deer Creek, MIL = Mill Creek, COL = Coleman Hatchery, USR = Upper Sacramento River.

Table 3.1: Table indicating number of samples collected and the known source population and run timing, if available. Abbreviations for the various sites are the same as Figure 1.

LOCATION	ABBREVIATION	Fall	Late Fall	Winter	Spring	Unknown
Coleman Hatchery	COL	30	-	-	-	-
Mill Creek	MIL	20	-	-	16	-
Deer Creek	DER	15	-	-	27	-
Butte Creek	BUT	21	-	-	19	-
Feather River Hatchery	FRH	27	-	-	7	-
Nimbus River Hatchery	NIM	30	-	-	-	-
Mokelumne River Hatchery	MKH	28	-	-	-	-
Tuolumne River	TOU	23	-	-	-	-
Merced River Hatchery	MRH	30	-	-	-	-
Merced River	MER	31	-	-	-	-
Upper Sacramento River	USR	-	21	26	-	-
Rangitata River	NZ_RG	-	-	-	-	29
Rakaia River	NZ_RK	-	-	-	-	28
Waitaki River	NZ_WT	-	-	-	-	32

Genotyping

We processed and quality filtered the genetic data in preparation for alignment. CCV samples were de-multiplexed using the "process_radtags" program in STACKS (Catchen et al. 2013; Rochette and Catchen 2017). NZ samples were pre-processed for quality by LGC using their standard procedures for their SeqSNP projects (LGC Group 2023). We repeated the LGC procedures on all CCV samples, which we explain here. First, reads were clipped to remove adapter sequence and then quality trimmed. Quality trimming consisted of removing reads containing Ns with trimming at the 3'-end over a window of 10 bases to get a minimum average

Phred quality score of over 30. Reads with less than 65 bases were discarded. Because later analyses would require reads to be all of the same length and the CCV sequences were longer than NZ reads, we trimmed the end of the reads in all CCV samples to a length of 75 bp and to allow for comparison to NZ samples, we only used the forward reads from this point on. We then assessed sequence quality using fastqc (Babraham Bioinformatics - FastQC A Quality Control tool for High Throughput Sequence Data).

After all samples were processed and quality filtered, we aligned them to the reference genome. We then aligned all reads using the bwamem program (Li and Durbin 2009), aligning to the newest published version of the Chinook reference genome (GCA_018296145.1)(Oncorhynchus tshawytscha genome assembly Otsh_v2.0). We completed SNP discovery using the populations program in gstacks portion of Stacks v2.64, via the creation of a catalog (Rochette and Catchen 2017; Rivera-Colón and Catchen 2021).

To investigate the dataset multiple ways, we created two catalog datasets to begin genotype filtering because the NZ samples were such a small proportion of the total samples in the NZ/CCV comparison. In order to meaningfully compare NZ populations and minimize the amount of SNPs from NZ populations that would be lost to stringent filters in the NZ/CCV comparison, we created catalogs for both the NZ samples on their own, and the NZ and CCV samples together. The first dataset contains only individuals from NZ, and we will hereafter refer to that dataset as NZO. The second dataset contained both the NZ and CCV populations and will hereafter be referred to as NZCA.

After SNP discovery, we then filtered the genotypes in NZO and NZCA for quality using VCFtools (Danecek et al. 2011). The methods for filtering between the two datasets diverged at this point, with more stringent filters applied to the NZO dataset, and then separate filtering parameters applied to the NZCA dataset. For the NZO only dataset, we first filtered out genotypes missing in 50% of individuals, a minor allele count of 3, minimum genotype quality value of 30, and a minimum depth of read of 5. We then filtered and removed SNPs that were missing up to 10% of genotypes, and removed individuals with 50% or more missing data. For the final step of quality filtering on the NZO dataset, we filtered out genotypes with minor allele frequencies < 0.01 to remove possible monomorphic loci.

To mitigate the possibility of losing too many SNPs from either population, we manually divided the NZCA populations file produced from the catalog into NZ and CCV samples for

initial quality filtering, the NZ individual dataset we will hereafter refer to as NZ1 and the CCV individual dataset we will refer to as CA1. Both datasets were filtered separately for max missing genotypes of 0.2, minimum depth of reads of 3, and minimum genotype quality of 30. After that, we generated a list of SNPs still present in both the NZ1 and CA1 VCFfiles, and then used that list to contain only those SNPs in the original populations file generated by gstacks, generating the initial NZCA dataset. To ensure that no low quality reads were left in this newly filtered NZCA dataset, we filtered this dataset with VCFtools to remove any alleles with a minor allele frequency lower than 0.01 and removed individuals with more than 50% missing data (this resulted in the removal of 48 CCV individuals and 2 NZ individuals).

After initial quality filtering, both the NZO and NZCA datasets underwent the same final filtering steps. These reads were then filtered for paralogous genes using HDplot due to the whole genome duplication present in *Oncorhynchus* species(McKinney et al. 2017). After that, we examined genotypes to retain only one SNP per rad tag or region, eliminating those SNPs that were close together (on the same RAD locus) to minimize the possibility of linkage disequilibrium. To explore relatedness, we calculated pairwise **φ**, using the relatedness2 function in VCFtools (Manichaikul et al. 2010; Danecek et al. 2011). Individuals with a relatedness value ≥ 0.2 (indicative of excessive levels of relatedness such as half-siblings or closer) were removed from the analysis.

Statistical analysis

To investigate population level relationships between groups, we performed a principal component analysis (PCA) and a discriminant analysis of principal components (DAPC) to infer overall genetic variation among individuals using the "adegenet" package in R (Jombart 2008; Jombart and Ahmed 2011). The number of clusters was determined by running 25 iterations of the find.clusters module, with 30 possible clusters set as the maximum for the NZCA dataset, one group for each river in NZ and one for each river and run timing iteration in CCV. We ran the same analysis on the NZ samples only, this time setting the maximum clusters at 10 to capture any variation among drainages. We then inferred the most appropriate value of K from the Bayesian information criterion (BIC) values. The most likely value of K was then used to define genetically distinct populations within which genetic diversity was explored in the NZO dataset. For NZCA, we used the most likely value of K as a starting point, but also made some assumptions about groups based on already known genetic differentiation within the CCV.

Because the low level of SNPs did not resolve population level differences already known in the CCV, we separated those groups by run-timing and one group for NZ populations.

To investigate patterns of genetic variation within the NZ populations in the NZO dataset, we used populations defined by the PCA and DAPC. To calculate observed and expected heterozygosities, we used both of the R packages dartR and poppR (Nei 1978; Kamvar et al. 2014; Gruber et al. 2018). DartR was also used to evaluate each population for the presence of private alleles. We utilized the R package 'PopGenReport' to analyze levels of allelic richness(Adamack and Gruber 2014). To assess levels of genetic differentiation between the NZ populations and CCV populations in the NZCA dataset as well as within NZ populations within the NZO dataset, we calculated Fst in dartR with 10,000 bootstrap iterations (Gruber et al. 2018).

Table 3.2: Individuals and SNPs retained after initial and final filtering steps based on what dataset the individuals were processed in. Note that NZ individuals were both evaluated on their own and with the larger dataset including CCV individuals.

Dataset	Subset and individuals contained	n before filtering	SNPs before filtering	n after initial filtering	SNPs after initial filtering	Final n	Final SNPs after all filtering
NZO (NZ only)	N/A	89	127,103	89	4007	82	1774
NZCA (NZ and CCV)	NZ1 (NZ only)	564	312,596	517	28,187	593	131
	CA1 (CA only)	89	312,596	87	19,037		

RESULTS

Genetic population structure

The unfiltered NZO dataset exported from Stacks contained genotypes for 127,103 SNPs in 89 individuals (Table 3.2). After final quality filtering, we retained 1774 SNPs in 82 individuals. The unfiltered dataset for the NZCA dataset exported from Stacks contained genotypes for 312,596 SNPs in 653 individuals (Table 3.2). Unfortunately, due to the amount of SNPs only present in the CCV populations, many of these SNPs had to be removed. When

comparing the overlap between the NZ1 and CA1 outputs for the NZCA data set, only 170 SNPs remained. After removing the additional SNPs for the various quality filtering steps described in the methods, only 131 SNPs remained. Because of the low amount of SNPs in the NZCA dataset, we were not able to accurately determine values of relatedness among individuals. We removed 2 individuals from the NZO dataset due to a relatedness value of ≥ 0.2, and removed those same individuals from the NZCA dataset.

Results from the PCA based on the NZO dataset revealed that there was some separation along PC axes and that they were likely two or more genetically distinct populations (Fig. 3.2). When performing DAPC, we identified groups of individuals that separated very clearly into at least 2 (BIC = 427) or 3 groups (BIC = 429) (Fig. 3.3). When examining the posterior probabilities for these groups by NZ river, individuals did not show evidence for admixture among groups (Fig. 3.4). In the K = 2 scenario, one group consisted of all Rangitata individuals, the majority of Waitaki individuals, and roughly half the Rakaia individuals, while the other group was almost entirely Rakaia individuals. With a K = 3 scenario, we began to see one group of almost entirely Waitaki origin individuals, one group of Rangitata individuals with roughly half the Rakaia individuals, and another group almost entirely Rakaia individuals. The K = 4 scenario shows signs of overfitting our model as it does not appear the genetic groupings are no longer biologically meaningful. This shows that the models showing K = 2 or 3 are the most likely biologically accurate.

Results from the PCA and DAPC NZCA dataset revealed that the CCV populations and NZ populations were genetically differentiated from each other, based on our limited SNP set. The PCA resulted in three groups, with some overlap, showing NZ and winter run CCV individuals beginning to separate out, with some overlap (Fig. 3.5). This was also apparent in the results from the DAPC, which found that a K value of five (BIC = 905) was the most optimal during K means clustering (Fig. 3.6). Although there was overlap in the DAPC , group one consisted entirely of phenotypic winter run samples from the CCV, while group three consisted entirely of NZ origin samples. Groups two, four, and five were largely a mix of phenotypic spring, fall, and late fall run from the CCV (Fig. 3.6). The limited resolution of groups 2-5 is likely due to the low number of SNPs in the dataset. When examining the posterior probabilities of the DAPC, we saw a clear demarcation between winter run, NZ rivers, and the other groups at K = 3 (BIC = 909). As we increased K, the fall, spring, and late fall run groups became less

clear, however the separation of winter run and NZ groups remained, and we began to see the fall run groups looking distinct from spring run groups (Fig. 3.7).

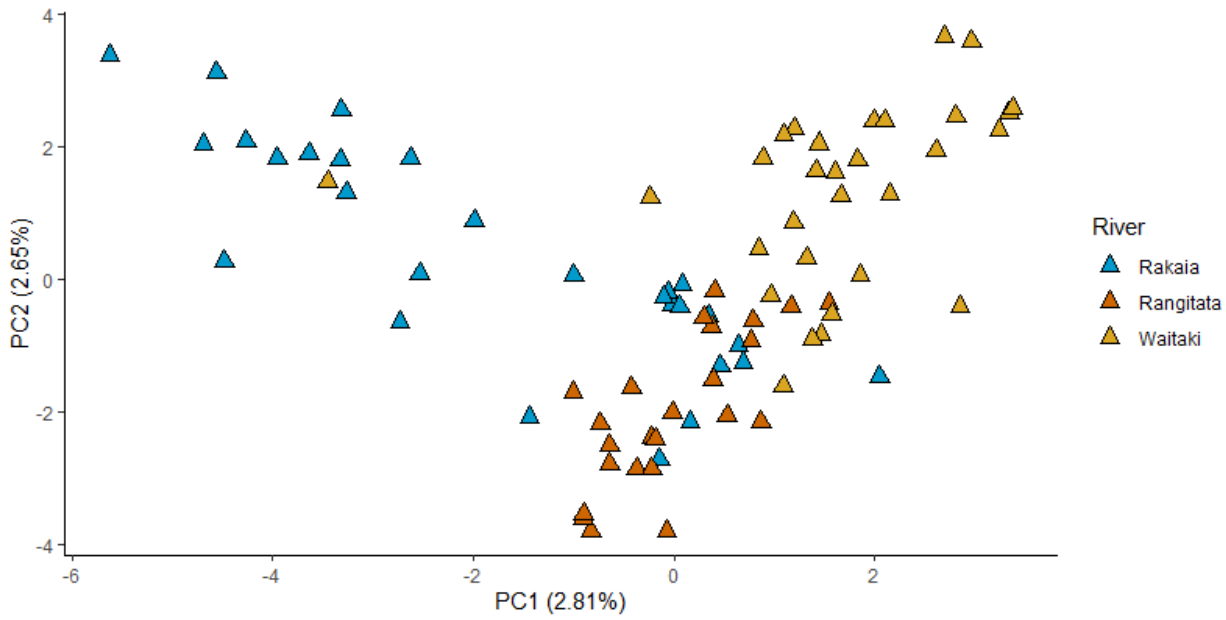

Figure 3.2: Results from a PCA of the Chinook salmon from NZ analyzed in this study, with axes corresponding to PC 1 and 2. Individuals are colored based on river origin using a dataset with filtered SNPs for only NZ (n = 82, SNPs = 1774).

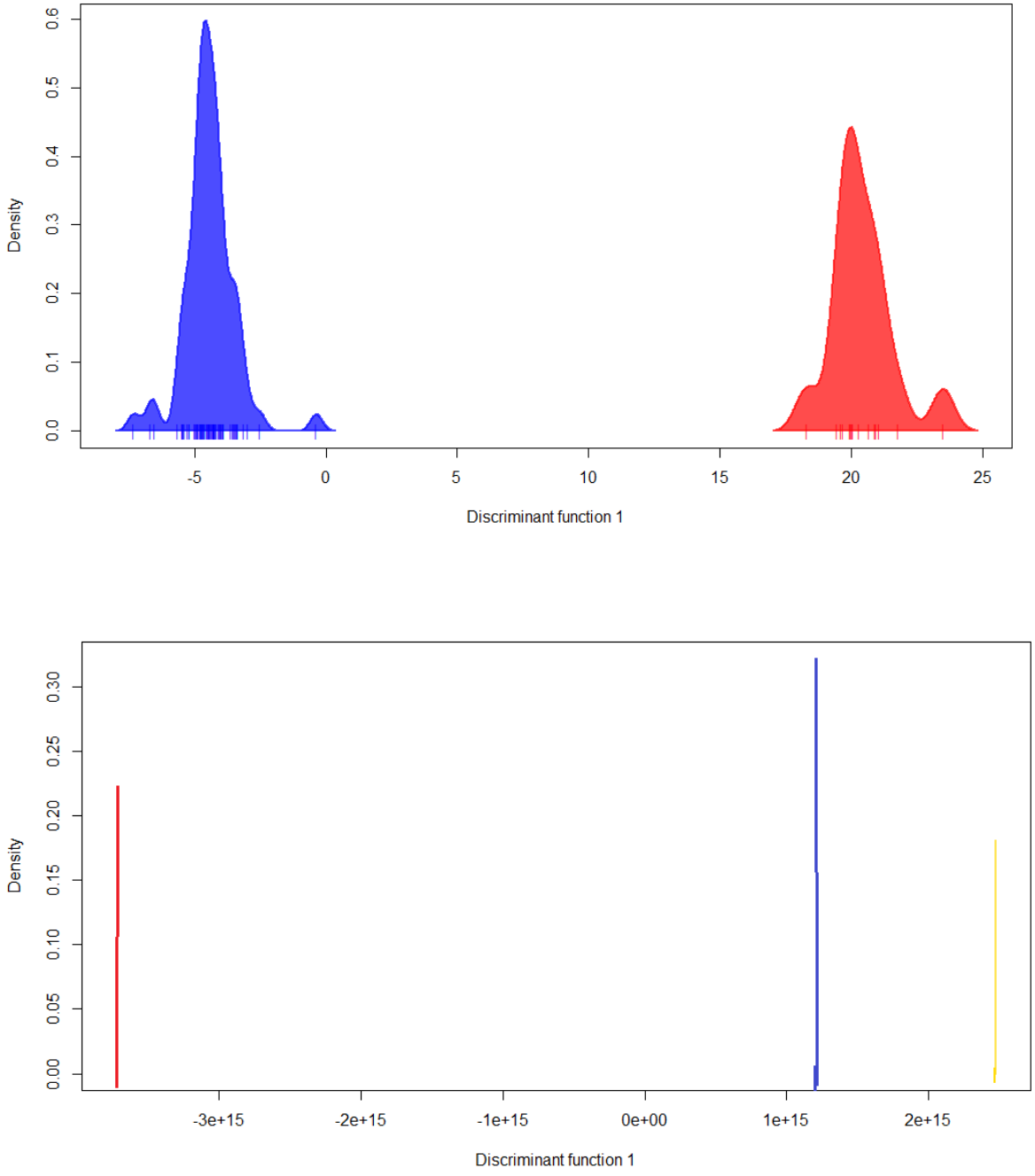

Figure 3.3: Results from a DAPC comparing NZ Chinook salmon. The figure shows separation based on the most informative linear discriminant for the top panel (K = 2) and the bottom panel (K =3).

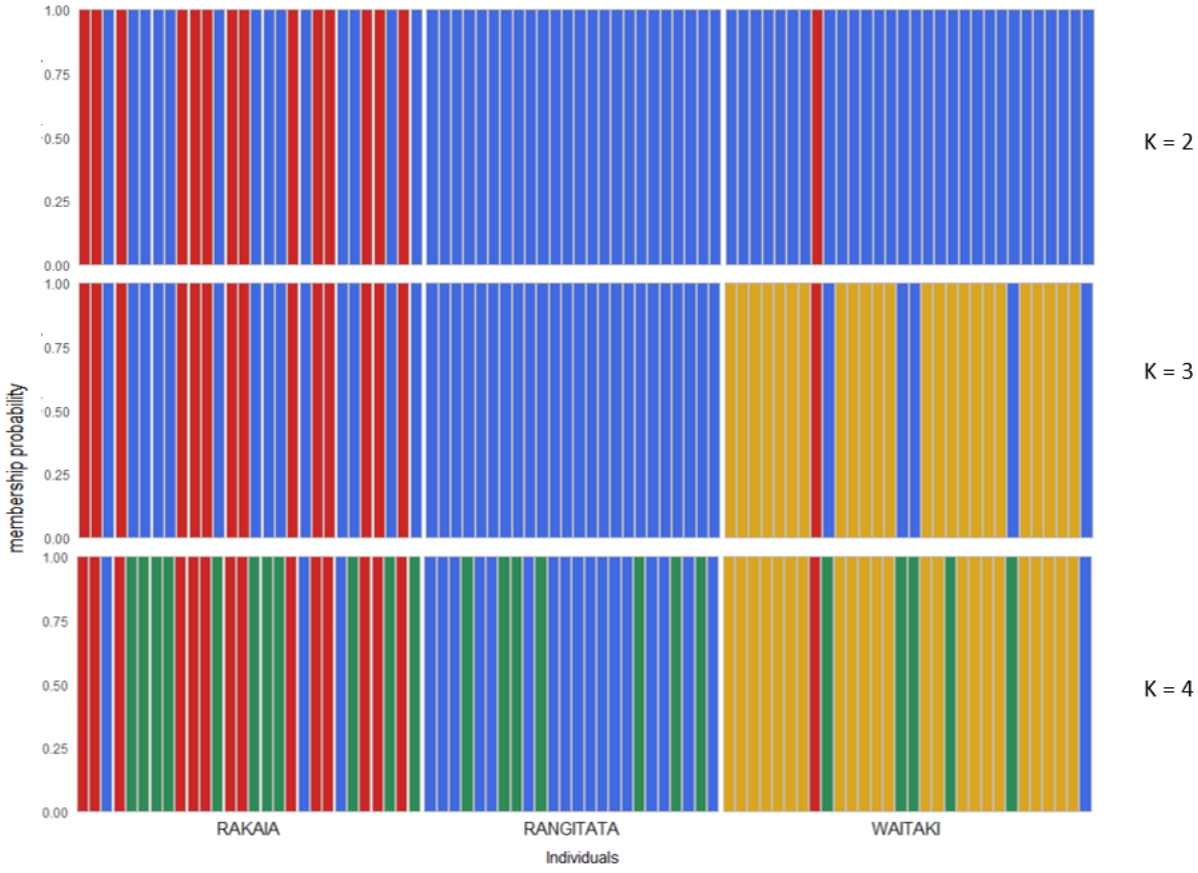

Figure 3.4: Results from posterior probabilities of DAPC analysis comparing NZ Chinook salmon. The figure shows results for K = 2 (top panel), K = 3 (middle panel), and K = 4 (bottom panel). Vertical bars represent individuals and are color coded based on their proportion of membership to a particular DAPC group as illustrated in Figure 3. Individuals are organized by the NZ river of origin.

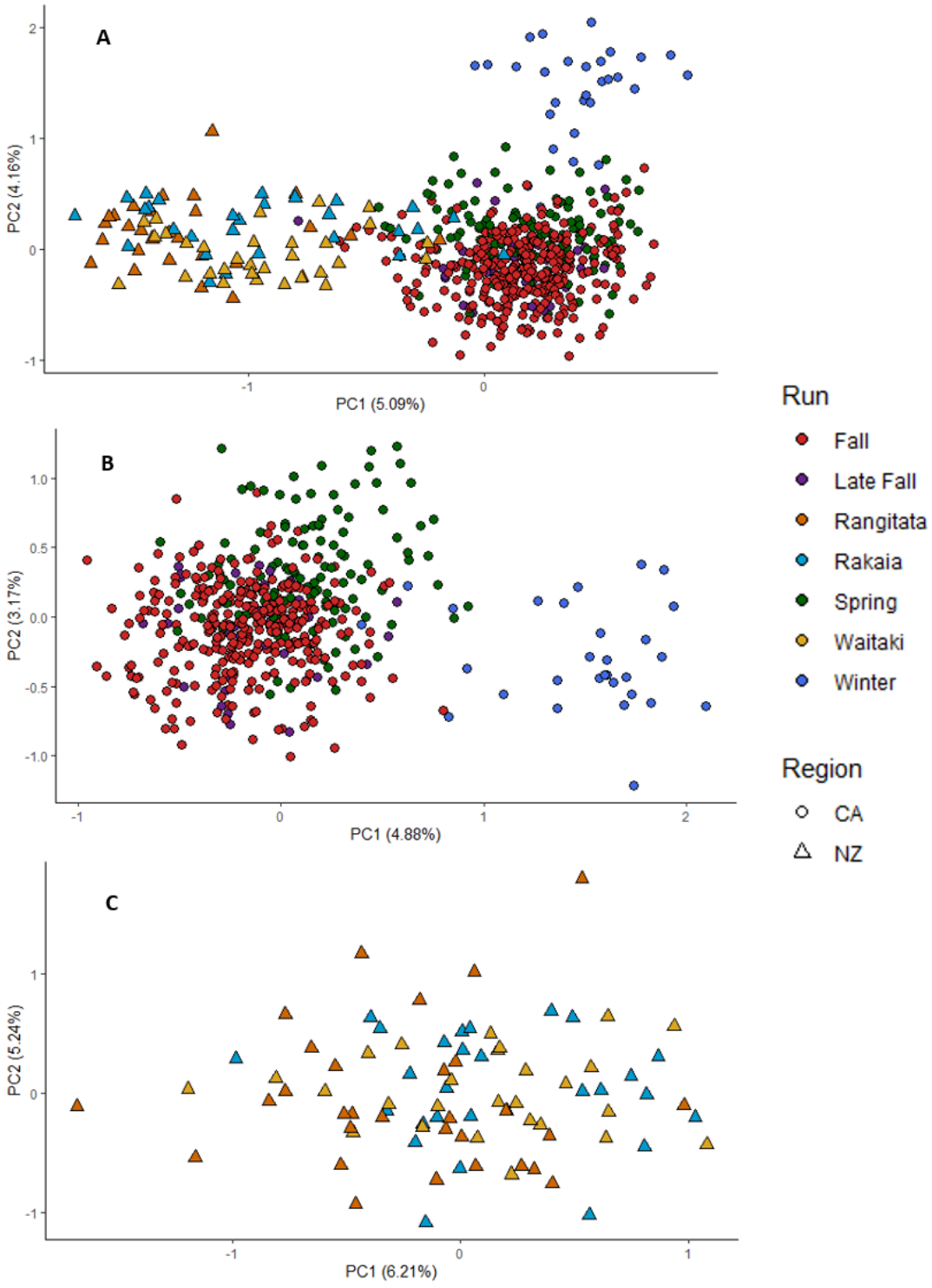

Figure 3.5: Results from a PCA of the Chinook salmon from NZCA dataset, with axes corresponding to PC 1 and 2. Individuals are colored based on run-timing or river origin (NZ individuals) filtered SNPs comparing NZ and CCV (n = 593, SNPs = 131). A) contains all individuals from the NZCA dataset in PC space together B) CCV samples, and C) NZ samples.

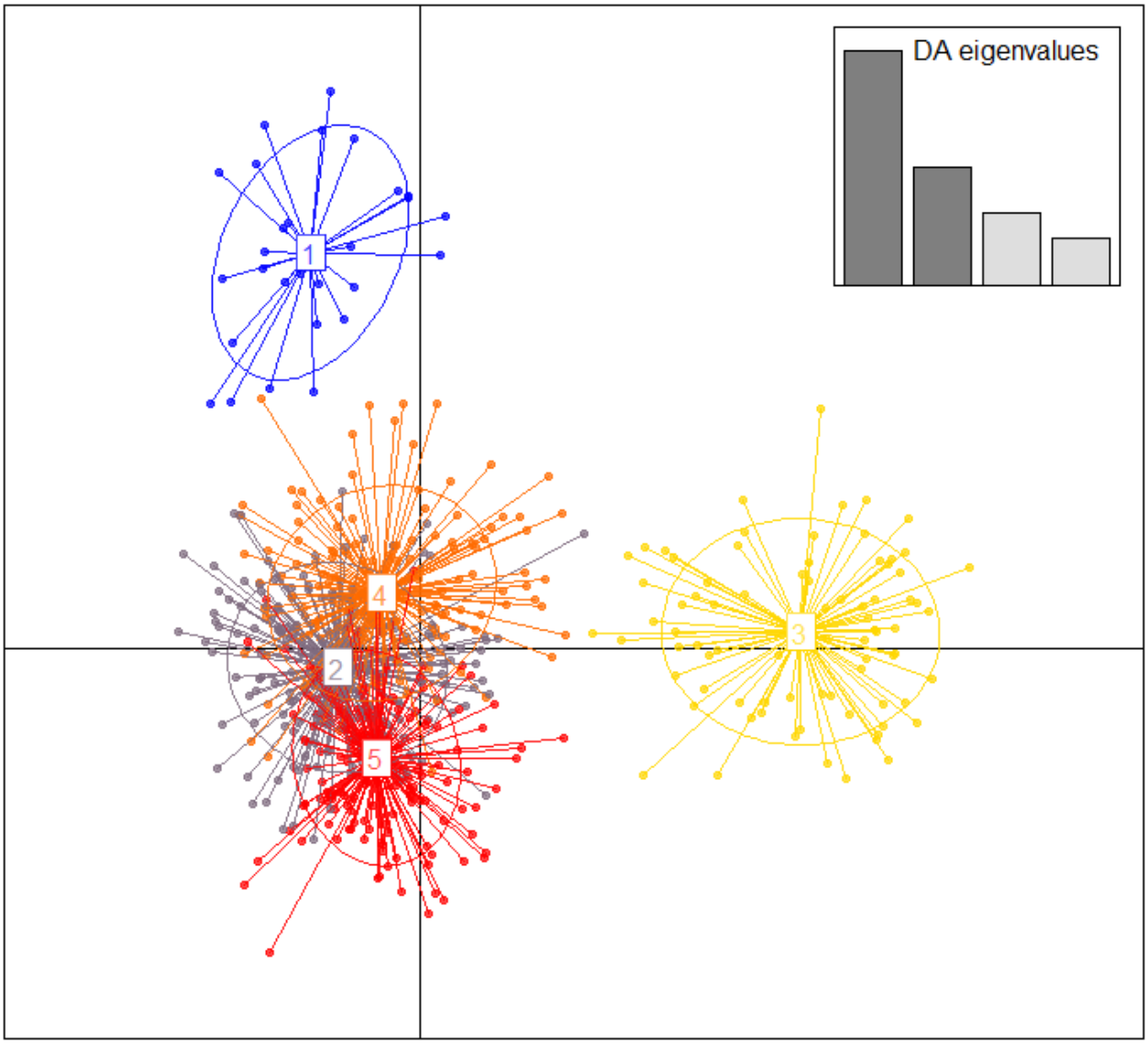

Figure 3.6: Scatter plot results of a DAPC comparing the NZ vs CCV samples. Group 1 is composed entirely of NZ individuals while group 3 is composed entirely of Winter Run CCV samples. Groups 2, 4, and 5 are a mix of Spring, Fall, and Late Fall run individuals.

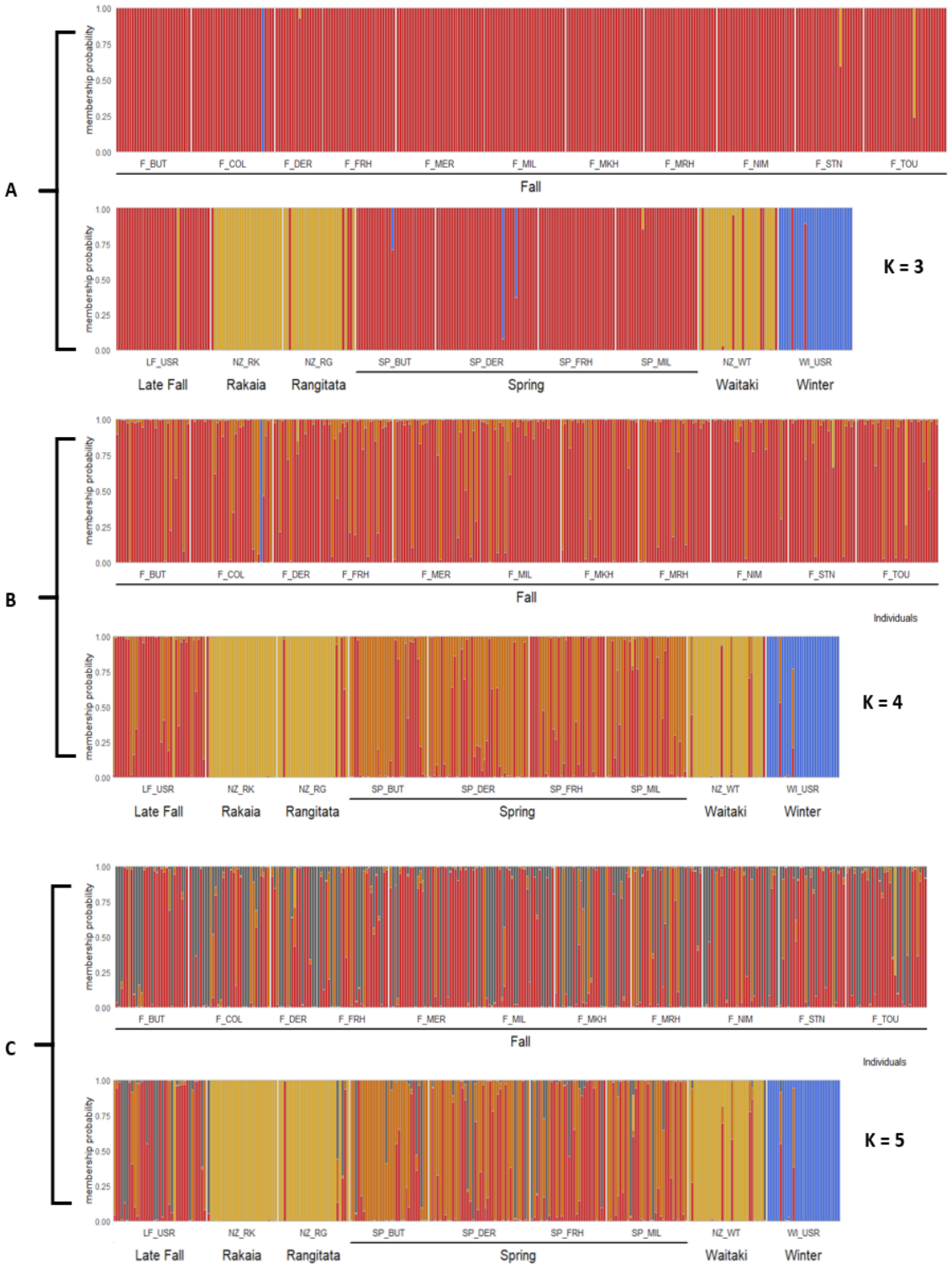

Figure 3.7: Results from posterior probabilities of DAPC analysis comparing NZ and CCV Chinook salmon. The figure shows results for A) K = 3, B) K = 4, and C) K = 5. Vertical bars represent individuals and are color coded based on their proportion of membership to a particular DAPC group, organized by river origin, and where available, run-timing (CCV individuals).

Metrics of genetic diversity among populations and drainages

Measures of genetic diversity among all populations in NZ were largely very similar (n = 89, SNPs = 1774). Populations in New Zealand both grouped by river as well as the DAPC groups reported similar high allelic richness as well as observed and expected heterozygosity (Table 3.3). Estimates of the inbreeding coefficients were also very similar and all were low and negative. When comparing statistics of genetic diversity in the NZCA dataset, we see different trends. Because genetic information was limited and did not illustrate the fine scale population structure that is known to exist in the CCV, we first examined populations as defined by known run timings (except in the case of NZ salmon, which were retained as their own group) (Meek et al. 2019b). This showed that Winter run had the lowest heterozygosity and allelic richness, and the highest inbreeding coefficient values, which is as expected given the low contemporary population size of Winter run (Table 3.4). New Zealand had the second lowest heterozygosity and allelic richness values, although those values were much higher than the Winter run group.

When comparing levels of genetic differentiation among groups where K = 3, (Table 3.5), we observed relatively low Fst between the groups that contained mainly Rangitata and Waitaki individuals, with a higher level of genetic variation between Rakaia and both the Waitaki and Rangitata rivers. When comparing DAPC groups of K = 2, the differentiation between NZ groups was very similar, largely driven by the difference between some Rakaia individuals and all the other samples (Table 3.6). No private alleles were found in any of the populations in either analysis on either dataset, NZO or NZCA.

When comparing NZ and CCV populations and drainages (n=593, SNPs = 131), there were a range of population differentiation scores. In each comparison, Winter run diverged most from NZ drainages, but also from Spring, Fall, and Late Fall run (Table 3.7). The smallest Fst value was between Fall and Late Fall groups, while the 2nd largest Fst values existed between NZ, Late Fall run, and Spring run (after Winter run comparisons).

Table 3.3: Heterozygosity (H_O=observed and H_e=expected), inbreeding coefficients (F_{IS}), and allelic richness (a_R) for NZO dataset with n=82 and 1774 SNPs. Groups were based first on the K = 3 DAPC groups, named by their majority composition of the 3 river sites in NZ and second on the groups K = 2 groups DAPC assigned, followed by the total metric when the population was considered as a whole. N = sample size.

Group	**N**	**a_R**	**H_O**	**H_e**	**F_{IS}**
Rangitata (K = 3)	43	1.99	0.369	0.355	-0.039
Rakaia (K = 3)	15	1.99	0.344	0.342	-0.005
Waitaki (K = 3)	24	1.99	0.360	0.350	-0.030
Rakaia (K = 2)	15	1.97	0.363	0.341	-0.027
Rangitata & Waitaki (K = 2)	67	1.98	0.362	0.352	-0.030
Total	82	1.99	0.344	0.331	-0.005

Table 3.4: Heterozygosity (H_O,=observed and H_e,=expected), inbreeding coefficients (F_{IS},), and allelic richness (a_R) for NZCA dataset n = 593 and 131 SNPs. Groups were based on known run-timing phenotype in the CCV compared to one group of all NZ individuals. N = sample size.

Group	**N**	**a_R**	**H_O**	**H_e**	**F_{IS}**
Fall	315	1.61	0.188	0.184	-0.019
Late Fall	37	1.59	0.185	0.183	0.005
Spring	127	1.62	0.191	0.190	-0.002
Winter	29	1.45	0.139	0.140	0.019
New Zealand	85	1.52	0.178	0.166	-0.063

Table 3.5: Fst Estimates for the NZ rivers where n=89 and 1774 SNPs. Groups for Fst statistics are based on NZ samples grouped by DAPC K = 3 but named as the major tributaries that the majority of the individuals originated from.

NZ Fst Estimates - K =3 groups			
River	Rangitata	Rakaia	Waitaki
Rangitata	-	0.021	0.0125
Rakaia	-	-	0.0289

Table 3.6: Fst Estimates for the NZ rivers where n=89 and 1774 SNPs were used. The Fst statistic is based on NZ samples grouped by DAPC (K = 2) but named as the major tributaries that the majority of the individuals in that group originated from.

NZ Fst Estimates - 2 Groups	
Group	**DAPC Group 2 (Rangitata and Waitaki)**
DAPC Group 1 (Rakaia)	0.021

Table 3.7: Fst Estimates for CCV vs NZ groups where n = 593 and 131 SNPs were used. Groups were based on known run-timing phenotype in the CCV as compared to one group of all NZ individuals.

NZ vs CCV Fst Estimates - 5 groups					
Group	Fall Group	Late Fall Group	Spring Group	Winter Group	NZ Group
Fall Group	-	0.014	0.019	0.153	0.093
Late Fall Group	-	-	0.031	0.165	0.102
Spring Group	-	-	-	0.126	0.096
Winter Group	-	-	-	-	0.223

DISCUSSION

Our results show that NZ Chinook salmon have diverged from each other and from CCV Chinook salmon while also maintaining relatively high levels of heterozygosity and allelic richness. Previous work has explored the divergence between NZ and CCV populations, but this is the first time divergence has been demonstrated amongst all run-timing populations within the CCV and with new higher resolution genomic tools (Kinnison et al. 2002). Additionally, this is the first study to include Rangitata to explore differentiation among NZ populations. Our results show there is some evidence for differentiation among NZ river drainages, and that all drainages have similar levels of allelic richness, and heterozygosity compared to CCV spring and fall runs, and low levels of inbreeding.

We found that all NZO groups have similar levels of heterozygosity and allelic richness to one another. There was some genetic differentiation between NZ rivers based on Fst values, although it was relatively low. Our results show that Chinook from the Rangitata and Waitaki rivers are most genetically similar, which is perhaps not unexpected as these rivers are next to

each other geographically and the Waitaki is where the populations were first introduced. The Rakaia is the furthest north and appears to be the least genetically similar to both the Rangitata and Waitaki. Therefore, there may be a pattern of isolation by distance occurring along the NZ coastline, which will also be an interesting avenue for future study by incorporating additional NZ sampling sites.

Using a limited SNP dataset, we found evidence for genetic distinction between CCV and NZ Chinook salmon, though our analyses were limited by the small SNP panel size and were not able to identify which population from the CCV served as the source for the NZ introduction. We do see evidence for distinction of NZ Chinook from CCV Chinook, and that there may be genetic diversity present in these populations that is not found in the CCV, highlighting their potential usefulness in future genetic rescue efforts. In order to further explore which runs and populations served as the source for the original NZ introduction, further study should be done that includes a much greater number of SNPs that are common among the NZ Chinook and CCV Chinook populations and use samples from other individuals in other rivers. This will be an exciting avenue for future study.

Because our analysis of the NZCA dataset only contained 131 SNPs, it is possible we did not have the statistical power to distinguish populations, especially considering what is already known about genetically distinct populations in the CCV. However, when comparing the NZ and the CCV, we also found that there is a high level of differentiation between these populations, but most strikingly, that differentiation of Winter run Chinook could still be detected at a low number of loci. The differentiation was highest between Winter run and the combined NZ populations, perhaps indicating preliminarily that the NZ fish did not originate from Winter run, or at least are the least genetically similar to current day populations of Winter run. Interestingly, the lowest Fst value between any CCV group and the NZ group was between the demographically Fall run group and NZ, which is not unexpected as Winter run have been shown to be the most genetically divergent from other CCV run types (Meek et al. 2014; Thompson et al. 2020; O'Leary et al. 2021). Additionally, Winter run was the most divergent population, showing the largest Fst values when compared to any populations, even when NZ and other CCV populations were compared. This indicates that although they have diverged, they are not so diverged that they are substantially different from CCV populations and are a good candidate for exploring rematriation and genetic rescue.

Although we did not have the resolution to fully address the cause of these patterns in the NZ Chinook, they are likely driven by genetic drift, adaptation, or a combination of both. At the time of the introduction into NZ, populations of Chinook in the CCV were likely more genetically diverse than today, since they have since faced massive population declines due to anthropogenic factors such as overfishing, dams, and extensive urbanization (Fisher 1992; Yoshiyama et al. 1998; Fisher 2016). This also means CCV populations of Chinook salmon may have diverged simply due to genetic bottlenecks, since population declines have been so severe (Bartley and Gall 1990; Meek et al. 2016). This is particularly true in the case of Winter run, as they may have diverged because they have undergone the most extreme bottleneck, leading to effective population size estimates as low as 174 (well below the recommended value of 500 needed to reduce genetic drift, and swiftly approaching the value of 50 recommended to avoid inbreeding depression) (Franklin 1980; M 1980; Hedrick et al. 2000; O'Leary et al. 2021). It is additionally possible that NZ populations underwent a founder effect due to a small founding population, causing them to look genetically dissimilar to modern CCV populations, which there is some evidence to support this from prior research (Nei et al. 1975; Barton and Charlesworth 1984; Quinn et al. 2001). All of these factors could be leading to the pattern of divergence we see between the populations in this dataset.

It is possible that divergence in NZ Chinook means they have evolved different coadapted gene complexes due to their isolation, potential effects of drift, and the novelty of the NZ rivers. If this has occurred, it may limit their utility to recover CA populations. Strong signatures drift and/or selection could mean that these populations are less genetically viable, or have locally adapted to a different environment that will not benefit them when translocated (Templeton et al. 1986; Burton et al. 1999). Not only is NZ in a different hemisphere, meaning the introduced populations would have had to respond to new environmental cues, the rivers are all much shorter than the Sacramento River (Biggs et al. 1990; Jowett and Richardson 1996). Adaptation to a novel environment could mean that introduced NZ Chinook could have decreased survival and fitness or that cross breeding with native Chinook would result in outbreeding depression (Tallmon et al. 2004; Edmands 2007). However, the risks of outbreeding depression are markedly low for these populations given they have been separated for less than 500 years and have no fixed chromosomal differences (Frankham et al. 2011). In fact, for many species (including salmonids), the benefits of avoiding inbreeding and therefore lower fitness far

outweigh the risks and effects of outbreeding depression, even among subspecies (Hedrick and Fredrickson 2010; Johnson et al. 2010; Lehnert et al. 2014; Wells et al. 2019; Pregler et al. 2023). The benefit of NZ rivers, however, is that they have been much less impacted by other anthropogenic changes such as damming and heavy urbanization compared to CCV Chinook. This is particularly relevant, as many Chinook populations in their native range have faced massive declines due to anthropogenic changes to their environment, resulting in less genetic variation within populations and therefore less adaptive capacity to respond to change (Weeder et al. 2005; Janowitz-Koch et al. 2019; Thompson et al. 2019). Collectively, this highlights the value in exploring NZ Chinook as possible sources for rematriation in the CCV.

Adaptation can happen on scales more rapid than previously assumed (within one to two generations), and understanding how imperiled and introduced species can respond to change is of the utmost importance if we are going to manage them effectively (Christie et al. 2012; Willoughby et al. 2018). Although Chinook Salmon in NZ presumably started from relatively small founding populations highly susceptible to drift, they have colonized and maintained populations in several NZ rivers for over a century and exhibit a diverse set of phenotypes, including unique run–timing (Quinn et al. 2001). This is particularly relevant to locations in North America where some run-types are threatened and/or have become extirpated, and have undergone great losses of genetic diversity since being used for the NZ Chinook introduction (Healey 1994; O'Leary et al. 2021). Further understanding how NZ populations compare genetically to CCV populations can help inform how to proceed with future management efforts to restore CCV populations from their present day small population sizes.

It will be important to select NZ populations with the best chance of success for rematiration efforts into historic spawning grounds above the Shasta Dam. Populations with a wide array of genetic diversity will likely be the most resilient to challenges faced as a result of rematriation (Whiteley et al. 2015). Populations with high genetic diversity are often more successful, largely due to increased size and higher reproductive success, but more diverse populations can also provide enhanced ecosystem services (Reynolds et al. 2012; Robinson et al. 2017; Fitzpatrick et al. 2020). We have shown here that the NZ drainages have important pockets of diversity that may allow them to be successful in a system where Chinook are imperiled and genetic diversity is dwindling. This genetic diversity is one important piece of the puzzle that can be used when selecting a source population for rematriation.

Our research shows that there is genetic diversity in NZ drainages that has so far been unaccounted for in other analyses. This diversity may be critical for restoring the genetic diversity of imperiled CCV Chinook, but also means that it is possible rapid adaptation and diversification may have taken place in New Zealand. This is promising for the success of imperiled populations in the CCV because this influx of genetic diversity could recover genetic health. Because Chinook genetic diversity has allowed for long-term species resilience and persistence in the face of ecosystem changes, maintaining that diversity is of paramount importance. By understanding the genetic diversity of populations, management agencies can gear objectives towards maintenance of genetic diversity in these systems to maximize biodiversity and overall species resilience.

FUTURE DIRECTIONS

This work paves the way for exciting next steps to compare populations in NZ and the CCV. Although the same enzyme was used at some point during the design of both populations, the SNPs used for the NZ samples were not explicitly designed at SNP positions with CCV populations in mind. The SNPs were designed for polymorphic sites found across the entire species native range of Chinook, and while potentially informative, did not match a high percentage of the sites found in the CCV populations. A greater amount of genomic information may allow us to tease apart the relationships and further understand the genetic diversity found in both populations and how it relates to each other. Therefore, our next steps are to conduct whole genome resequencing to further explore both signals of rapid adaptation across NZ populations, and also to further disentangle the possible source origin for the NZ introduction.

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