Comparison of genetic versus Delta Model Length-at-Date race assignments for juvenile Chinook Salmon at state and federal south Delta salvage facilities

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Executive Summary

This report provides documentation and a summary of results for genetic identification of late-fall, fall, winter, and spring-run juvenile Chinook Salmon salvaged from the federal Central Valley Project (CVP) and California State Water Project (SWP) pumping operations in the southern Sacramento-San Joaquin Delta. The genetic race identification database for fish sampled at the salvage facilities was developed by the California Department of Water Resources (DWR), the Department of Fish & Game (DFG) Tissue Archive, and geneticists at Bodega Marine Laboratory and Oregon State University. In addition to providing an overview of the sampling and genetic methodology used through the history of the Central Valley Chinook Salmon genetics program, we used genetic race assignments to evaluate the accuracy of assignments based on the Length-at-Date approach, currently the major tool used to estimate entrainment loss and take of endangered Chinook Salmon races at the state and federal salvage facilities. The Length-at-Date approach assigns race to sampled juvenile Chinook Salmon based on fork length and the sample date. The years used to evaluate the Length-at-Date approach were limited to 2004-2010, because fork length distributions suggested these years represented the most unbiased subset of CVP/SWP samples taken during the genetic study period (1996 – 2010). Genetic assignments from this same subset of migration years were also used to describe the yearly timing and biweekly fork length distributions of salvaged juveniles from each of the four Central Valley Chinook Salmon races. These distributions were then used to test the validity of two central assumptions of the Length-at-Date approach: 1) juvenile fork length ranges for the four races are segregated from each other on any given day of the calendar year, and 2) salvaged juveniles exhibit similar constant apparent growth rates. Finally, to evaluate the assumption stated in the National Marine Fisheries Service (NMFS) Biological Opinion for CVP-SWP operations (NMFS), that genetic winter-run loss is roughly 50% of winter-run loss based on Delta Model size criteria, we calculated loss of naturally spawned winter run from 1997 to 2010 using both genetic and Length-at-Date race assignments.

Over years 2004-2010, 11,069 salvaged Chinook Salmon juveniles of unknown origin were assigned a race based on genetics. Of these, 86.7% were genetically assigned to fall run, 7.1% to winter run, 4.7% to late-fall run and 1.4% to spring run. Nearly half of these genetic assignments differed from the corresponding Length-at-Date assignments. Only genetic winter run consistently fell within the Length-at-Date fork length range for the appropriate race. However, many genetic non-winter run also fell within the winter-run Length-at-Date size range. In addition, two central assumptions of the Length-at-Date approach were not supported by fork length data of genetically assigned Chinook Salmon at the south Delta salvage facilities: (1) juvenile Chinook Salmon fork length ranges from the different races were not segregated, and (2) empirical fork lengths trends for all races failed to exhibit the constant apparent growth rates used to generate Length-at-date size criteria. In fact, in at least half the years evaluated for each race, older juveniles did not exhibit any significant positive apparent growth, with significant negative fork length trends in several years.

The yearly ratio of genetic winter-run loss compared to winter-run loss based on Length-at-Date size criteria averaged 44% over all study years (1997-2010). However, prior to 2005, the average yearly ratio was only 35%, while after 2005 yearly genetic loss averaged 60% of Length-at-Date loss. Since 2005, genetic winter-run loss was most similar to Length-at-Date loss between mid-February and mid-March,

with an average genetic to Length-at-Date loss ratio of 71%. This was also the time of year when the greatest density of genetic winter-run salvage occurred.

These results suggest that genetic tools have the potential to improve estimation of population sizes and corresponding take of endangered and threatened Chinook Salmon races. We expect that genetic race assignment will be integral in the development of future and more accurate take estimation procedures, either directly through near real-time genetic race assignment, or indirectly as a measure of accuracy for non-genetic methods. This report serves as a starting point for salmon biologists and resource managers to consider alternative methods for race classification and take estimation not only for water export operations, but also for a plethora of other sampling programs in the Delta that are required to identify and enumerate listed winter and spring-run Chinook Salmon.

Introduction

Chinook Salmon in the Sacramento and San Joaquin River drainages were historically abundant and behaviorally diverse, with reproductively isolated subpopulations exhibiting four distinct life histories defined by the timing of adult spawning migrations, known as runs (Yoshiyama et al. 2001). Although these four runs, fall run, spring run, winter run, and late-fall run, were originally classified by their distinct behavioral patterns, genetic analyses have since confirmed reproductive isolation of the four subpopulations (Banks et al. 2000). Human activities since the arrival of Europeans, particularly extensive land and water development, have altered the ecological conditions these Chinook Salmon races evolved to exploit, resulting in a great reduction in Chinook Salmon abundance.

Following the federal listing of Sacramento River winter-run Chinook as threatened in 1990 and then endangered in 1994 under the Endangered Species Act (59 Federal Register 440), and with the establishment of incidental take limits for the joint operations of the federal Central Valley Water Project (CVP) and State Water Project (SWP), the California Department of Water Resources (DWR) required a method to distinguish winter-run Chinook Salmon juveniles from juveniles of other races entrained at the SWP pumping facility near Tracy (NMFS 1993, 1995). As mandated by the National Marine Fisheries Service initial 1993 Biological Opinion and all subsequent Biological Opinions for the joint CVP-SWP operations (NMFS 1993), DWR adopted a “Length-at-Date” approach. The Length-at-Date approach is based on the observation that salmon races have different peak spawning periods. This observation became the basis for the first assumption underlying the Length-at-Date approach, that spawning periods for the races are segregated in time. This assumption, coupled with an assumption that all four races share a similar growth rate, lead to the third main assumption supporting the Length-at-Date approach, that juveniles from the separate races should exhibit distinct, non-overlapping fork length ranges at any given date. Following this logic, sampling programs, such as salvage, should be able to assign the race of a juvenile Chinook Salmon by determining which fork length range it fell within. The original Length-at-Date size criteria was developed by Frank Fisher (1992) of the California Department of Fish and Game (DFG) to designate race for out-migrant juvenile Chinook Salmon in the upper Sacramento River. Fisher (1992) extrapolated the upper and lower size limits for juveniles of each race at any given day of the year by applying a growth rate to the average emergence size of juveniles at the earliest and latest typical emergence dates of each race. A version of Fisher’s size criteria became known as the River Model.

At the time of its adoption, the Length-at-Date approach was expected to have reduced accuracy in the Delta relative to the Upper Sacramento River because, over the time it took juveniles to migrate downstream, growth rate variability of individuals within each race would cause overlap in the size ranges of the different races (Juvenile Salmon Monitoring/Loss Group 1993, Williams 2006). In addition, the growth rate used to calculate juvenile fork length ranges for all races was based on a single tenuous estimate of fall-run apparent growth in artificial rearing channels adjacent to the Tehama-Colusa Canal. Therefore, modified winter-run size limits for specific use at the south Delta pumping facilities (known as the Delta Model) were later developed by Mark Pierce (USFWS) for a sub-committee of the inter-agency Winter-run Monitoring and Loss Group under the lead of the United States Fish and Wildlife Service (Gibbons 1994, Holsinger 1995). Delta Model size criteria were adopted for use at the south Delta pumping facilities in April, 1997 (see Appendix A for a detailed description of development and implementation history of Length-at-Date size criteria in the Sacramento River and the Delta).

The primary evidence DWR presented to NMFS to support adoption of the new winter-run size criteria was genetic test results showing many non-winter-run juveniles were classified as winter run by River Model size criteria, leading to inflated take estimates (Hogarth 1997). Development of genetic tests had been underway at the UC Davis Bodega Marine Laboratory (BML) under contract to DWR since 1991 to inform the winter-run captive breeding program (Banks et al. 1999, Hedrick et al. 2000). Upon adoption of the Length-at-Date approach to establish take at the south Delta pumping facilities, and in recognition of the potential to misclassify juvenile race with the approach, genetic research at BML was redirected to construct genetic tests to distinguish Sacramento River winter-run Chinook Salmon juveniles from other Chinook Salmon races at the facilities (Hedgecock et al. 2001, 2002). The objective of this research was to evaluate the accuracy of the Length-at-Date approach and possibly provide a more accurate alternative for assessing take and loss of winter run at the south Delta pumping facilities. Improvement of genetic tests and provision of race assignments for salvaged fish continued through the drafting of this report. In 2001, genetic analyses were discontinued at BML, moving with Dr. Michael Bank's Marine Fisheries Genetics Laboratory to Oregon State University Hatfield Marine Science Center.

Under the auspices of the Interagency Ecological Program (IEP), a Genetics subcommittee of the Salmon Project Work Team (Salmon Genetics PWT) was established in the early days of the genetics program to define research objectives and evaluate research progress. By 1997, research objectives included the development of "real-time" monitoring of juvenile take. With the listing of Central Valley spring-run Chinook Salmon as a threatened species and classification of Central Valley fall run and late-fall run as species of concern in 1999 (64 Federal Register 50393), research objectives expanded to define genetic markers that could differentiate both winter run and spring run, and eventually to distinguish all four Chinook Salmon races in the Central Valley, as per the terms and conditions imposed by Biological Opinions issued by National Marine Fisheries Service (NMFS 2000, 2004, 2009).

As of the 2010 juvenile migration year (September 2009-August 2010), genetic approaches have been used to characterize the race of nearly 20,000 individual Chinook Salmon juveniles from tissue samples collected at south Delta fish salvage facilities, though not all characterizations are to the level of a specific race. However, more than 11,000 of these have been assigned by the more advanced genetic tests employed since 2003 that resolve to one of the four recognized races.

Since the inception of the IEP salmon genetics program, only one official report has been issued describing the progress of the genetics project. The report described methods and results up to the 2001 juvenile migration year, including development and verification of genetic methods, detailed laboratory and statistical practices, brief accounts of field sampling design, and seasonal timing of genetically assigned winter-run occurrence in salvage and Delta-wide samples (Hedgecock 2002).

In this, the second report on the IEP salmon genetics project, we provide an updated description, including methods compiled from diverse sources (e.g., journal articles, draft reports, memoranda, and email communications) and database construction. We also present preliminary analyses summarizing genetic assignments of Central Valley Chinook Salmon races. Specifically, this report had five primary objectives. First, to document the various field, laboratory, and computational methods that have been used to produce genetically assigned race for salvaged Chinook Salmon juveniles since the inception of the IEP salmon genetics project. Second, to identify years when genetically tested Chinook Salmon juveniles appeared to compose a representative (i.e. unbiased) sample of the population of salvaged

Chinook Salmon juveniles with regard to fork length distribution, by comparing fork length distributions between records in the genetic database (hereafter SALMGEN) and the CVP/SWP salvage database (hereafter SALVAGE). Third, to use the genetic race assignments from the unbiased subsets of records to describe yearly timing and biweekly fork length distributions of juvenile occurrence in the salvage for each of the four genetically assigned Central Valley Chinook Salmon races (fall, spring, winter, late-fall). Fourth, to use the same unbiased subsets of records to evaluate the accuracy of the Delta Model and to test two key assumptions of "Length-at-Date" approach, the current method used to identify the race of salvaged juveniles at the Skinner Delta Fish Protective Facility and the Tracy Fish Collection Facility. These assumptions are i) juvenile fork length ranges for the four races are segregated from each other on any given day of the calendar year, and ii) salvaged fall, late-fall, and spring-run juveniles exhibit the same daily exponential apparent growth rate of 0.00657, which was derived from apparent growth of fall-run juveniles raised in the Tehama-Colusa artificial rearing channels, while salvaged winter-run juveniles exhibit a heightened growth rate of 0.00816 based on juvenile winter-run catch data in the Delta. Our fifth and final objective was to reexamine the evidence accepted by NMFS that resulted in a doubling of the take allowance at the south Delta export facilities in 1997, a decision prompted by early genetic tests that suggested roughly half the winter-run sized juveniles in salvage were not genetic winter run; to evaluate whether this determination was supported by subsequent years of data, we used genetic assignments to estimate the proportion of winter-run salvage and loss (based on Delta Model size criteria) that were true (i.e. genetic) winter run.

Over the course of the 15-year salmon genetics project, data in the genetics database had been altered during quality assurance/control procedures to resolve discrepancies between source databases at the California Department of Fish and Wildlife (hereafter DFW) Tissue Archive (hereafter TISSUE ARCHIVE) and salvage facilities. This database showed that over the 15 plus years of the project, different personnel had applied different and sometimes conflicting procedures to resolve discrepancies (e.g. using SALVAGE data versus TISSUE ARCHIVE data to determine fork length when values differed between databases). SALVAGE and TISSUE ARCHIVE database managers were typically notified when discrepancies were found and were requested to resolve discrepancies among themselves. The expectation was that corrected source data would be incorporated into the genetics database when it was periodically recompiled from updated source databases. However, we found that most previously identified discrepancies remained unresolved between current SALVAGE and TISSUE ARCHIVE databases. For this reason, prior to conducting our analyses, we entirely reconstructed the genetics database from original data files provided by the salvage facilities, DFG Tissue Archive, and Oregon State University Marine Fisheries Genetics Laboratory. The reconstructed database (SALMGEN) was fully evaluated for quality assurance/control based on consistent criteria that are documented in this report, and a complete record of data corrections was attached to the database.

This report is not intended to be a comprehensive evaluation of the data, but rather serves as a starting point for more sophisticated analyses that may be used, among other things, to determine a more appropriate method for estimating take at the CVP and SWP facilities. Since full documentation of the sampling methodology and database structure over the 15-year project had never been carried out, compilation of this information was necessary prior to use of the database for research purposes. We expect researchers will find this document useful not only as a source of background information for

future analyses, but also for determining which analyses constitute appropriate or inappropriate uses of the SALMGEN database.

Sample Acquisition, Preparation, and Storage

Chinook Salmon juvenile migration year

Although Chinook Salmon race is identified by differences in the seasonal timing of adult upstream migration, the four races are also distinguished by differences in adult fresh-water residency prior to spawning, spawn timing, spawning habitat, and the duration of juvenile fresh-water residency prior to migrating to the ocean. There may also be small differences in the timing of juvenile out-migrations through the Delta. However, the majority of juveniles from all four races pass through the Delta from December through June. Over the course of the Central Valley Chinook Salmon genetics project, sampling seasons and reporting of data have traditionally been subdivided by funding year (August 1 – July 31). However, we found there was a consistent lull in Chinook Salmon salvage between late August and early September, which provided a convenient point to separate juvenile migration years. Therefore, in this document, juvenile migration year refers to the time span from September of the previous year to the following August. For example, juvenile migration year 2008 refers to salvage patterns from September 2007 to August 2008.

Salvage facilities

Diversions of water from the southern Delta are thought to create flows that attract migrant juvenile Chinook Salmon away from ocean migration routes and toward the south Delta pumping facilities of the federal Central Valley Water Project and the California State Water Project (Brandes and McLain 2001, Newman 2008, Newman and Brandes 2010). The Tracy Fish Collection Facility and the Skinner Delta Fish Protective Facility were constructed to divert fish from the intake channels of the Delta-Mendota Canal and California Aqueduct pumping plants and began operations in 1957 and 1968. These two facilities have similar designs. Vertical louvers placed diagonally across the pumping plant intake channels create a turbulence field that initiates an avoidance response from fish and diverts them toward bypass pipes, which discharge into secondary channels. A second set of louvers diverts fish from the secondary channels into holding tanks where fish are collected. Holding tanks are then drained through a sieve screen in order to concentrate the fish into a large bucket. The bucket is mechanically lifted to a tanker truck which transports the fish to release locations in the western Delta.

Salvage count and tissue sampling

Salvaged fish were sampled for identification and enumeration at two hour intervals throughout the day and night. At the SWP, salvage counts were also conducted immediately preceding any change in pumping rate. During a salvage count, all fish entering the facilities were collected in a holding tank for a portion of the two-hour salvage period (usually for 30 minutes). At the end of the sampling period, the holding tank was dewatered. Fish were concentrated into a bucket and transferred to a smaller tank, which served as a count station. Fish in this sample were identified to species and counted. Fork length was measured for all salmon and steelhead salvaged since 1993, and in more recently for all smelt and sturgeon. Fork lengths of other fish species were only measured during “length counts” which occurred

four times per day. Count data was recorded on salvage count data sheets and subsequently logged into the SALVAGE database. When fish were salvaged at unusually high densities, the state facility reduced the collection period incrementally to maintain a sample size between 100 and 150 total fish; the federal facility retained 30 minute collection periods, estimated abundant species in the count (usually a single species) from the weight ratio of a subsample to the total sample of that species, and counted each individual of other species in the sample.

During the study period, all non-adipose clipped Chinook Salmon in the salvage sample were put in an isolated bucket after being measured and logged onto datasheets for the normal salvage count. A small 3 mm x 3 mm section of tissue was clipped from the tip of the caudal fin lobe of each Chinook Salmon and placed in prepared vials of tissue storage buffer. Each tissue sample received a unique archive record code which was preprinted on the tissue sample container and was immediately logged onto the tissue archive datasheet along with the date and time of the salvage count, fork length, fin clip status, staff on duty and special comments regarding the sampling event, such as whether the sample was collected as part of a special study or during flushing of the intake pipes. Tissue samples were frozen until transfer to the tissue archive facility for processing. Datasheet information was logged into the TISSUE ARCHIVE database.

No defined protocol was ever implemented to ensure that the fork length for a given fish on the TISSUE ARCHIVE datasheet would match the fork length for that fish on the SALVAGE datasheet. This is important because quality assurance and control procedures for the salmon genetics database historically used a comparison of fork length, salvage time, and salvage date between the SALVAGE and TISSUE ARCHIVE databases to verify the veracity of a database entry. No unique identifier was established to track fish between the SALVAGE and TISSUE ARCHIVE databases. Although there was no established protocol to ensure a fish received the same fork length measurement in both databases, facility managers indicated that fork lengths were measured and logged onto TISSUE ARCHIVE datasheets, and then copied from TISSUE ARCHIVE datasheets onto SALVAGE datasheets. However, salvage facility staff currently responsible for conducting salvage counts and tissue sampling stated that they take two separate fork length measurements for each juvenile salmon, one for the SALVAGE datasheet and another for the TISSUE ARCHIVE datasheet. Therefore a single millimeter difference in these two measurements would cause a mismatch during quality assurance/quality control procedures.

Tissue collection for genetic race assignment was limited to non-adipose clipped juveniles because the race of adipose clipped salmon was already identifiable by coded wire tag. Non-adipose clipped juveniles sampled for genetic tests were not necessarily wild, as many hatchery origin fish were released unclipped. However, the purpose of genetic tests was to identify race of unidentified juveniles whether of hatchery origin or wild since both hatchery and natural origin winter-run individuals were part of the endangered winter-run population. Sampling and genetic tests were not designed to differentiate between hatchery and naturally produced Chinook Salmon juveniles.

Size-stratified, non-random, non-uniform sampling

From the initial stages of the IEP Chinook Salmon genetics program, a completely random sampling procedure was recognized as optimal for statistical analyses of genetic assignment data. However, several constraints and concerns caused Genetics PWT members to adopt non-random sampling strategies as early as the 1999 juvenile migration season. The primary concern that influenced sampling design was

that fully random sampling would miss most of the larger smolts in the winter-run size range due to their small numbers relative to the numerous small, fall-run size juveniles in salvage samples. Ideally, every Chinook Salmon juvenile in the salvage sample would have been tissue sampled, but the large number of juveniles salvaged at peak migration, along with limited personnel and funding for genetic tests, precluded this option. Therefore, starting with the 1999 migration season, a size-stratified sampling regime was agreed upon to obtain an adequate genetic profile from the winter-run sized pulse of juveniles at the salvage facilities. Through March 1st of that year, most samples were taken from juveniles greater than 55 mm. After this date, all fish sizes were supposed to be randomly sub-sampled from the salvage sample for tissue collection. However, numerous large juveniles continued to be salvaged after March 1st, prompting a revision in the protocol to continue sampling all fish in the Delta Model winter-run size category and to subsample smaller juveniles. Although the Genetics PWT recognized that size stratified sampling would produce non-representative samples, the primary purpose of genetic testing in this period was to accurately identify the number of ESA listed Chinook Salmon in the salvage, which were expected to occur at larger fork lengths. Therefore the limited funds available for genetic analyses were directed toward larger juveniles to maximize detection of listed Chinook Salmon. Similarly, during the late 1990s and early 2000s, budget constraints usually prompted a halt to tissue sampling in June when winter-run Chinook Salmon juveniles were no longer expected in the salvage. Tissue sampling resumed each August. After 2003, reduced numbers of Chinook Salmon in the salvage enabled tissue sampling of nearly all juveniles, with subsampling rarely necessary.

Although the threshold for size-stratified sampling was initially set at 55 mm, this threshold varied through the years, and sometimes within years. Initially the threshold was set to ensure maximum detection of ESA-listed Chinook Salmon juveniles. In later years, the limit was set to minimize mortality of sampled juveniles and was left to the discretion of the personnel performing the sampling based on their ability to collect samples from small fish without jeopardizing their survival.

Subsampling

Since original datasheets do not indicate whether tissue samples for a given sample reflect the entirety or a subsample of a salvage count, and since sampling regimes and dates of implementation were not systematically documented, DWR staff traditionally relied on a comparison of fork lengths between the TISSUE ARCHIVE and SALVAGE databases to indicate whether sub-sampling occurred. However, these comparisons do not resolve the sampling strategy or whether discrepancies between database fork length counts were due to sub-sampling or data entry errors. Most archived memoranda and email communications between work team members describe the sub-sampling strategy prior to the 2000 season as taking tissue samples from the first five juveniles in the salvage count below a size threshold, and every tenth juvenile thereafter. In other archived communications the protocol is variously described as tissue sampling the first ten juveniles in the count, or sampling every fifth juvenile after the initial five or ten. In all the above cases, all fish larger than the size threshold were tissue sampled.

At some point in the 2000s a more uniform approach was established. This protocol (in current use) is to tissue sample every non-adipose clipped Chinook Salmon juvenile in the salvage count throughout the year. However, during rare salvage counts with high juvenile densities, protocol allows tissue to be collected from the first 10 juveniles in the count, and then from every 5th juvenile thereafter.

DFG Tissue Archive

Staff at the DFG Tissue Archive was responsible for labeling and filling tissue sample vials with buffer solution, delivering prepared vials to the salvage facilities, and then retrieving used sample vials and associated datasheets. After retrieval, sample data was logged into the TISSUE ARCHIVE database and checked for quality assurance and control. Tissue samples were stored frozen. To prepare for shipment to the genetic research laboratory, samples were briefly thawed, removed from buffer, and if large enough, the sample was split with DNA-sterile utensils on a clean surface. Half of the sample was sent to the genetics laboratory, while the other half remained in the Tissue Archive freezer. If the sample was not large enough to split, the entire sample was sent. Laboratory bound samples were stored frozen until shipment and then shipped on dry ice. Samples sent to the genetics laboratory were labeled only by Tissue Archive identification number, with no other sample data included.

During the 2005 migration year, all samples collected until late May, 2005 failed to amplify. The cause of this massive failure was traced to faulty buffer solution.

Genetics Approaches

In this section we describe the development and basic components of the genetic approaches used over the history of the Salmon Genetics Project (Table 1). Where available, we reference peer-reviewed publications that provide a more comprehensive description of individual approaches or genetic markers. Table 2 describes the number of Chinook Salmon juveniles that were analyzed by each genetic approach in each year.

Table 1. Details of genetic approaches used to assign race to salvaged juvenile Chinook Salmon. EPS/Bayes indicates whether assignment probability calculations assumed equal population size (EPS) of races in the salvage sample, or used Bayes priors generated with an Excel macro or GMA software. Races are late fall (L), winter (W), spring-Butte Creek (SB), spring-Mill/Deer creeks (SMD) and fall (F). See Genetic Approaches in text for further details regarding genetic approaches.

Method Code	*Software*	*EPS/Bayes*	*# Loci*	*Stringency*	*Race Resolution*	*Lab*	*Loci*
ONCOR-16	ONCOR	n/a	16	n/a	L,W,SB, SMD,F	OSU	Ots104, Ots107, Ots209, Ots201b, Ots211, OtsG83b, OtsG249, OtsG253b, OtsG311, OtsG409, OtsG422, Ots215, Ots208b, Ots212, Ots515, OtsG78b
GMA-16	GMA 1.0	n/a	16	n/a	L,W,SB, SMD,F	OSU	Ots104, Ots107, Ots209, Ots201b, Ots211, OtsG83b, OtsG249, OtsG253b, OtsG311, OtsG409, OtsG422, Ots215, **Ots208b**, **Ots212**, **Ots515**, **OtsG78b**
GMA-12b	GMA 1.0	n/a	12	n/a	L,W,SB, SMD,F	OSU	Ots104, Ots107, Ots209, Ots201b, Ots211, OtsG83b, OtsG249, OtsG253b, OtsG311, OtsG409, OtsG422, **Ots215**
GMA-12a	GMA 1.0	n/a	12	n/a	L,W,SB, SMD,F	OSU	Ots104, Ots107, Ots209, Ots201b, Ots211, Ots213, OtsG83b, OtsG249, OtsG253b, OtsG311, OtsG409, OtsG422

Table 1, Continued

Method Code	*Software*	*EPS/Bayes*	*# Loci*	*Stringency*	*Race Resolution*	*Lab*	*Loci*
WR4.0B-12a	WHICHRUN 4.0	Bayes (GMA)	12	2	L,W,SB, SMD,F	OSU	Ots104, Ots107, Ots209, Ots201b, Ots211, Ots213, OtsG83b, OtsG249, OtsG253b, OtsG311, OtsG409, OtsG422,
WR4.0E-12a	WHICHRUN 4.0	EPS	12	2	L,W,SB, SMD,F	OSU	Ots104, Ots107, Ots209, **Ots201b**, **Ots211**, **Ots213**, **OtsG83b**, **OtsG249**, **OtsG253b**, **OtsG311**, **OtsG409**, **OtsG422**
WR3.2E-9	WHICHRUN 3.2	EPS	9	2	W,S,NWS	OSU	Ots2, Ots3, Ots9, Oneμ13, Ots104, Ots107, **Ots209**, **Ots212**
WR3.2B-7	WHICHRUN 3.2	Bayes (macro)	7	0	W,NW	BML	Ots2, Ots3, Ots9, Ots10, Oneμ13, Ots104, Ots107
WR3.2E-7	WHICHRUN 3.2	EPS	5	0	W,NW	BML	Ots2, Ots3, Ots9, Ots10, Oneμ13, **Ots104**, **Ots107**
WR3.2E-5	WHICHRUN 3.2	EPS	5	0	W,NW	BML	Ots2, Ots3, Ots9, Ots10, Oneμ13

Genetic approaches to distinguish winter-run Chinook Salmon from other races (collectively referred to as non-winter) were initiated in the early 1990s to avoid hybridization and inbreeding in a conservation hatchery program designed to enhance survival probability of the dwindling winter-run subpopulation (Banks et al. 1999, Hedrick et al. 2000). With the 1990 listing of winter run as threatened under the federal Endangered Species Act, and subsequent restrictions on juvenile winter-run take at state and federal pumping facilities (NMFS 1993), genetic research turned to the task of developing genetic methods to quantify take at the pumping plants and evaluate the accuracy of the Length-at-Date approach.

An initial baseline survey of genetic diversity within and between phenotypically-defined Chinook Salmon races found substantial divergence between races that matched traditional phenotypic race designations, including two distinct lineages of spring run, one from Butte Creek and the other from Mill and Deer creeks (Banks et al. 2000). In the survey, winter run were the most genetically divergent from the other races. Fall and late-fall runs were most closely related, and the two spring runs were more closely related to fall and late-fall runs than to winter run. Genetic relationships were based on ten genetic markers, known as loci (singular locus), that researchers developed or adapted from existing salmonid loci.

Table 2. Chinook Salmon juveniles successfully tested by each genetics method. Numbers in parentheses reflect fish retested by more advanced method (each fish is counted only once in open numbers). Bold type indicates data used in dna race - length run comparisons.

Year (Sept-Aug)	*Genetic Method*										*Year Total*
	WR 3.2E5	***WR 3.2E7***	***WR 3.2B7***	***WR 3.2E9***	***WR 4.0E12a***	***WR 4.0B12a***	***GMA 12a***	***GMA 12b***	***GMA 16***	***ONCOR 16***	
1996-1997	890	0	9								899
	(9)	(9)									
1997-1998	1,032	0	31								1,063
	(31)	(31)									
1998-1999	1,573	0	70								1,643
	(70)	(70)									
1999-2000	1,081	0	40				2				1,123
	(42)	(40)									
2000-2001	604	72	525				361				1,563
	(954)	(731)	(52)								
2001-2002				335		0	120				456
				(120)		(120)					
2002-2003				2	0	0	783				785
				(204)	(579)	(783)					
2003-2004						**1**	**124**	**195**	**582**		**902**
						(123)	(1)				
2004-2005									279		**279**
2005-2006									**2,932**		**2,859**
2006-2007									**1,055**		**1,055**
2007-2008										**2,859**	**2,859**
2008-2009										**1,728**	**1,728**
2009-2010										**1,832**	**1,832**
Method Total	*5180*	*72*	*675*	*337*	*0*	***1***	*1,266* ***124***	***195***	*279* ***4,569***	***6,419***	*7,809* ***11,308***

A locus is a specific location of a gene or nucleotide sequence along a chromosome. Although individuals within a species usually have the same sequence subset that identifies a given locus, the full nucleotide sequence associated with that locus may vary between individuals or between homologous chromosomes within an individual. Variant forms of the nucleotide sequence at a locus are called alleles. Geneticists found microsatellite loci were particularly powerful for distinguishing between closely related Chinook Salmon races in the Central Valley (Banks et al. 2000). Microsatellite loci are regions of genetic code that mutate rapidly and are non-coding and therefore not under selective pressure that would eliminate

mutations from the population. For these reasons, microsatellite loci accumulate many allelic variants. In order to distinguish between Central Valley Chinook Salmon subpopulations, geneticists searched for microsatellite loci with alleles that were common within subpopulations, but different between subpopulations. Statistical algorithms could then be applied to compare allelic frequencies in a sample of unidentified juveniles to allelic frequencies in samples of known race (baseline sample), and thereby estimate the fraction of the unidentified sample belonging to each subpopulation. This is called a mixed stock analysis.

The original plan proposed by Dr. Hedgecock of BML and the Genetics PWT was to use mixed stock analysis to assess the fraction of winter-run Chinook Salmon in the population of all Chinook Salmon salvaged at the SWP/CVP pumping facilities (Hedgecock 2002). This fraction could then be projected through a series of established equations to estimate take and loss numbers resulting from pumping operations. The sample size necessary for the mixed stock analysis required salvaged Chinook Salmon tissue samples to be pooled over a continuous series of salvage counts spanning a week. However, mixed stock analysis also requires a sample to be random and uniform in order to accurately estimate allele frequencies in the mixed population. This requirement could not be met by a pooled series of salvage counts because of variability in pumping rates, salvage count procedures, and other factors in loss estimates that could not be held constant over the course of the series of salvage samples.

Instead, an alternative approach was adopted in which race was assigned to each individual juvenile taken at salvage. Similar to the mixed stock analysis, an individual's race assignment was obtained by comparing the individual's allele composition at target microsatellite loci with the allele composition of baseline samples from the four Central Valley races. However, assessing take and loss estimates from individual race assignments was fraught with many of the same difficulties as the mixed stock analysis due to non-uniform sampling at the salvage facilities. Therefore take and loss calculations based on genetic race identification were never formally adopted. The tests used for individual-based assignments were continually modified through the years as new genetic markers and assignment algorithms were developed. Following are brief descriptions of these tests.

WHICHRUN

The program WHICHRUN was developed to calculate the frequency with which an individual's alleles occurred in the baseline for each race, and therefore the likelihood of an individual belonging to each race (Banks and Eichert 2000). WHICHRUN was used to analyze genotype data and produce race assignments from samples collected from 1996 to 2004. WHICHRUN-derived likelihood scores for each race were then divided by the highest likelihood score from among the other races. The ratio for each race was referred to as the "odds" of belonging to that race, and the $\log_{10}$ of the ratio was called the Log of the Odds, or LOD score. An LOD score greater than zero indicated the most probable race.

Initial genetic methods that assigned race to individual fish assumed equal proportions of each race occurred in the source population from which the individual was drawn (i.e. the salvage population); however, race proportions in the salvage population are seldom equal. For instance, fall-run juveniles in the salvage population can outnumber other races by as much as 500 to 1 at certain times of the year. Under these conditions, a genetic test that assumes equal race proportions will misidentify many more fall-run juveniles as belonging to another race than it will correctly identify the race of juveniles from the

rare races, even if the test has a very small error rate for individual race assignments. In other words, the majority of individuals assigned to the rare races will actually belong to the more abundant race.

To account for unequal race proportions in the salvage population, geneticists began using Bayesian priors to modify assignment probability scores and to reduce assignment errors, where "prior" refers to prior information about actual race proportions in the sampled population (Hedgecock 2002). Race proportions in the sampled population were estimated by mixed stock analysis. Although the salvage sampling regime deviated from the assumptions of mixed stock analysis and precluded the use of mixed stock analysis for estimating endangered species take (as discussed above), deviations from these assumptions were considered less problematic when estimating Bayesian priors. Initially, Bayesian priors for individual assignment were determined by analyzing 100 juveniles with similar fork length as the individual in question using the mixed stock analysis in Statistics Program for Analyzing Mixtures (Debevec et al. 2000). For the mixed stock analysis, fish were selected with salvage dates close the individual in question, but not from the same year to preserve independence from the individual. Later, Bayesian priors were estimated with General Mixture Analysis software (GMA, Kalinowski 2003), described in more detail below.

While WHICHRUN was still in use for estimating salvage race assignments, a final change was made to minimize race-assignment errors caused by unequal race proportions in the source population. LOD scores for the probability of an individual belonging to each race were calculated by dividing the modified race probability by the *average* of the modified probability for all the other races, rather than by the probability of the next highest single race.

During the period when WHICHRUN was used to designate winter run from non-winter run, five loci were found to adequately distinguish between these two classifications. Later, individuals receiving an LOD greater than 0 but less than 2 based on the initial five loci test (equivalent to an odds ratio between 1 and 100) were further tested at two additional loci. With the listing of spring run under the federal Endangered Species Act, two more loci were added to the analysis (loci = 9) to distinguish winter run and spring run from non-winter-spring run. Before switching from WHICHRUN to GMA during the 2004 juvenile migration season, the WHICHRUN-based approach included twelve loci and distinguished between fall run, Butte Creek spring run, Mill/Deer Creek spring run, winter run and late-fall run.

GMA

GMA was used to analyze samples collected from 2004 through 2007. In addition, many samples from earlier years that were originally analyzed with WHICHRUN were reanalyzed using GMA. GMA software generated individual race-assignments in a similar fashion as the latest version of WHICHRUN by using equation 10 from Ranala and Mountain (1997) with Baysian priors estimated by mixed stock analysis using algorithms from Pella et al. (1996) and Smouse et al. (1990). The major differences between the latest version of WHICHRUN and GMA were that Bayesian priors were estimated directly from the mixed race group of tissue samples from which the individual was drawn, which equated to samples collected within a similar time frame. Also, GMA output was in the form of assignment probabilities, rather than LOD scores (Kalinowski 2003, release notes). The same twelve loci used with the last incarnation of WHICHRUN were initially used with GMA to produce race-assignment probabilities (Table 1). Then one locus was exchanged for another. By the end of the first season that

GMA was in use, four more loci were added to the analysis, bringing the total number of loci to 16. The same 16 loci continued to be used through the replacement of GMA software with ONCOR in 2008 until 2010, the latest year discussed in this report.

ONCOR

ONCOR (Kalinowski 2007) is the updated version of GMA and was used to analyze all samples collected from the 2008 migration season until 2010. ONCOR uses the same general approach to estimate race-assignment probabilities as GMA (and by extension the latest version of WHICHRUN), with one major exception. WHICHRUN (Bayes) and GMA use mixed stock analysis to calculate Bayesian priors. ONCOR uses the expectation-maximization (EM) algorithm to calculate a conditional maximum likelihood estimate of prior race proportions (Millar 1987). The EM process begins by calculating race-assignment probabilities for individual fish in a sample using equation 10 from Ranalla and Mountain (1997) and assuming equal race proportions in the sampled mixed stock population. The resulting individual assignment probabilities provide an estimate of race proportions in the mixed stock sample. The estimate is used as priors in a second iteration of individual race assignment probability estimates. This process is repeated until the change in race proportions between successive iterations, summed across all possible races, is less than 10^{-6}. Individuals are assigned to the race with the greatest probability estimate after the final iteration.

Genetic Test Assignment Accuracy

WHICHLOCI (Banks et al. 2003) was used to evaluate the accuracy of the genetic approach that used seven loci and WHICHRUN software (Hedgecock 2002), referred to as WR3.2B-7 in Table 1. WHICHLOCI generated simulated mixed race populations based on genotypes in the baseline and then used WHICHRUN to assign simulated individuals to race to assess assignment accuracy.

A Leave-One-Out test and a separate Blind Test were used to evaluate the accuracy of ONCOR-16, the latest genetic approach used to make race assignments for salvaged Chinook Salmon (as of the writing of this report). For the Leave-One-Out test, each genotype in the baseline was removed sequentially from the baseline (with replacement) and then reassigned to race using the remaining fish in the baseline. For the Blind Test, blind samples from known-race Chinook Salmon not in the baseline were analyzed by the genetics laboratory (see Banks et al., for details of the blind test). Leave-One-Out tests have been shown to overestimate test accuracy in cases where the baseline sample has also been used to select loci for the test (as occurred for ONCOR-16), while Blind Tests with non-baseline samples are considered the most rigorous method for evaluating genetic test accuracy (Anderson 2010).

Controlling for genetic assignment error rates

Typically when using genetic assignment data, it is desirable to control for assignment error rate. Each individual assignment has an associated probability of correct assignment. However, these probabilities do not accurately reflect test-wide error rate. We attempted to control for test-wide error rate by determining the assignment probability that corresponded to a desired rate of miss-assignment. For each race, baseline fish genetically assigned to the race by the Leave-One-Out evaluation (described above) were ordered by their associated assignment probability. Then, starting with the highest probability

assignment, we calculated the cumulative assignment error rate as fish with lower and lower assignment probabilities were included in the calculation, where error rate was the proportion of miss-assigned fish. The objective was to estimate the assignment probability value that corresponded to test-wide error rates of 5%, 10%, and 20%. Since many individual assignments had the same assignment probability as at least one other individual, we randomly reordered individual assignments that shared assignment probability 100 times to determine the average assignment probability that corresponded with each test-wide error rate. The same procedure was repeated using assignment probabilities from the Blind Test evaluation (described above).

Using the above procedure, we were not able to determine assignment probabilities that would allow control of test-wide error rate for most races. Regardless of the targeted error rate (5%, 10%, or 20%), the error rate was either never achieved for a race (i.e. too few fish were miss-assigned), or error rates were achieved at the highest possible assignment probability of 1.00, which would require exclusion of all fish with assignment probability ≤ 1.00 from the Length-at-Date analysis in order to maintain the desired minimum error rate (i.e. all fish in the database would be excluded). For example, due to a low rate of miss-assignment for spring run and winter run, Leave-One-Out assignment probabilities never achieved cumulative error rates of 5% or greater in most randomly ordered iterations (Table 3). In contrast, 5%, 10%, and 20% error rates for fall and late-fall runs were achieved at assignment probability of 1.00 in the majority of iterations, the highest possible assignment probability. Similarly, Blind Test assignment probabilities achieved an error rate of 5% for spring (including all Central Valley spring), fall, and late-fall runs at assignment probability of 1.00, and a 10% error rate for spring, fall, and late-fall runs at assignment probabilities of 0.99, 1.00, and 1.00. Therefore, we were unable to control for test-wide miss-assignment rate using genetic test assignment probabilities. Instead we included all genetically tested fish in our analyses, assigning juveniles to the race with the highest assignment probability, and relied upon genetic test accuracy evaluations (Leave-One-Out, Blind Test) to qualify the reliability of our results.

Table 3. Average genetic test assignment probability at which a 5% posterior assignment error rate was achieved for ONCOR-16, based on 100 test permutations, calculated using assignment probabilities from both Leave-One-Out test on baseline and blind test. The blind test results are considered a more accurate evaluation because the blind test sample contained relative proportions of Chinook Salmon races estimated for the Sacramento River, including Feather River Chinook Salmon.

Run	***Leave-One-Out Error Rate***			***Blind Test Error Rate***		
	5%	10%	20%	5%	10%	20%
Winter	n/a	n/a	n/a	n/a	n/a	n/a
Spring (Butte/Mill/Deer)	n/a	n/a	n/a	untested	untested	untested
Spring (All Sacramento)	untested	untested	untested	1.00	0.99	n/a
Fall	1.00	1.00	1.00	1.00	1.00	1.00
Late-fall	1.00	1.00	1.00	1.00	1.00	1.00

n/a = error rate never achieved for > 90% of iterations due to high accuracy of test.

Compilation of SALMGEN database

Following genetic analyses, the genetics laboratory sent analysis results to DWR in the form of data files with records referenced by tissue archive identification numbers. Test results describe race assignments and their associated assignment probabilities in descending order of probability, or notes describing genetic tests that failed and the reason (e.g., no tissue in vial, failed to amplify). Using Oracle SQL Developer software, data file records from the genetics laboratory were imported into the SALMGEN Oracle database and joined with records from the TISSUE ARCHIVE database by matching archive identification numbers. This combined the genetic race assignment with the fork length, sample date, sample time, and sampling location of each individual Chinook Salmon tested. Other information recorded on the tissue archive datasheet was also attached to the record representing the individual. Each genetics datasheet record could match only one tissue archive record (i.e. no duplicate data was allowed for any record). Successfully merged records form the SALMGEN database. Over the fourteen years of data compiled so far (through 2010 migration season), comprising over 21,000 genetically analyzed tissue samples (not all from salvage), only 16 records from the genetics laboratory have failed to attach fork lengths from the TISSUE ARCHIVE database. These failures were caused by wrongly entered archive identification numbers on datasheets from the genetics laboratory, or by omission of fork length data on the original tissue archive datasheets.

As an additional data quality control measure, successfully merged records in the SALMGEN database were compared to salvage sampling data in the SALVAGE database. Since SALVAGE records do not include archive identification numbers, a computer algorithm was used to compare fork length, sample date, sample time, and sample location of SALMGEN records with non-adipose clipped Chinook Salmon SALVAGE records. In the process, each SALVAGE record (i.e. each non-clipped Chinook Salmon juvenile recorded in SALVAGE database) was assigned a unique identification number so that each SALVAGE record could match only one SALMGEN record.

From the 1996 through the 2010 migration season, 1091 SALMGEN records from salvaged Chinook Salmon had no initial matching record in the SALVAGE database. These unmatched records were investigated and resolved as described in the following paragraphs. A log of all database record changes was recorded in a separate table attached to the SALMGEN database. The log includes original and any revised data values. Notes were also placed in note fields in the SALMGEN main table and a copy of the SALVAGE database table attached to SALMGEN. Finally, a column (archsal_match) was added to the main SALMGEN table to quickly identify with flagging codes the records that did or did not meet specific matching criteria. Flag code definitions are listed in Table 4.

Table 4. Archsal_match column flagging codes in SALMGEN database with associated record counts through the 2009/2010 migration season. Numbers in parentheses are juveniles with fork length < 270 mm that were sampled at salvage facilities.

Flag	*Definition*	*Records*
M	Match was found between SALMGEN and SALVAGE databases and part of a salvage sample count.	21,102 (21,082)
P	Match was found between SALMGEN and SALVAGE databases and was collected during predator removal (secondary flush).	431 (428)
D	Tissue collection datasheet indicates part of a salvage count, but not recorded in salvage database.	130 (130)
O	Tissue sampler note indicates was part of predator removal, but record of predator removal sample missing (omitted) from salvage database.	65 (64)
S	Collected as part of a predator removal or special study and NOT part of the facilities statistical count or predator removal.	214 (212)
C	Adipose fin clipped (Adclip=Y).	95 (94)
E	Irresolvable discrepancy, (e.g. two sampled fish on the datasheets shared same archive identification number, fork length not recorded and could not be determined from salvage records).	25 (13)

Data entry errors

Obvious data entry errors were corrected based on original, hand-written, tissue archive datasheets. In cases other than obvious data entry errors on tissue archive datasheets (e.g., listing archive ID as time) or other than the time/date errors discussed below, tissue archive datasheets were considered the original documentation of Chinook Salmon salvage data. Therefore discrepancies between the TISSUE ARCHIVE and SALVAGE databases were resolved by changing SALMGEN or our internal copy of SALVAGE to match tissue archive datasheet values (changes were not made to the sourced original SALVAGE database maintained by DFG).

Time and date errors

Sample time or sample date were changed for individual records in SALMGEN by up to one hour (for sample time) or one day (for sample date) when an obvious mistake was made on the tissue archive datasheet (based on adjacent datasheet entries). Occasionally, a set of tissue archive records were recorded on a datasheet with a sample time that did not match a sample event in the salvage records, yet

all fork lengths for that sample matched fork lengths for a salvage sample event with a slightly different collection time (+/- one hour or less). In almost all cases errors of this sort were caused by a discrepancy in entry of time for midnight samples, recorded variously as 0:00, 23:59 and 24:00. To resolve this issue, SALMGEN sample times were altered to match the sample time recorded in the SALVAGE database for that sampling event so that future analyses could use environmental variables for the sampling event recorded in the SALVAGE database. In these instances, SALMGEN sample date was also altered when a time shift from 23:59 to 0:00/24:00 caused a date shift.

Fork length errors

Fork length changes were only made in SALMGEN or SALVAGE databases to match original tissue archive datasheets. Usually these errors reflected a slight discrepancy (1-2 mm) between the TISSUE ARCHIVE database and the SALVAGE database. There are several possible reasons for these discrepancies, but we assume that the majority were caused by the practice of measuring the fork length of each tissue-sampled juvenile twice, once for the salvage datasheet, and then again for the tissue archive datasheet. In these cases SALMGEN fork length was set to the value in the TISSUE ARCHIVE database. When the original tissue collection sheets clearly indicated a fish was non-adipose clipped (adclip = N), but the SALVAGE database listed the fish as adclip = Y, a record was added to our internal copy of the SALVAGE database table in order to match the SALMGEN record with SALVAGE sampling event data.

Missing SALVAGE records

Some records in the tissue archive datasheets were clearly listed as part of an official sample count, but are not in the SALVAGE database. These fish were almost always the first or last in a list from a specific salvage count sampling event, a clear reason why they may have been overlooked when data was copied between tissue archive and salvage datasheets. These SALMGEN records were flagged for inclusion or exclusion from future analyses, as seen fit by future researchers, if a matching SALVAGE database sample event was clearly determinable.

Where no obvious correction could produce a TISSUE ARCHIVE-SALVAGE record match, the record was flagged so that it could be removed from future statistical analyses linked to official salvage counts, such as take and loss estimates. Many of the TISSUE ARCHIVE records that did not match records from the SALVAGE database represented tissue-sampled fish that were part of a special study or were taken dead off the sieve and were not part of an official salvage count sample.

Data Analyses

Prior to evaluating Length-at-Date size criteria with genetics, we conducted distribution comparison tests on all years of data to ensure that the fork lengths of genetically tested juveniles composed a representative sample of salvaged juvenile fork lengths. It was necessary to make these distribution comparisons because, as previously discussed, size stratified, non-random sampling was employed at the salvage facilities, particularly when large numbers of small juveniles occurred in the salvage. This sampling strategy was adopted to maximize detection of listed fish, the primary purpose of genetic testing. However, for the purpose of evaluating the accuracy of Length-at-Date size criteria, a sample that reflected the fork length distribution of all salvaged Chinook Salmon juveniles was necessary. Since the

Length-at-Date approach is by its very nature based on the length of juveniles, and since juveniles of the different races were not evenly distributed among the size range of juveniles collected at the salvage facilities, pronounced size-biased samples would not have provided an accurate appraisal of the Length-at-Date approach for those samples.

To understand this concept, consider a hypothetical salvage sample taken on March 2nd in which all juveniles ≥ 55 mm were genetically tested, while only a sub-sample of juveniles < 55 mm were genetically tested. Since fall-run size criteria range from 0-59 mm on March 2nd, sampling would be biased toward larger genetic fall run that fell outside fall-run size criteria, leading to a deflated estimate of size criteria accuracy for fall run. Conversely, since spring and winter-run size criteria range from 60-94 mm and 95-252 mm on March 2nd, sampling would be biased toward larger spring-run and winter-run juveniles that fell inside their respective size criteria, thereby inflating estimated accuracy for spring and winter-run size criteria.

If the size limits for size-stratified sampling were consistently documented (e.g. 55 mm in the example above), then accuracy estimates could account for a size-stratified sampling regime. However, size limits were changed at the facilities without consistent documentation because changes were usually prompted by rapidly changing and arduous conditions at the salvage facilities when documentation of size limits was not a priority. In order to assess the degree of size biased sampling, we compared fork length distributions of non-adipose clipped, genetically tested Chinook Salmon juveniles with the fork length distributions of non-adipose clipped juveniles from the SALVAGE database. Based on these comparisons, we identified migration years when genetically-tested juveniles appeared to form a representative sample of the salvage count with respect to fork lengths. Further analyses were restricted to this subset of data.

Percent rank fork length distribution comparisons

To conduct the distribution comparison, only SALMGEN records with fork length less than 270 mm and archive-salvage match flags of M or D were used (see Table 4 for flag descriptions). SALMGEN and SALVAGE records were grouped separately by sample month. We chose to pool samples by month in order to balance our desire for temporal resolution with the sample size required for statistical distribution comparisons. Records were further separated by sample location (i.e. CVP and SWP) because salvage facilities did not always follow identical sampling protocols. We then compared salvage versus genetically tested fork length distributions within each record group using Kolmogorov-Smirnov (KS) and also Anderson-Darling K-sample (ADK: Scholz 2011) tests, both implemented in R (R Development Core Team 2012). The KS test is sensitive to differences between the centers of distributions, while the ADK test is more sensitive to differences between the tails of distributions. Since KS and ADK tests assume untied data (i.e. no duplicate data values in each distribution), tied data in each record group were converted to untied data by adding or subtracting a small value to each tied data point such that the average of a set of untied data equaled the original data value. For example, if there were five occurrences of 65 mm fork length in a distribution, these values were converted to 64.998, 64.999, 65, 65.001 and 65.002, while four occurrences of 65 mm would be converted to 64.9985, 64.9995, 65.0005 and 65.0015. KS and ADK tests also require at least five fork lengths from both SALMGEN and SALVAGE for each record group, which excluded many months from comparison.

We also plotted rank distribution plots for each group for visual comparison. Rank distribution plots represent fork length for each juvenile (record) against its rank percentile in the record group. Rank percentile was calculated as R-1/N-1 with R the ascending rank (by fork length) and N the number of records in the record group. Rank percent is essentially the same as a cumulative rank distribution adjusted so that the lowest rank percentile equals zero and the highest rank percentile equals one. Plots for a given month were plotted in the same graph.

Based on the fork length distribution comparisons, we limited our further analyses to migration years 2004 to 2010 (excluding 2005). Migration year 2005 was not considered because there was a massive failure of genetic tests in this migration year caused by faulty tissue storage solution. Fortunately, the 2004 migration year also marked the introduction of genetic tests that distinguished between all four Central Valley races (previous years grouped races under designations of non-winter or non-winter-spring).

Evaluation of Delta Model Length-at-Date criteria

To evaluate the accuracy of the Length-at-Date approach we assigned each genetically tested fish to a Length-at-Date race based on Delta Model size criteria (Delta Model size criteria are described in detail in Appendix A), and then for each Length-at-Date race, we calculated the proportion of fish assigned to each genetic race. We also examined the reverse, for each genetic race, the proportion of juveniles that fell within each Length-at-Date race size category. As described above, evaluations were only conducted for migration years where genetic tests resolved all four races and where SALMGEN records were representative of the fork length distribution of salvaged juveniles. Data were pooled across years for this comparison.

Next, we determined whether genetic assignment data supported two central assumptions of the Length-at-Date approach, (1) juvenile fork length size ranges of the different races are segregated on any given day of the calendar year, and (2) average fork length of salvaged juveniles increases with time and at the same rate for each of the Central Valley salmon races, except winter-run fork length, which increases at a faster rate due to early migration to the Delta where water temperature is warmer and food more abundant.

To determine whether fork lengths of the different races were segregated at any given point in the calendar year, we examined fork length distributions for each race as box plots, pooling fork lengths across years for each biweekly interval throughout a calendar migration year (September – August). The biweekly interval spanning February 29th in leap years included 15 days (rather than the normal 14) for continuity of biweekly date ranges between years. For each race, we also plotted fork length frequency using 3 mm bin intervals for each biweekly period. Lines depicting Delta Model size thresholds for each race were plotted over both box plot and length frequency graphs to examine genetic race fork length distributions in relation to Delta Model size criteria.

To test whether temporal trends in fork lengths conformed to the growth rate assumptions of the Delta Model, we regressed ln(fork length) against salvage date for each race and for each migration year. Since biweekly length frequency graphs (described above) exhibited more than one size class of out-migrants and also exhibited seasonal changes in median fork length trends (i.e. apparent growth rate) within each

size class, we performed separate regressions for each size class and for the date ranges within each size class that had distinctly different rates of apparent growth. Year classes were separated by visually inspecting biweekly length-frequency histograms for each race. When length-frequency histograms suggested distinctly different rates of apparent growth rate at different points in the juvenile migration season, the segmented package in R 2.15.0 (Muggeo 2003, 2008) was used for each race, using data pooled across years, to objectively determine dates of transition in apparent growth rate. These transition dates from data pooled across years were then used to separate data within each year for use in growth rate regressions. Regressions were only performed if segmented datasets contained ten or more fork lengths. Although data sets for individual ln-linear regressions did not all strictly conform to regression assumptions of linearity and homoscedasticity, we did not attempt alternative data transformations on non-conforming data sets so that we could maintain comparability between years, races and size classes, and more particularly with original growth rates used to develop Length-at-Date Models, which also used ln-linear transformations for regressions.

Calculation of expanded salvage and loss

We calculated salvage and loss (i.e. incidental take) associated with the operations of the south Delta export facilities for non-adipose clipped winter-run juveniles using both Delta Model and genetic race identifications. We did not include adipose clipped juveniles in our calculations because genetic testing was rarely done for these fish and because we were concerned that marked hatchery fish would not have the same size distribution as unclipped fish, which would bias loss extrapolations from genetic assignments. Salvage (sometimes called expanded salvage) was estimated for each salvage period by dividing the salvage period time by the duration of the sample period, and then multiplying this quotient by the number of juveniles in the salvage sample count.

$$\text{Salvage (a.k.a. Expanded Salvage)} = \text{sample count (salvage time} \div \text{sample time)} \quad (1)$$

Juveniles salvaged during predator removals received no expansion and juveniles observed during special studies were not counted toward salvage.

For salvage and loss based on genetic identifications, we used genetic assignments with archive-salvage match codes of M, D, P and O, which included juveniles caught in regular salvage sample counts and during predator removals. Since genetic race identification was not made for every juvenile Chinook Salmon in salvage samples, the number of genetically identified winter run in each salvage sample was divided by the fraction of the sample that was genetically tested to account for sub-sampling.

Loss calculations account for juvenile mortality resulting from entrainment by the pumps, predation in the vicinity of the pumping facilities and salvaged fish that die during transport to release locations in the western Delta. Specifically,

$$\text{Loss} = \text{Entrainment} - \text{Release} \quad (2)$$

$$\text{Entrainment} = \text{Encounter} \div (1\text{-P}) \quad (3)$$

$$\text{Encounter} = \text{Salvage/Efficiency} \quad (4)$$

$$\text{Efficiency} = a + b * \text{velocity} \quad (5)$$

$$\text{Release} = \text{Salvage} * c \quad (6)$$

where P = 0.75 for the SWP and 0.15 for the CVP, and is an estimate of juvenile loss prior to encountering salvage facility screens. Encounter is the estimated number of juveniles encountering the facility screens and accounts for screen efficiency at directing the fish away from the pump intake channels. Velocity is the water velocity in the intake channel upstream of the louvers, calculated by dividing channel flow by the cross-sectional area of water in the channel. The empirically derived parameters a and b are equal to 0.630 and 0.0494 for juveniles < 101 mm and 0.568 and 0.0579 for juveniles > 100 mm. The parameter c is an estimate of survival to release equal to 0.98 for juveniles < 101 mm and 1 for juveniles > 100 mm. Salvage and Loss expansions were calculated separately for each genetic winter-run juvenile, and for each Delta Model winter-run juvenile. Salvage and loss values were rounded to the ten thousandth decimal place to avoid rounding error and then summed for each salvage sample.

Distribution analyses showed pronounced size-biased sampling toward larger juveniles prior to 2003. To account for this, we conducted separate salvage calculations for winter-run size and larger juveniles and for spring-run size and smaller juveniles to minimize inflation of genetic-based winter-run salvage. To further minimize inflation caused by size biased sampling in the spring-run size and smaller group, we separately analyzed juveniles in this group that were 80 mm or smaller. Distribution analyses indicated that fork lengths of genetically tested juveniles within the two larger size groups were representative of the corresponding size groups in salvage samples. Although distribution analyses indicated size-biased sampling was still present for juveniles ≤ 80 mm, only seven genetic winter run were sampled in this smallest size group over the history of the genetics project. Following separate salvage and loss calculations for the three size groups in each salvage sample, results were summed across groups to determine total salvage and loss of genetic winter run for each salvage sample. Genetic-based salvage and loss calculated from individual salvage samples were then summed for each year to examine year to year variation in genetic winter-run salvage from 1997 to 2010. Salvage and loss were also summed across 14 day periods, and then were averaged within 14 day calendar periods across all years to determine average salvage at biweekly intervals through the calendar year. Following each final summation, salvage and loss values were rounded to whole numbers.

Results

Percent rank fork length distribution comparisons

Comparison of SALMGEN and SALVAGE fork length distributions by Kolgomorov-Smirnov and Anderson-Darling K tests yielded similar results (Appendix B). In migration years 1997 to 2003, more than 35% of genetically tested fish were sampled in months where fork length distributions were not representative of the SALVAGE population. In four of these years, more than 77% of genetically tested fish were sampled in months where fork length distributions were not representative of SALVAGE. Inspection of rank distribution plots for these years revealed that differences between SALMGEN and SALVAGE distributions were caused by pronounced size biased sampling of larger fork length juveniles for genetic testing, particularly in March and April when a large proportion of each year's cumulative salvage occurred (Appendix B: Figures B-1a-n).

In contrast, fork length distributions of genetically tested fish in migration years from 2004 and later (excluding 2005) were fairly representative of SALVAGE fish fork lengths. Only in two instances, March-CVP in 2004 and January-SWP in 2006, were significant differences found between SALMGEN and SALVAGE fork length distributions ($P < 0.05$). The rank distribution plot for March-CVP in 2004 demonstrated the difference in distributions for this month were slight (Figure B-1h), while January-SWP in 2006 had only nine genetically tested fish (Figure B-1j), a very small proportion of the 11,069 records used in the Length-at-Date evaluation.

In addition to differences between SALVAGE and SALMGEN, fork length distributions also show a repeated pattern of larger Chinook Salmon juvenile fork lengths in SWP salvage, compared to CVP salvage during January, February and March. This pattern is most pronounced in 1999-2009.

Genetic test accuracy

As previously reported in Hedgecock (2002), the evaluation of genetic test WR3.2B-7 (Table 1) using which Loci found greater than 99% accuracy for assignment of simulated winter run and around 2% error rate for simulated non-winter run that were wrongly assigned to winter run. Since WR3.2B-7 and earlier tests only distinguished between winter and non-winter run, this evaluation provided no accuracy evaluations for other races. It should be noted that accuracy evaluations such as those employed by WHICHLOCI have recently been shown to overestimate the accuracy of genetic tests because simulated samples are generated from the baseline under evaluation (Anderson et al. 2008).

The Leave-One-Out test we performed for genetic test ONCOR-16 found that 100% of baseline winter run and 99.5% of baseline spring run from Butte, Mill, and Deer creeks were correctly assigned (Figure 1a). Reassignment accuracy of baseline fall and late-fall runs were less accurate at 88.6% and 73.7%. Compared to the Leave-One-Out test, the Blind Test suggested ONCOR-16 had slightly lower accuracy for assignment of winter run (95.5%), greater accuracy for fall run (95.9%) and much lower accuracies for spring run (50%) and late-fall run (44.2%) (Banks et al. In Review). The discrepancy between Leave-One-Out and Blind Test results for spring run was largely caused by inclusion in the Blind Test sample of spring and fall-run Chinook Salmon from the Feather River. This contrasts with the baseline used for the Leave-One-Out test which included spring run from only Butte, Mill, and Deer creeks. Feather River spring and fall runs have hybridized following the construction of Oroville Dam on the Feather River, which forced spring-run and fall-run spawning habitat to overlap. Although Feather River Chinook Salmon still display the two phenotypic races, genetic tests cannot distinguish between individuals from each race. Genetic test ONCOR-16 assigned 92.9% of Feather River spring-run phenotype to fall run and 96.7% of Feather River fall-run phenotype to fall run in the Blind Test (Figure 1b). Only 7% of Feather River spring-run phenotype was correctly assigned to spring run by ONCOR-16. When Feather River samples were excluded from Blind Test results, spring-run accuracy for juveniles from other streams in the Central Valley increased from 50% to 87.5% and fall-run accuracy remained practically unchanged (95.5% to 94.5%), demonstrating that ONCOR-16 assigned the majority of both spring-run and fall-run Chinook Salmon from the Feather River to fall run, and also that ONCOR-16 is fairly accurate for non-Feather River spring run. The Blind Test is described in detail in Banks et al. (In Review.)

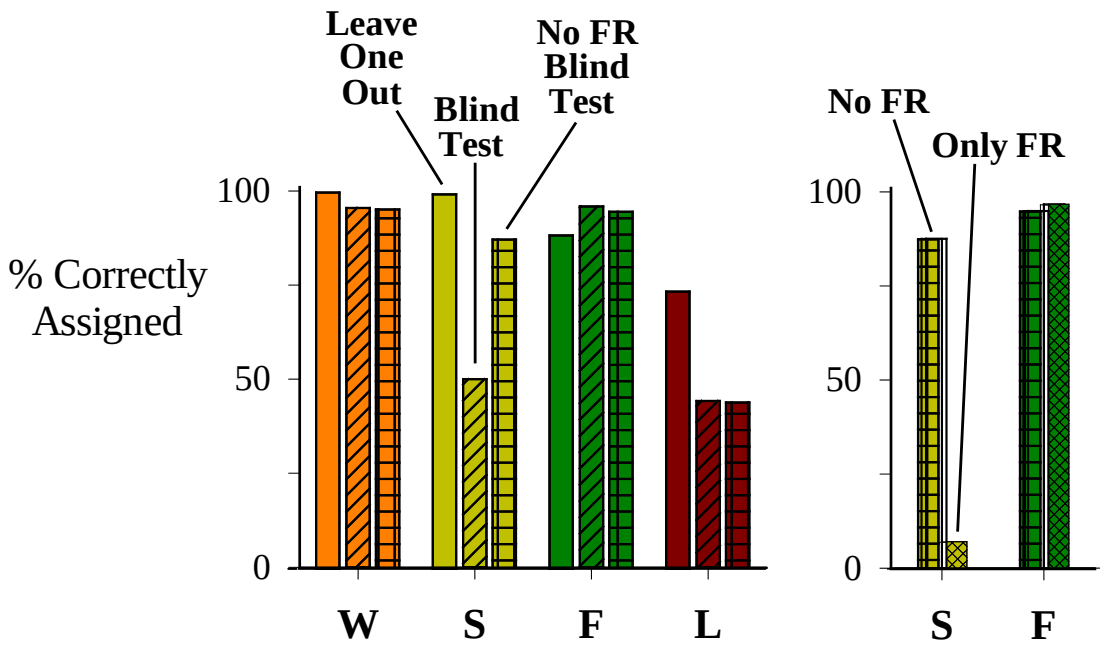

Figure 1. (A) Accuracy of genetic test ONCOR-16 based on Leave-One-Out (LOO) and the Blind Test with and Blind Test without inclusion of Feather River "FR" samples. (B) Spring-run and fall-run accuracy based on Blind Test for only non-Feather River and only Feather River samples.

Genetic versus Length-at-Date race assignments

For the 2004 and 2006 to 2010 migration seasons, genetic tests successfully assigned race to 11,069 individual Chinook Salmon juveniles salvaged at the state and federal fish protection facilities in the south Delta. Genetically designated fall run composed 86.7% of this number, winter run 7.1%, late-fall run 4.8%, and spring run (resolved for only Butte, Mill, and Deer creeks) just 1.4%.

Note: these values strictly reflect race proportions of genetically tested juveniles, not proportions of these races in salvage, because we used only raw, unexpanded genetic results for evaluation of the Length-at-Date approach.

We found that for the 11,069 successfully tested juveniles, 5446 (49%) of the genetic race assignments did not agree with Length-at-Date race assignments using the Delta Model. The greatest discrepancy by raw number was the 4,777 (47%) of genetic fall run that fell within the Delta Model spring-run size range (Table 5a-b; Figure 2a). These same 4,777 genetic fall run composed 95% of all juveniles with fork lengths in the Delta Model spring-run size range (Table 5c; Figure 2b). Other large discrepancies were 276 genetic fall run and 211 genetic late-fall run that fell within the Delta Model winter-run size range (composing 22% and 17% of Delta Model winter-run sized juveniles), 192 genetic late-fall run that fell within the Delta Model fall-run size range, and 115 genetic late-fall run that fell within the Delta Model spring-run size range. Although relatively few juveniles were genetically assigned to spring run (151 individuals), large proportions of the genetic Mill/Deer spring run fell within the Delta Model size ranges for fall run and winter run (38% and 20% of genetic Mill/Deer spring run; Table 5b).

Table 5a. September 2003 – August 2010; total numbers of salvaged and genetically tested Chinook Salmon assigned to each race by genetics and by Delta Model length-at-date size criteria.

	DNA Run					
Length Run	***Late-fall***	***Winter***	***Spring (Butte)***	***Spring (Mill-Deer)***	***Fall***	***Total Length Run***
Late-fall	**9**	0	0	0	2	*11*
Winter	211	**728**	3	19	276	*1237*
Spring	115	56	**45**	**39**	4522	*4777*
Fall	192	5	9	36	**4802**	*5044*
Total DNA Run	*527*	*789*	*57*	*94*	*9602*	

Table 5b. September 2003 – August 2010; proportion of each genetically assigned Chinook Salmon race that were assigned to each length-at-date race by Delta Model size criteria.

	DNA Run				
Length Run	***Late-fall***	***Winter***	***Spring (Butte)***	***Spring (Mill-Deer)***	***Fall***
Late-fall	**0.02**	0	0	0	< 0.01
Winter	0.40	**0.92**	0.05	0.20	0.03
Spring	0.22	0.07	**0.79**	**0.41**	0.47
Fall	0.36	0.01	0.16	0.38	**0.50**
Total DNA Run	*527*	*789*	*57*	*94*	*9602*

Table 5c. September 2003 – August 2010; proportion of each Delta Model Chinook Salmon race that were genetically assigned to each DNA race.

	DNA Run					
Length Run	***Late-fall***	***Winter***	***Spring (Butte)***	***Spring (Mill-Deer)***	***Fall***	***Total Length Run***
Late-fall	**0.82**	0	0	0	0.18	*11*
Winter	0.17	**0.59**	0	0.02	0.22	*1237*
Spring	0.02	0.01	**0.01**	**0.01**	0.95	*4777*
Fall	0.04	0	0	0.01	**0.95**	*5044*

Length-at-Date and genetic race assignments were also compared for individual juvenile migration years and are provided in Tables 6a to 6f. For four of the years in our analysis, more than 96% of genetic winter run fell within Delta Model winter-run size range, with 82% and 85% of genetic winter run falling within Delta Model winter-run size range for the other two years. However, large percentages of genetic late-fall and spring runs and large numbers (though a small percentage) of genetic fall run also fell within winter-run size range in most years, so that a sizable proportion of juveniles that fell within the winter-run size range (23%-50%) were genetically assigned to one of the three non-winter runs.

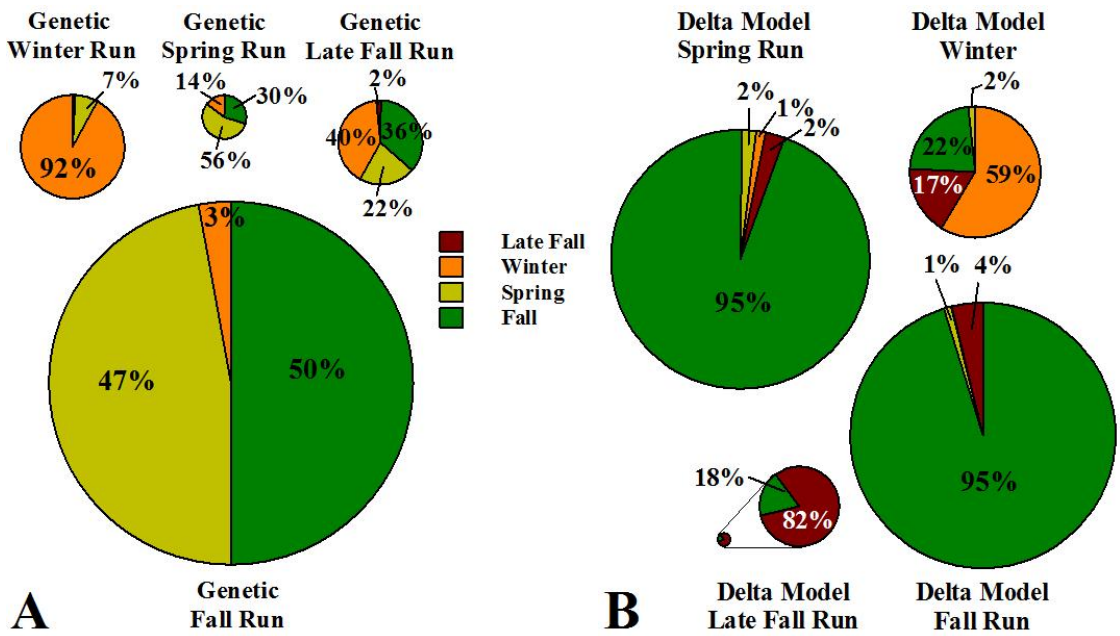

Figure 2. (A) Pies represent genetically tested juvenile Chinook Salmon assigned to each genetic race; slices are the proportion that fell within corresponding Delta Model size criteria. (B) Pies represent juvenile Chinook Salmon assigned to each Delta Model race by fork length; slices are the proportion of juveniles that were assigned to each genetic race. Pie size represents the relative number of juveniles in each category. This chart shows data from migration years 2004 and 2006-2010.

Temporal salvage distribution

Based on the salvage dates of genetically assigned juveniles from each race over the 2004 to 2010 migration seasons (excluding 2005), winter run exhibited the most consistent and constrained salvage date range with 90% of salvage occurring in the 40 day period spanning February 11th – March 22nd (Figure 3). For spring run and fall run, 90% of salvage occurred over 97 day periods spanning February 27th – June 1st and March 11th – June 16th. Late-fall-run salvage was the most dispersed with 90% of individuals salvaged over a 153 day period spanning January 7th – June 9th.

Table 6. Total numbers of salvaged and genetically tested Chinook Salmon assigned to each race by genetics and by Delta Model length-at-date size criteria for each migration year.

6a - 2004

	DNA Run					
Length Run	***Late-fall***	***Winter***	***Spring (Butte)***	***Spring (Mill-Deer)***	***Fall***	***Total Length Run***
Late-fall	2	0	0	0	0	*2*
Winter	21	116	0	8	32	*177*
Spring	11	17	15	7	88	*138*
Fall	108	4	4	15	447	*578*
Total DNA Run	*142*	*137*	*19*	*30*	*567*	

6b – 2006

	DNA Run					
Length Run	***Late-fall***	***Winter***	***Spring (Butte)***	***Spring (Mill-Deer)***	***Fall***	***Total Length Run***
Late-fall	4	0	0	0	0	*4*
Winter	40	69	1	0	28	*138*
Spring	12	3	0	6	690	*711*
Fall	37	0	0	10	1953	*2000*
Total DNA Run	*93*	*72*	*1*	*16*	*2671*	

6c - 2007

	DNA Run					
Length Run	***Late-fall***	***Winter***	***Spring (Butte)***	***Spring (Mill-Deer)***	***Fall***	***Total Length Run***
Late-fall	1	0	0	0	0	*1*
Winter	59	178	0	0	78	*315*
Spring	8	5	5	2	450	*470*
Fall	1	1	2	1	230	*235*
Total DNA Run	*69*	*184*	*7*	*3*	*758*	

Table 6, continued

6d – 2008

	DNA Run					
Length Run	***Late-fall***	***Winter***	***Spring (Butte)***	***Spring (Mill-Deer)***	***Fall***	***Total Length Run***
Late-fall	0	0	0	0	2	*2*
Winter	15	95	2	5	62	*179*
Spring	13	21	5	12	1317	*1368*
Fall	13	0	1	6	1206	*1226*
Total DNA Run	*41*	*116*	*8*	*23*	*2587*	

6e – 2009

	DNA Run					
Length Run	***Late-fall***	***Winter***	***Spring (Butte)***	***Spring (Mill-Deer)***	***Fall***	***Total Length Run***
Late-fall	0	0	0	0	0	*0*
Winter	17	112	0	3	13	*145*
Spring	58	4	19	2	1078	*1161*
Fall	22	0	0	3	374	*399*
Total DNA Run	*97*	*116*	*19*	*8*	*1465*	

6f – 2010

	DNA Run					
Length Run	***Late-fall***	***Winter***	***Spring (Butte)***	***Spring (Mill-Deer)***	***Fall***	***Total Length Run***
Late-fall	2	0	0	0	0	*2*
Winter	59	158	0	3	63	*283*
Spring	13	6	1	10	899	*929*
Fall	11	0	2	1	592	*606*
Total DNA Run	*85*	*164*	*3*	*14*	*1554*	

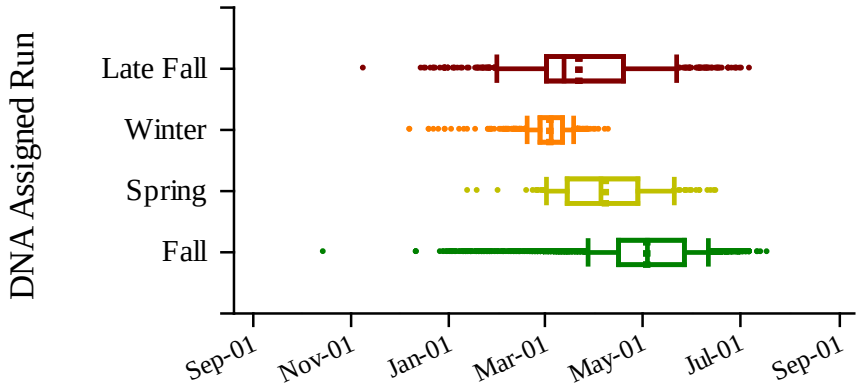

Figure 3. Distribution of salvage date for genetically-identified Chinook Salmon juveniles from CVP and SWP facilities, migration years 2004 and 2006-2010. Figure depicts 25th and 75th percentiles (box ends), median (solid centerline), mean (dotted centerline where different from median), 10th and 90th percentiles (whisker ends) and data points outside the 10th and 90th percentiles.

Biweekly fork length distributions

There was considerable overlap in fork length ranges between all four genetic races in most months that they co-occurred in the salvage (Figure 4). Late-fall run, spring run and particularly fall run were salvaged with widely distributed fork lengths between late December and the end of March, with distinctly narrower distributions from April through July. Length frequency charts (Figures 5a-d) show that the early broad distributions for spring, fall and late-fall runs were composed of two distinctly separate size classes: larger, older juveniles greater than 100 mm (early large), which probably included both yearling and early-spawned sub-yearling juveniles, and very small young-of-the-year (YOY) between 30 mm and 60 mm (early small). Winter-run juveniles were almost exclusively salvaged in the early large size class, with only four salvaged in the early small size range, and only five salvaged after March. Diminishing salvage of the early large and early small size classes during March coincided with the appearance of a mid-sized class of YOY (late mid-sized), which continued in the salvage through early June for spring run and through late June for fall and late-fall runs. Length-frequency charts suggest these three size classes had distinctly different fork length trends. In addition, overlapping fork length distributions of the early small and late mid-sized juveniles during March created a transition zone of rapid fork length increase.

Fork length trends

For spring, fall, and late-fall runs, segmented regression identified separate fork length trends for the early-season, small juvenile migrants and the late-season, older juvenile migrants, as well as a period between the early and late migration seasons when average fork length of juveniles rapidly transitioned from smaller to larger juveniles. The dates that segmented regression identified as break points in fork length trends between early season, transition period and late season were March 1st and April 1st for spring run, March 5th and March 31st for fall run and for late-fall run March 7th and March 30th.

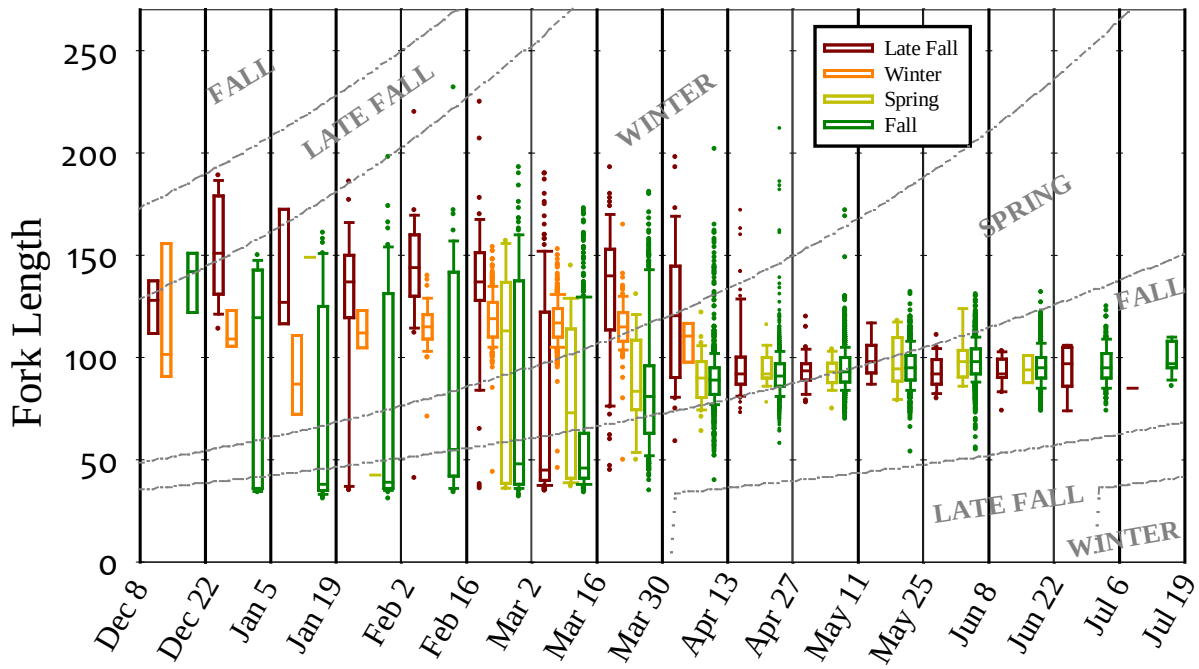

Figure 4. Fork length distributions of Chinook Salmon juveniles pooled biweekly and across migration years 2004 and 2006-2010. Percentiles are 25th/75th (box ends), median (center line), 10th/90th (whiskers) and outlying fork lengths (dots). Boxes without whiskers did not have adequate data to estimate these percentiles.

The most consistent feature of fork length trends for early-migrating large juveniles of all races and for late-migrating, mid-sized juveniles of spring, fall, and late-fall runs was a striking lack of consistency (Figures 6a-d). Across all races and years, only five of the eighteen regressions for large, early-migrating juveniles had significant trends ($P < 0.05$), and two of these five years had significantly negative fork length trends. Similarly, only five of fifteen regressions for late-migrating, mid-sized juveniles had significant fork length trends, one of them negative, while the four with significant positive fork length trends had apparent exponential growth rates averaging 0.00443 (compare to Delta Model 0.00657 for non-winter runs). Three of the five regressions for small, early-migrating juveniles had significant fork length trends, all positive, with an average apparent growth rate of 0.0156. This was more than double the growth rate used to derive Delta Model size criteria for non-winter-run juveniles. In the March transition period between small early migrants and late mid-sized migrants, fork length trends were significantly positive for all eight regressions performed, with a 0.0237 average exponential rate of fork length increase, more than three and a half times the Delta Model growth rate for non-winter runs. Since growth rates are exponential, the differences between expected Delta Model fork lengths and empirical fork lengths associated with these growth rates are likewise exponential.

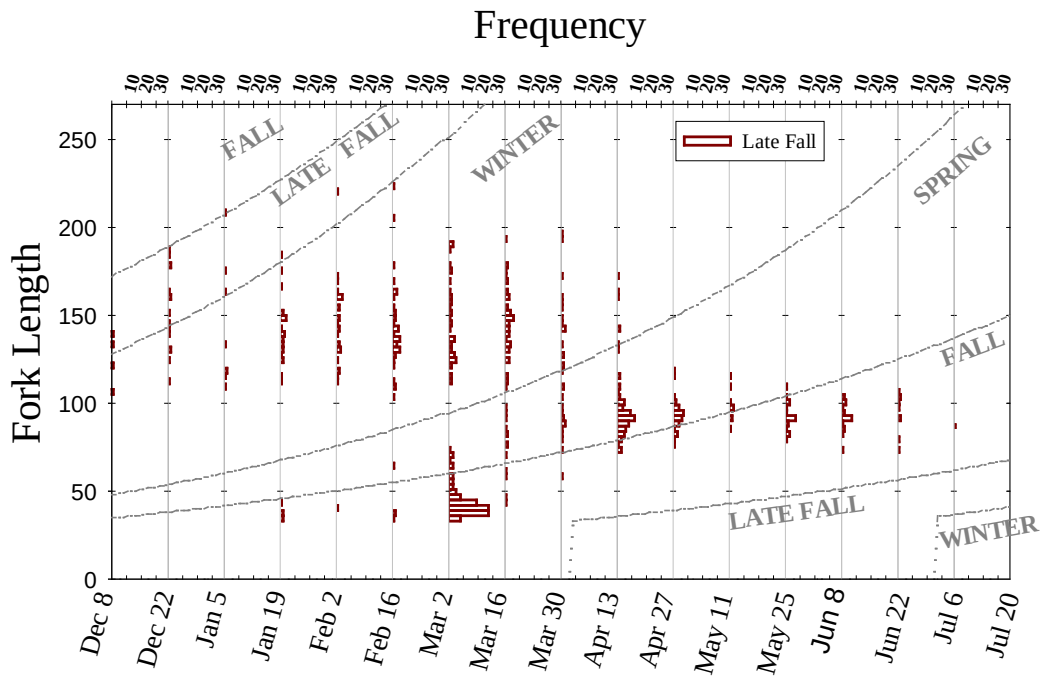

Figure 5a. Genetic late-fall-run Chinook Salmon fork length frequency distribution, pooled at biweekly intervals and across years for fish salvaged in migration years 2004 and 2006-2010. Dashed lines depict Delta Model length-at-date size criteria.

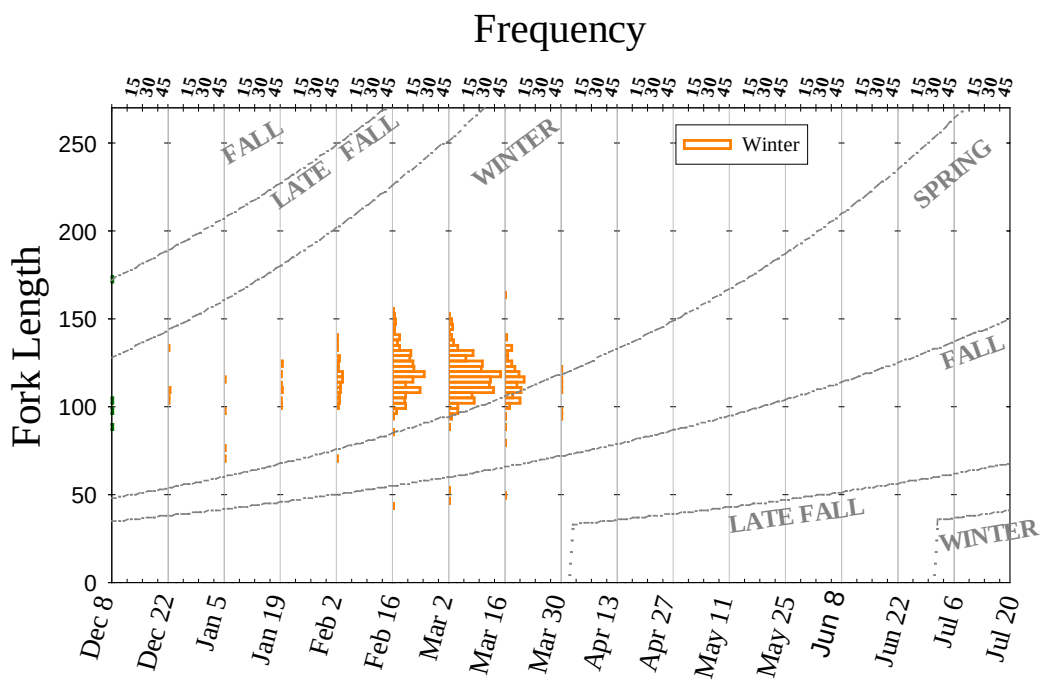

Figure 5b. Genetic winter-run Chinook Salmon fork length frequency distribution, pooled at biweekly intervals and across years for fish salvaged in migration years 2004 and 2006-2010. Dashed lines depict Delta Model length-at-date size criteria.

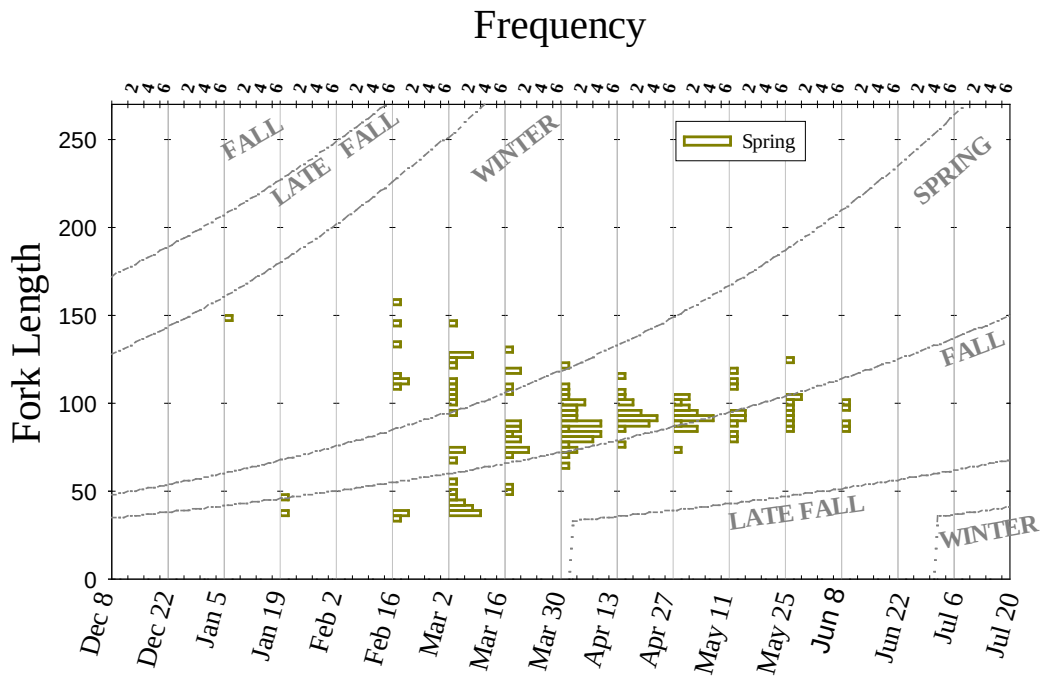

Figure 5c. Genetic spring-run Chinook Salmon fork length frequency distribution, pooled at biweekly intervals and across years for fish salvaged in migration years 2004 and 2006-2010. Dashed lines depict Delta Model length-at-date size criteria.

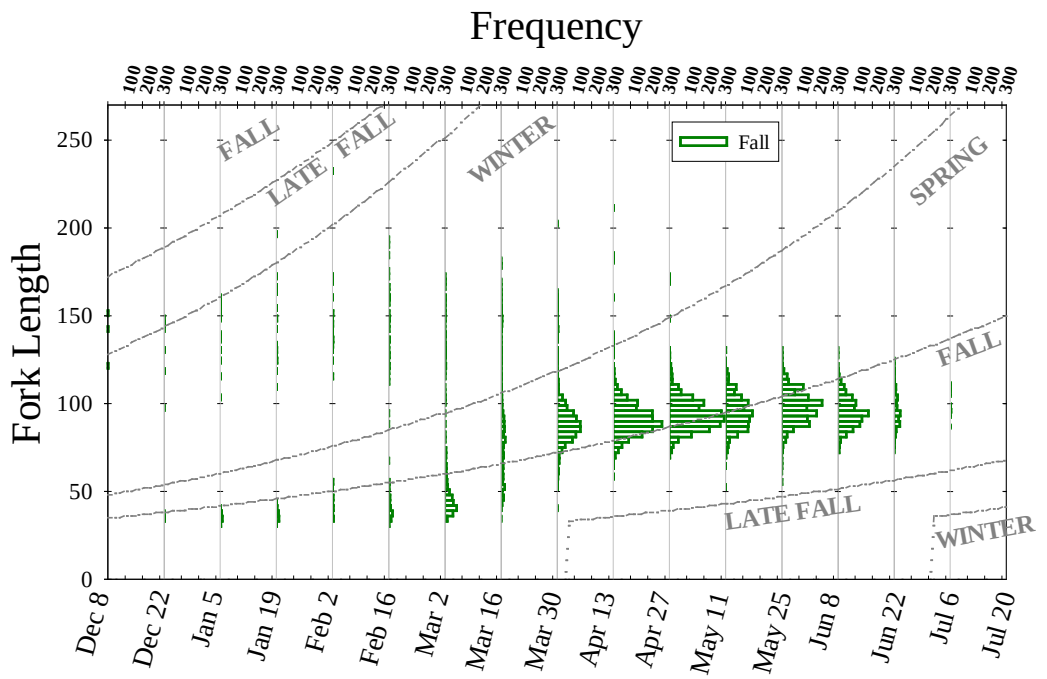

Figure 5d. Genetic fall-run Chinook Salmon fork length frequency distribution, pooled at biweekly intervals and across years for fish salvaged in migration years 2004 and 2006-2010. Dashed lines depict Delta Model length-at-date size criteria.

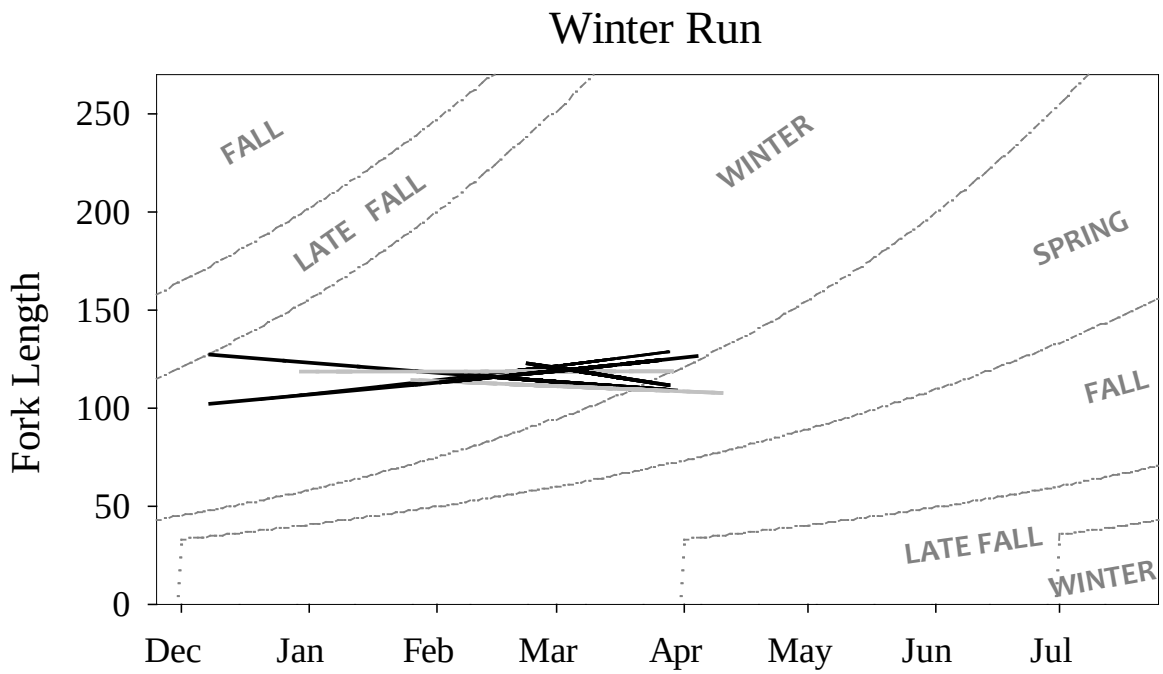

Figure 6a. Regression lines for early-migrating older winter-run Chinook Salmon salvaged in migration years 2004 and 2006-2010. Black lines depict regression P < 0.05.

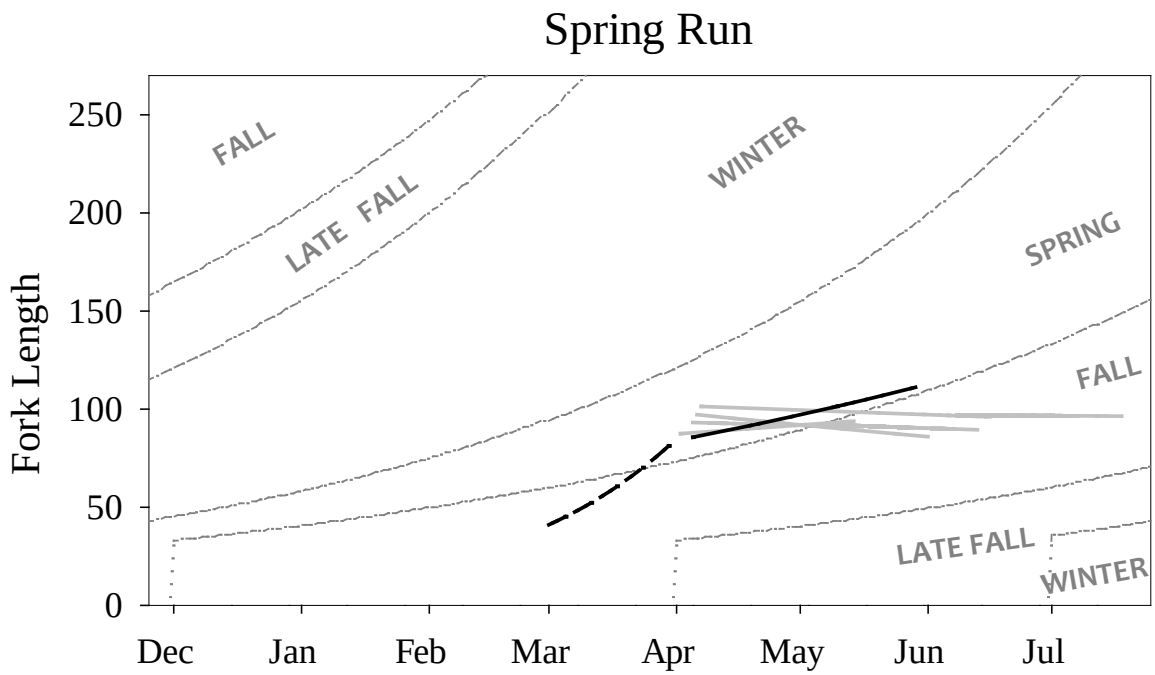

Figure 6b. Regression lines for transition period (dashed) and for late-migrating older spring-run Chinook Salmon salvaged in migration years 2004 and 2006-2010. Black lines depict regression P < 0.05.

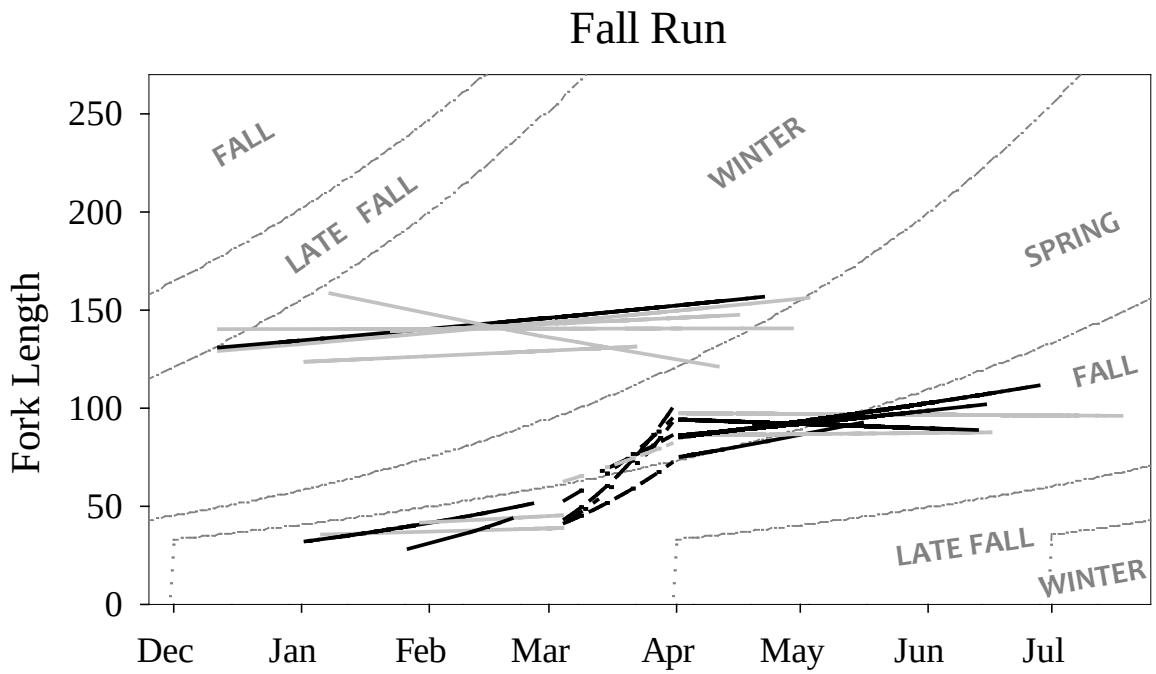

Figure 6c. Regression lines for early-migrating young and older, for transition period (dashed) and for late-migrating older fall-run Chinook Salmon salvaged in migration years 2004 and 2006-2010. Black lines depict regression P < 0.05.

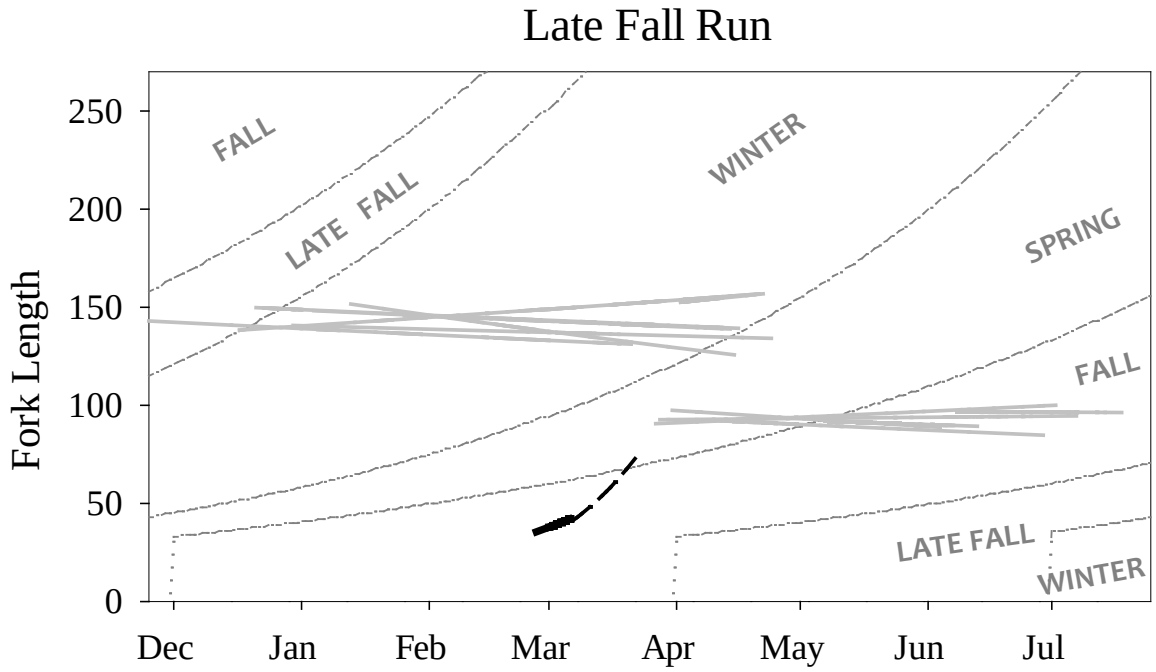

Figure 6d. Regression lines for transition period (dashed), and for early and late-migrating older late-fall-run Chinook Salmon salvaged in migration years 2004 and 2006-2010. Black lines depict regression P < 0.05.

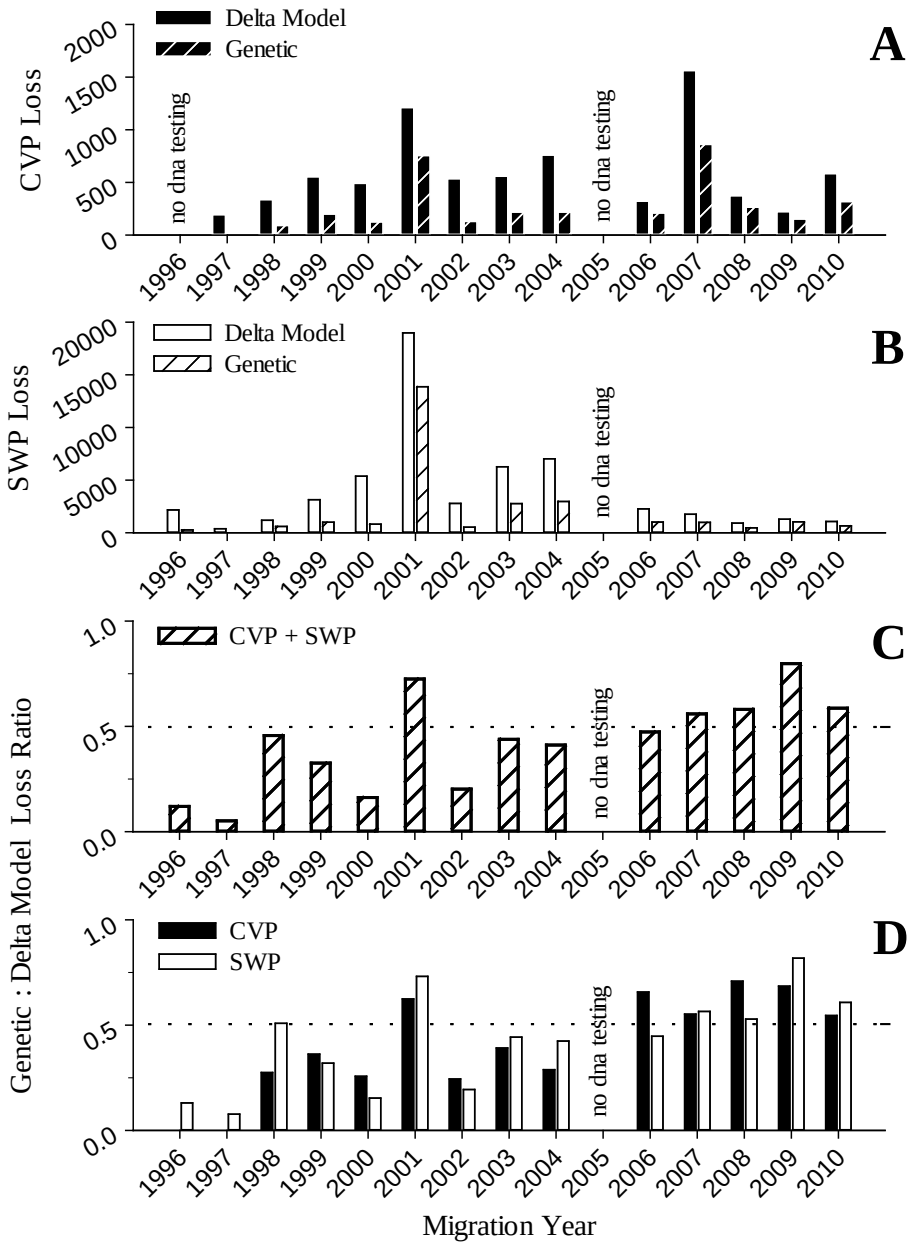

Figure 7. Yearly loss based on Delta Model and on Genetic identification for (A) CVP and (B) SWP. Yearly ratio of genetic and Delta Model based loss for CVP and SWP (C) combined and (D) individually. Note order of magnitude difference in scale between CVP and SWP loss axes.

Delta Model versus genetic salvage and loss estimates

Yearly salvage and yearly loss for non-adipose clipped winter-run Chinook Salmon were highly correlated ($r^2 > 0.95$, $p < 0.001$ for combined facilities, for CVP and SWP individually, and for both genetic and Delta Model based estimates). Winter-run average annual loss at the SWP was about seven times CVP average annual loss over the 1997 to 2010 period, ranging from about equal to 18 times CVP

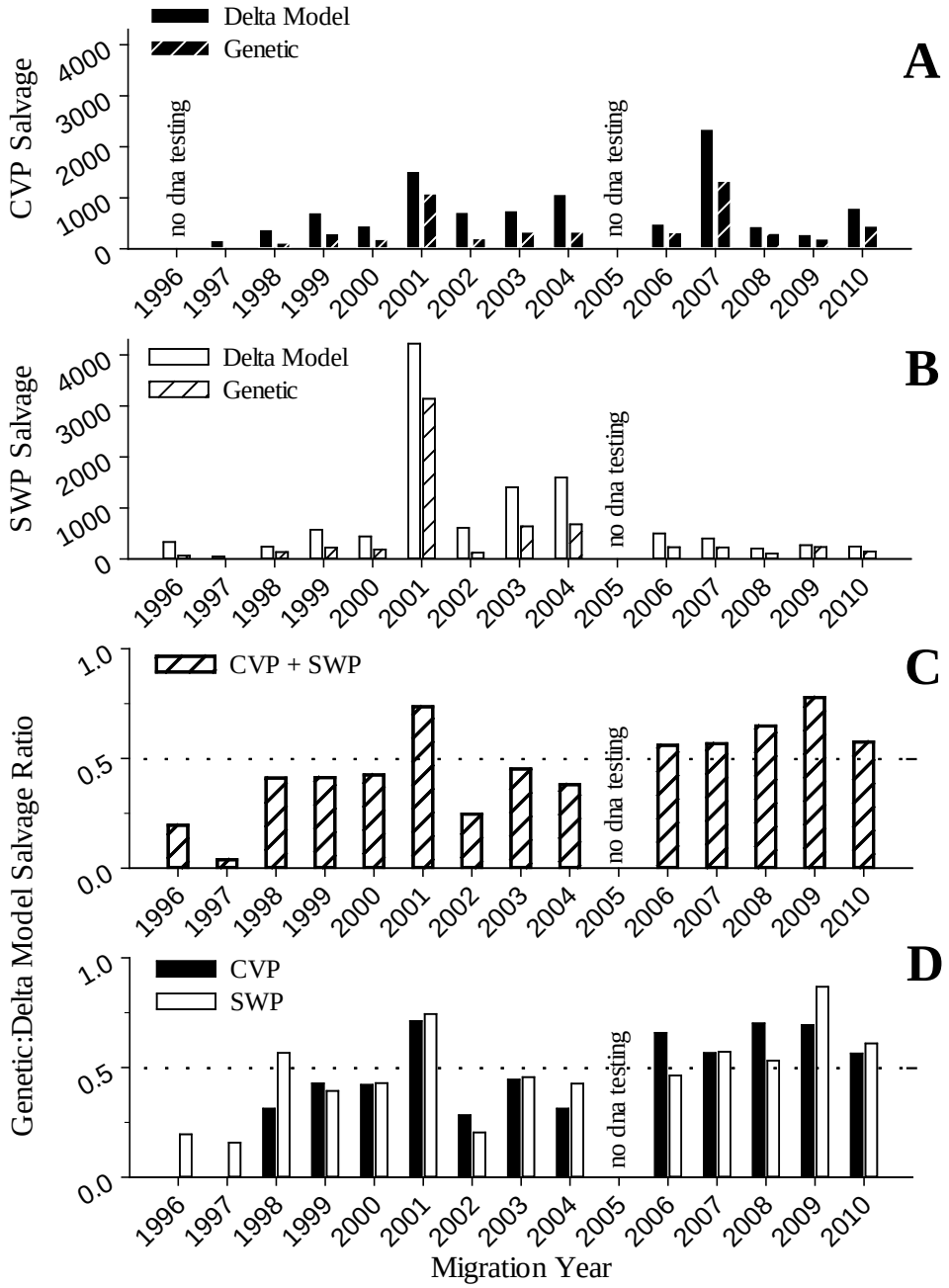

Figure 8. Yearly salvage based on Delta Model and on Genetic identification for (A) CVP and (B) SWP. Yearly ratio of genetic and Delta Model based salvage for CVP and SWP (C) combined and (D) individually.

annual loss (Figures 7a-b). This was true whether loss was based on genetic or Delta Model race designations. The difference between the facilities reflected primarily the difference in the loss equation expansion factor that accounts for prescreen loss (e.g., predation), which is more than four times larger for SWP than for CVP for most sampling events. Average annual expanded salvage over this period was nearly identical between the facilities, with SWP annual salvage ranging from less than a fifth to nearly three times CVP annual salvage (Figure 8a-b).

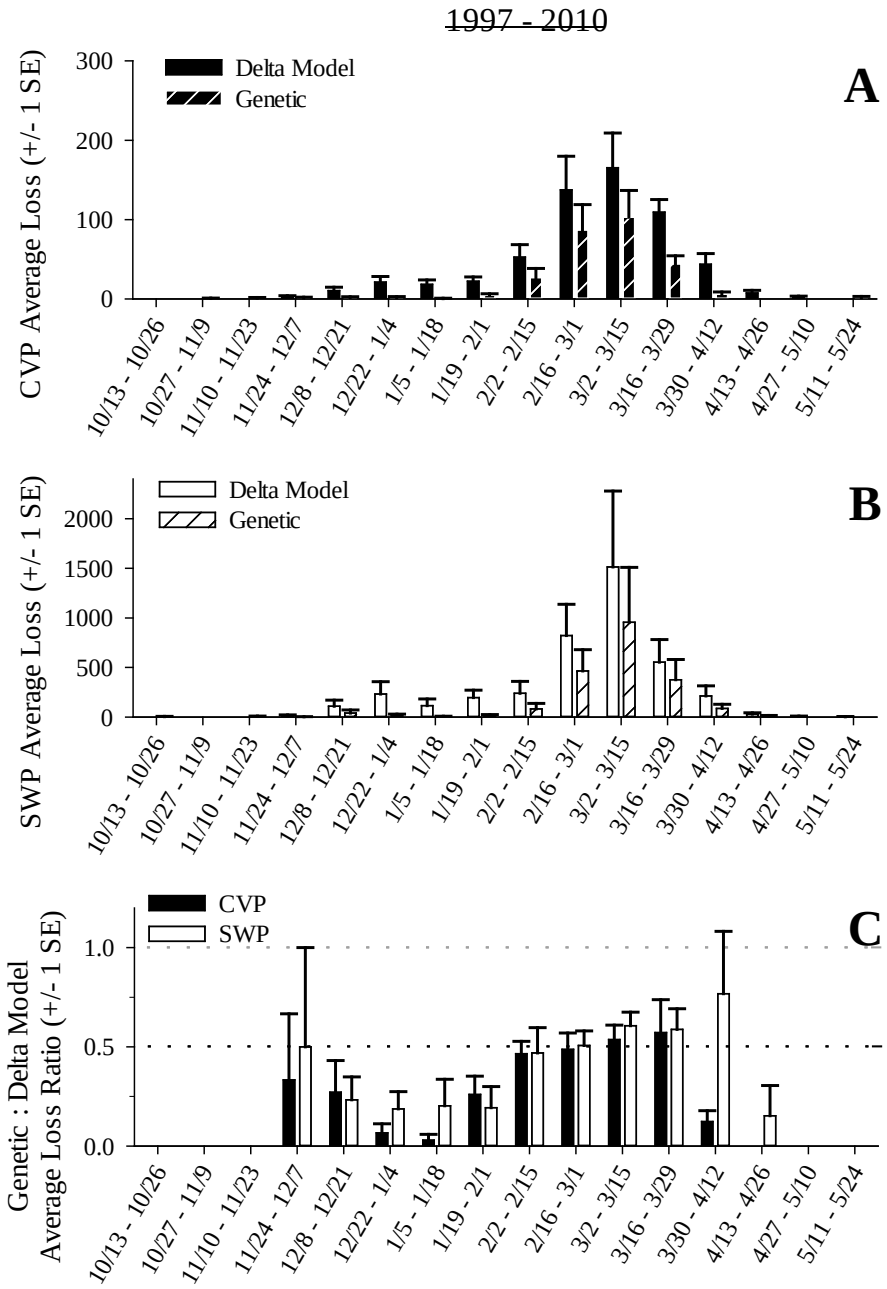

Figure 9. Delta Model and genetic assignment based biweekly winter-run loss, average of migration years 1997 - 2010 for CVP (A) and SWP (B), and CVP and SWP average genetic:Delta Model loss ratio (C).

The yearly ratio of genetic winter-run loss compared to winter-run loss based on Length-at-Date size criteria averaged 44% over all study years (1997-2010). However, prior to 2005, the average yearly ratio was only 35%, while after 2005 yearly genetic loss averaged 60% of Length-at-Date loss. Since 2005, genetic winter-run loss was most similar to Length-at-Date loss between mid-February and mid-March, with an average genetic to Length-at-Date loss ratio of 71% (Figure 10a). This was also the time of year when the greatest density of genetic winter-run salvage occurred (Figure 10b-c).

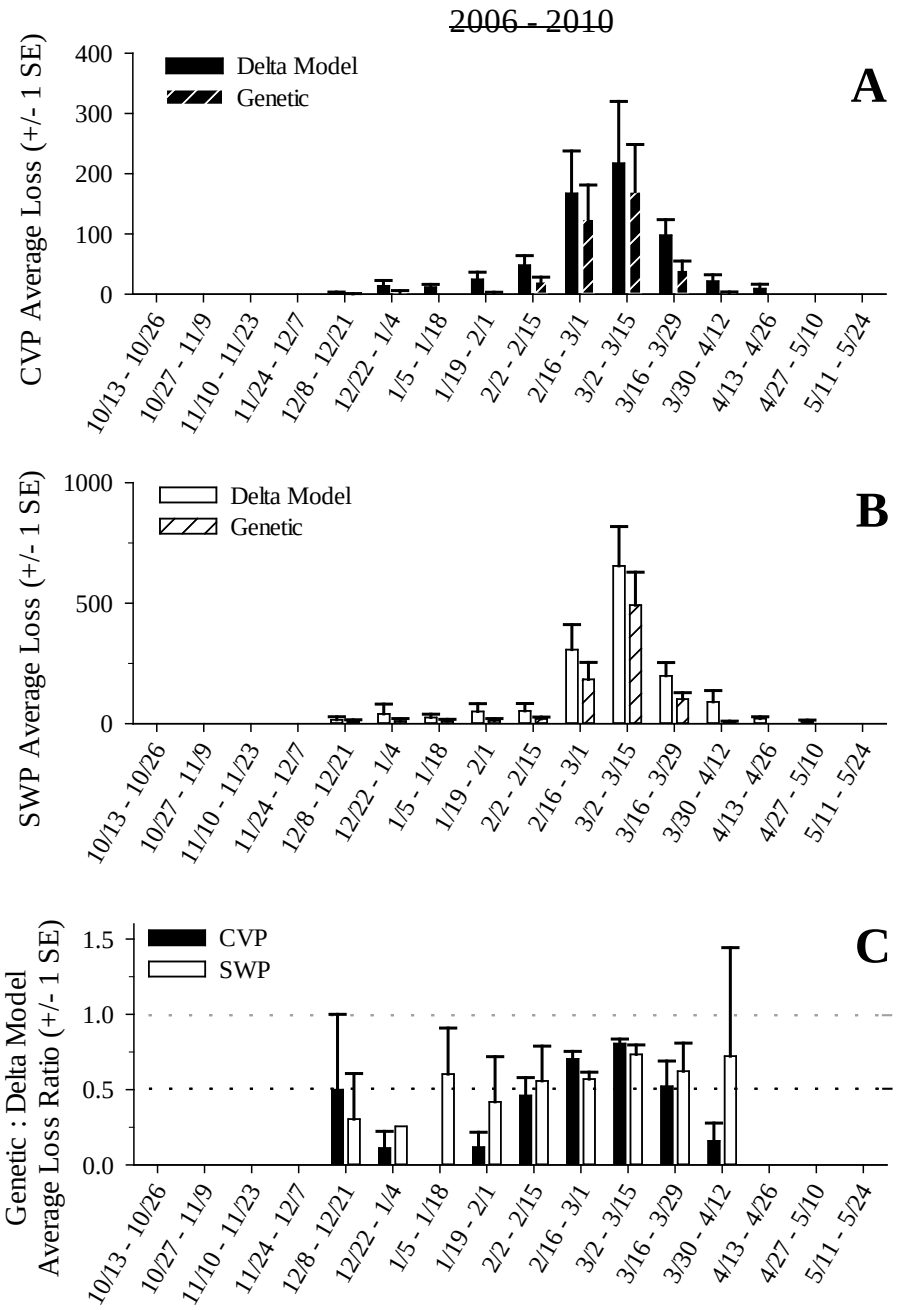

Figure 10. Delta Model and genetic assignment based biweekly winter-run loss, average of migration years 2006 - 2010 for CVP (A) and SWP (B), and CVP and SWP average genetic:Delta Model loss ratio (C).

Discussion and Conclusions

Genetic test accuracy

When considering analyses and results based on genetic race assignments in SALMGEN, it is important to bear in mind that genetic race assignments are not absolute. Evaluations of genetic tests found varying levels of assignment accuracy for each race (Figure 1) and demonstrated that the "probability of correct assignment" generated by genotyping software for each individual juvenile were not a reliable indicator of the actual probability of assignment error (Table 3). According to blind tests (Banks et al. In Review), genetic tests provided highly accurate race assignments for winter run, fairly accurate assignments for fall run and for spring run from Butte, Mill, and Deer creeks, and poor accuracy assignments for late-fall run. Individual assignment tests were not able to distinguish between spring-run and fall-run phenotypes in the Feather River, with both genetically assigned to fall run (Figure 1b). Although hatchery-produced spring-run juveniles from the Feather River are coded-wire tagged, natural spawning spring-run phenotypes in the Feather River produce substantial numbers of untagged juveniles (Ryon Kurth, DWR, personal communication). Therefore, an unknown proportion of salvaged juveniles that were genetically assigned to fall run were progeny of Feather River Chinook Salmon that displayed spring-run migration and spawn-timing behavior; in other words, they were spring-run juveniles.

Why did the genetic tests fail to differentiate between Feather River spring-run and fall-run Chinook Salmon? Differentiation of the four Central Valley Chinook Salmon races was originally based on four generally observed behavioral patterns of adult migration and spawn timing, and population-level genetic tests subsequently confirmed clear genetic differentiation between adult populations displaying these four behavioral patterns (Banks et al. 2000). However, accurate assignment of an individual to a specific race requires a higher level of differentiation than population level assessments. Historically, spring-run Chinook Salmon adults migrated to the upper reaches of the Feather River and its tributaries in late spring, and later in the early-fall season, during the overlapping spawning seasons of Feather River spring-run and fall-run Chinook Salmon, genetic differentiation between the races was maintained by low river flows that spatially segregated spring-run spawning grounds in the upper watershed from fall-run spawning grounds in the lower watershed. In the Feather River, the construction of Oroville Dam prevented spring-run migration above the lower Feather River and removed the spatial segregation that reproductively isolated spring run from fall run. This allowed the exchange of genetic material between the races so that individual assignment tests can no longer distinguish between races. A similar situation has been documented for spring-run and fall-run Chinook Salmon in the Trinity River below Lewiston Dam (Kinziger et al. 2008).

If the genetic markers for tests discussed in this report had been developed from genetic material actively involved in the regulation of migration and spawn-timing behavior, then selective pressure on this behavior (such as enforced in the hatchery) would have quickly reestablished genetic differences between the hybridized races at these genetic markers. However, the genetic tests used to develop SALMGEN employed genetic markers from "non-coding" regions of genetic material (i.e. inactive genetic material). For these non-coding regions of genetic material, re-divergence between populations occurs by random mutation, a relatively slow process compared to divergence of genetic material under selective pressure. Therefore, even if hatcheries match spawning pairs based on run-timing behavior, and thus maintain the

genetic integrity responsible for behavioral differences between races, this integrity will not be reflected in the immediate future by genetic tests with markers from non-coding regions of the salmon genome.

In recent years, geneticists pursued two different courses of research to allow individual assignment of Feather River salmon to the proper race. The first course of research attempted to identify genetic markers from genes associated with photoperiod-detection, because changes in day length were hypothesized to trigger migrations. Since genes associated with migration behavior are under active selection for both hatchery and wild populations, researchers expected genetic markers associated with photoperiod detection would allow differentiation between Feather River spring and fall runs. Although much progress was made investigating these photoperiod genes (O'Malley et al. 2007, O'Malley and Banks 2008b, 2008a, O'Malley et al. 2010), researchers did not find a relationship between genetic markers from these genes and Chinook Salmon run timing that could distinguish between Feather River races.

The second course of genetic research pursued to differentiate between hybridized stocks is called parental-based tagging (PBT). With PBT, a juvenile's race is obtained by genetically identifying one or both of its parents, thus requiring that the juvenile's parents were previously identified to race based on run-timing behavior, and that the parents were also genetically tested. Technically, no "tag" is actually applied with PBT because the tag is inherent in the DNA that a juvenile inherits from its parents. Recently initiated PBT programs for Central Valley Chinook Salmon and steelhead trout hatchery stocks appear to be a successful and effective means of identifying race and hatchery origin of juveniles, because the entire parent population can be easily and dependably sampled (Eric Anderson, NMFS, personal communication). Parent-based tagging has also been applied to wild populations in small stream systems in Washington State where researchers were able to sample a large and known proportion of the spawning adult population (Scott Blankenship, Cramer Fish Sciences, personal communication).

DWR is currently implementing two parallel pilot projects using genetic-based identification at the salvage facilities. The first project will use established genetic tests to monitor salvage of winter-run Chinook Salmon, for which the tests are highly accurate. The second pilot project will use winter-run salvage estimates from the established tests to evaluate PBT-based salvage estimates. If PBT proves accurate for non-hatchery winter run, DWR may explore PBT for estimating salvage populations of other salmon stocks that are not as accurately identified by established genetic tests. However, the ability of a PBT system to monitor large, wild populations in open systems remains uncertain due to logistical difficulties in sampling an appropriate proportion of the adult spawning population and in estimating the proportion of the adult population that has been sampled, both necessary elements of a PBT identification system for non-hatchery stocks. Some of the genetic and statistical techniques being applied in the pilot project are not fully vetted and in some instances are in the experimental stages. As such, it is uncertain whether these approaches will fully resolve race identification for all Central Valley salmonids. Genetic approaches for discriminating the race of California's Central Valley salmon have evolved and improved considerably since genetic research was initiated. Eventually, multiple complimentary approaches may be needed to sufficiently resolve Central Valley salmonid stock identification.

Size selective sampling

Besides error associated with genetic test accuracy, we were also concerned about possible size-selective sampling of salvaged juveniles for genetic testing, which can bias evaluations of Delta Model accuracy as

well as salvage and loss estimates based on genetic assignments. In fact, we found that prior to 2004 the fork lengths of genetically tested fish were biased toward larger juveniles, while from 2004 to 2010 genetically sampled juveniles composed a representative sample of salvage count fork lengths (Appendix B). The reason larger juveniles were disproportionately sampled in the early years of genetic studies was to ensure that winter run would be detected, since winter run in salvage samples tend to occur at larger fork lengths. During this period, only a limited number of juveniles could be genetically tested and random sampling would have selected a small proportion of larger juveniles, which were scarce relative to smaller juveniles.

In our comparison of Length-at-Date and genetic race assignments, we limited our analysis to juvenile migration years that provided a representative sample of the salvage count (according to fork length distributions) to avoid bias from size-selective sampling. To minimize bias in our calculation of winter-run salvage and loss, which included years in which size-selective sampling was apparent, we separated genetic winter-run juveniles into three fork length ranges and then calculated separate expansion factors for each fork length range. These precautions, along with the fact that genetic tests were highly accurate for winter run, ensured that salvage and loss error rates based on genetic winter run would not differ substantially from error rates of traditional salvage and loss estimates, which were based on Delta Model race assignments. We did not attempt to calculate these error rates. However, an examination of loss calculation error was reported in Jahn (2011).

Length-at-Date

In general, the Delta Model Length-at-Date size criteria are a poor indicator of salvaged juvenile Chinook Salmon race. Based on samples collected from 2004 to 2010, we found that Length-at-Date race assignments conflicted with genetic race assignments for half (49%) of genetically tested fish (Figure 2, Table 5a). Length-at-Date criteria for winter run were the most successful at “capturing” the corresponding genetically assigned juveniles, with 92% of genetic winter-run samples falling within winter-run size criteria (Table 5b , Figure 2a). This is not surprising, given that size criteria were initially established to protect winter run. On the other hand, many non-winter run were also captured by winter-run size criteria, such that 41% of juveniles falling within winter-run size criteria were not genetic winter run (Table 5c, Figure 2b). Similarly, spring-run size criteria did a moderately good job of capturing genetic spring run from Butte Creek (79%), but also captured nearly half of the relatively abundant genetic fall run – so that 95% of juveniles salvaged in the spring-run size range were genetically assigned to fall run. Some of these genetic fall run in the spring-run size range were likely Feather River spring-run phenotype that were genetically assigned to fall run (due to inaccuracy of genetic tests for the Feather River spring-run population, as previously discussed). In addition, inaccuracy of genetic tests for late-fall run may also account for a small part of the discrepancy between genetic and Length-at-Date assignments for these races. Nevertheless, the number of juvenile Chinook Salmon with conflicting genetic and Delta Model race assignments far exceeded the error rate suggested by blind test evaluations of the genetic tests. For example, blind test evaluations suggest about a 50% error rate for late-fall-run genetic assignment, while 98% of genetic late-fall run were salvaged outside of late-fall size criteria, indicating that the poor performance of Delta Model size criteria cannot be explained by inaccuracies of the genetic tests we used to evaluate the Delta Model.

The Delta Model's poor performance was more likely a reflection of the inaccuracies of its underlying assumptions. Two of the three central assumptions of the Length-at-Date approach, first that the size ranges of juveniles from the different races are mostly segregated, and second that juvenile fork lengths for all races increase at a similar rate throughout the season, were not supported by biweekly fork length distributions, or by fork length trends of salvaged juveniles through the juvenile migration season. Fork length ranges of the four races overlapped considerably (Figure 4), and no race demonstrated a consistent positive fork length trend amongst the early-migrating fry, the early-migrating larger juveniles, or the late-migrating larger juveniles (Figure 6a-d). In some years, fork length trends were significantly negative. Hedgecock (2002) found a similar lack of consistent fork length trend for winter run in his analysis of juvenile migration years 1996 to 2001. The lack of segregation between fork length distributions of the four races brings into question the veracity of the third central assumption of the Length-at-Date approach, that spawning dates of the different races are segregated in time, in that the assumption of fork length segregation stems directly from the assumption of temporal segregation of spawning times. The lack of support for the central assumptions of the Length-at-Date approach implemented at the south Delta export facilities indicates that a simple change in size criteria will not produce more accurate race assignment results.

When Length-at-Date criteria were initially adopted for identifying and managing Central Valley Chinook Salmon, those involved with the decision were keenly aware that the assumption of constant growth rate was likely wrong and therefore the Length-at-Date assignments were potentially inaccurate. However, with no other viable alternative for distinguishing between races, fisheries managers expected that the criteria would be at least moderately protective of winter run (Michael Lacy, DFG, personal communication). These results verify those assumptions to some extent and exemplify how resource agencies must often adopt imperfect protocols based on insufficient data to address urgent management needs.

Juvenile migratory behavior

One marked feature of fork length trends was a general convergence after mid-April to a modal fork length around 95 mm and a range between 80-110 mm (Figures 5a to 5d). Earlier in the season, up to early March, salvaged salmon were either very small fry-sized juveniles (25-60 mm) or juveniles that were 100 mm or larger. If salvage samples are accepted as an indicator of juvenile migration timing, the lack of trend and near constant modal fork length for Chinook Salmon after mid-April suggests that juvenile migration behavior affecting the timing of Delta entry, such as migration initiation or migration rate, depends more on juvenile size or perhaps juvenile age than time of year.

Implications of genetic race assignment for estimation of take

In March of 1997, winter-run take authorization for the operation of the CVP and SWP was doubled from 1% to 2% of estimated juvenile production based on evidence that roughly half of the juveniles salvaged in the Delta Model winter-run size range were not genetic winter run (NMFS 2009). Although our evaluation of the Delta Model found that genetic winter run since 2004 composed 50% –77% (never less than half) of genetically tested juveniles in the winter-run size range (Table 6a-f), these tested fish represented winter run sub-sampled from salvage counts, not juvenile loss. To provide a better indication of the accuracy of the assumption in the Biological Opinion, we compared yearly winter-run loss

estimates based on Delta Model assignments to loss based on genetic assignments, and we extended these estimates back to 1997 when consistent genetic testing began. For the most part, genetic winter run composed less than 50% of yearly unclipped loss from 1997 to 2004 (Figure 7c and 7d). After 2005, genetic winter run consistently composed more than 50% of unclipped winter-run loss, with the exception of loss at the SWP in 2006. This suggests that take restrictions were generally more stringent than assumed prior to 2005, and generally less stringent than assumed after 2005. Since winter-run escapement has been relatively low since 2007, the recent increase in the relative proportion of genetic winter run in the winter-run size range may signal a decline in the abundance of early migrating large juveniles from other races.

Conclusion

The Length-at-Date method is not an accurate indicator of juvenile race for salvaged Chinook Salmon. Although current genetic tests are highly accurate for winter run and spring run from Butte, Mill, and Deer creeks, they are not able to distinguish spring-run juveniles from the Feather River. Therefore any proposed modifications to the accepted assignment method at the salvage facilities that are based on genetic tests will have to account for these inaccuracies. Adoption of an improved race classification system in the near future is not optional. The Biological Opinion for CVP-SWP operations requires the Bureau of Reclamation to develop and implement an alternative technique to assess and minimize take of listed anadromous salmonids at the CVP-SWP export facilities, or risk losing take authorization (NMFS 2009). In addition to the SWP and CVP, there are other programs and operations that are required to use Length-at-Date size criteria to assess take of both winter and spring-run juveniles, including IEP studies. Although these other programs use River Model size criteria rather than the Delta Model size criteria used at the salvage facilities, it is reasonable to assume that adoption of a new race classification system for estimating population size and tabulating CVP-SWP take will project beyond water export operations. Alternative approaches under current consideration or under development include the new genetic approaches discussed above, which may be implemented in a near real-time framework, evaluation of fine-scale differences in morphological features between races, and analyses of multiple environmental variables in relation to daily salvage patterns of Chinook Salmon juveniles to identify potential environmental cues predicting arrival of juvenile pulses at pumping facilities. The SALMGEN database will be an important resource for determining how best to proceed with incorporation of genetic analyses into the CVP-SWP operations as well as many IEP sampling programs. Whether as a direct tool in the form of real-time genetic assays of salvaged Chinook Salmon juveniles, or as an indirect tool used to measure the accuracy of non-genetic alternative classification systems, genetic methods will clearly be integral in development of future take estimation procedures, and in the assessment of Central Valley Chinook Salmon race population statuses in general.

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References

Anderson, E. C. 2010. Assessing the power of informative subsets of loci for population assignment: standard methods are upwardly biased. Molecular Ecology Resources 10(4):701-710.

Anderson, E. C., R. S. Waples and S. T. Kalinowski. 2008. An improved method for predicting the accuracy of genetic stock identification. Canadian Journal of Fisheries and Aquatic Sciences 65(7):1475-1486.

Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon (Oncorhynchus tschawytscha). Journal of Heredity 90(2):281-288.

Banks, M. A. and W. Eichert. 2000. WHICHRUN (version 3.2): A computer program for population assignment of individuals based on multilocus genotype data. Journal of Heredity 91(1):87-89.

Banks, M. A., W. Eichert and J. B. Olsen. 2003. Which genetic loci have greater population assignment power? Bioinformatics 19(11):1436-1438.

Banks, M. A., D. P. Jacobson, C. A. Greig, V. K. Rashbrook, C. Ardren, I. Smith, J. Meusnier, J. Bernier, J. Van Sickle and K. G. O'Malley. In Review. Testing advances in molecular discrimination among California's Chinook salmon life-histories: evidence from a blind test. Animal Genetics.

Banks, M. A., V. K. Rashbrook, M. J. Calavetta, C. A. Dean and D. Hedgecock. 2000. Analysis of microsatellite DNA resolves genetic structure and diversity of chinook salmon (Oncorhynchus tshawytscha) in California's Central Valley. Canadian Journal of Fisheries and Aquatic Sciences 57(5):915-927.

Brandes, P. L. and J. S. McLain. 2001. Juvenile Chinook salmon abundance, distribution and survival in the Sacramento-San Joaquin Estuary. Pages 39-138 *in* Contributions to the biology of Central Valley salmonids, Volume 2 of 2. R. L. Brown, Department of Water Resources, editor. California Department of Fish and Game, California Department of Fish and Game Fish Bulletin 179. Sacramento, CA.

Debevec, E. M., R. B. Gates, M. Masuda, J. Pella, J. Reynolds and L. W. Seeb. 2000. SPAM (Versin 3.2): statistics program for analyzing mixtures. Journal of Heredity 91(6):509-510.

Fisher, F. W. 1992. Chinook salmon, Oncorhynchus tshawytscha, growth and occurrence in the Sacramento-San Joaquin River system. California Department Fish and Game.

Gibbons, B., Director, Department of Fish and Game. January 18, 1994. Memo to Water Policy Council (executive officer level council of state agencies). RE: Response to Department of Water Resources memorandum to the Water Policy Council, dated January 5, 1994.

Hedgecock, D., UC Davis Bodega Marine Laboratory. 2002. Microsatellite DNA for the management and protection of California's Central Valley Chinook salmon (*Oncorhynchhus tshawytscha*): final report for the amendment to Agreement No. B-59638. California Department of Water Resources.

Hedrick, P. W., V. I. Rashbrook and D. Hedgecock. 2000. Effective population size of winter-run Chinook salmon based on microsatellite analysis of returning spawners. Canadian Journal of Fisheries and Aquatic Sciences 57(12):2368-2373.

Hogarth, W. T., Acting Regional Administrator, National Marine Fisheries Service. April 7, 1997. Letter to R. Patterson, Regional Director Bureau of Reclamation and D. Kennedy, Director Department of Water Resources.

Holsinger, L., NOAA National Marine Fisheries, Southwest Region. January 11, 1995. Intradepartmental Memorandum. RE: Recommendations for the size criteria.

Jahn, A., Kier Associates. 2011. An alternative technique to quantify incidental take of listed anadromous fishes at the federal and state export facilities in the California Delta. Draft Report Prepared for National Marine Fisheries Service. April 2011.

Juvenile Salmon Monitoring/Loss Group. 1993. A review of the Sacramento River and Delta juvenile salmonid monitoring/loss program for 1992-93 and board recommendations for the 1993-1994 sampling year.

Kalinowski, S. T. 2003. Genetic Mixture Analysis 1.0. Department of Ecology, Montana State University, Bozeman MT 59717. http://www.montana.edu/kalinowski

Kalinowski, S. T. 2007. ONCOR: a computer program for genetic stock identification. Department of Ecology, Montana State University, Bozeman MT http://www.montana.edu/kalinowski/Software/ONCOR.htm

Kinziger, A. P., E. J. Loudenslager, D. G. Hankin, E. C. Anderson and J. C. Garza. 2008. Hybridization between Spring- and Fall-Run Chinook Salmon Returning to the Trinity River, California. North American Journal of Fisheries Management 28(5):1426-1438.

Millar, R. B. 1987. Maximum likelihood estimatin of mixed stock fishery composition. Canadian Journal of Fisheries and Aquatic Sciences 44:583-590.

Muggeo, V. M. R. 2003. Estimating regression models with unknown break-points. Statistics in Medicine 22:3055-3071.

Muggeo, V. M. R. (2008). segmented: an R Package to Fit Regression Models with Broken-Line Relationships. R News, 8(1). http://cran.r-project.org/doc/Rnews/

National Marine Fisheries Service. 1994 Endangered and threatened species; threatened status for two Chinook salmon Evolutionarily Significant Units (ESUs) in California; final rule Federal Register 440. 59(2). 4 January, 1994.

National Marine Fisheries Service. 1999 Endangered and threatened species; threatened status for two Chinook salmon Evolutionarily Significant Units (ESUs) in California; final rule Federal Register 50393. 64(179). 16 September, 1999.

Newman, K. B. 2008. An evaluation of four Sacramento-San Joaquin River Delta juvenile salmon survival studies. United States Fish and Wildlife Service, 00127144.

Newman, K. B. and P. L. Brandes. 2010. Hierarchical modeling of juvenile Chinook salmon survival as a function of Sacramento-San Joaquin Delta water exports. North American Journal of Fisheries Management 30(1):157-169.

NMFS. 1993. Biological opinion for the operation of the Federal Central Valley Project and the California State Water Project long term operations. National Marine Fisheries Service, Southwest Region.

NMFS. 1995. Amendment to the February 12, 1993 biological opinion for the operation of the Federal Central Valley Project and the California State Water Project long term operations. National Marine Fisheries Service, Southwest Region.

NMFS. 2000. Biological opinion for the operation of the Federal Central Valley Project and the California State Water Project from December 1, 1999 to March 31, 2000. National Marine Fisheries Service, Southwest Region.

NMFS. 2004. Biological opinion on the operation of the long-term Central Valley Project and State Water Project operations criteria and plan. National Marine Fisheries Service, Southwest Region.

NMFS. 2009. Biological opinion and conference opinion on the long-term operations of the Central Valley Project and State Water Project. National Marine Fisheries Service, Southwest Region.

O'Malley, K. G. and M. A. Banks. 2008a. Duplicated Clock genes with unique polyglutamine domains provide evidence for nonhomologous recombination in Chinook salmon (Oncorhynchus tshawytscha). Genetica 132(1):87-94.

O'Malley, K. G. and M. A. Banks. 2008b. A latitudinal cline in the Chinook salmon (Oncorhynchus tshawytscha) Clock gene: evidence for selection on PolyQ length variants. Proceedings of the Royal Society B-Biological Sciences 275(1653):2813-2821.

O'Malley, K. G., M. D. Camara and M. A. Banks. 2007. Candidate loci reveal genetic differentiation between temporally divergent migratory runs of Chinook salmon (Oncorhynchus tshawytscha). Molecular Ecology 16(23):4930-4941.

O'Malley, K. G., M. J. Ford and J. J. Hard. 2010. Clock polymorphism in Pacific salmon: evidence for variable selection along a latitudinal gradient. Proceedings of the Royal Society B-Biological Sciences 277(1701):3703-3714.

Pella, J. J., M. Masuda and S. Nelson. 1996. Search algorithms for computing stock composition of a mixture from traits of indiciduals by maximum likelihood. NMFS-AFSC-61. U. D. o. Commerce.

R Development Core Team. 2012. R: A language and environment for statistical computing. http://www.R-project.org/

Ranalla, B. and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Science 94:9197-9201.

Scholz, F. 2011. adk: Anderson-darling K-sample test and combinations of such tests. http://CRAN.R-project.org/package=adk

Smouse, P. E., R. S. Waples and J. A. Tworek. 1990. A genetic mixture analysis for use with incomplete source population data. Canadian Journal of Fisheries and Aquatic Sciences 47:620-634.

Williams, J. G. 2006. Central Valley salmon: a perspective on Chinook and steelhead in the Central Valley of California. San Francisco Estuary and Watershed Science 4(3):Article 2.

Yoshiyama, R. M., E. R. Gerstung, F. W. Fisher and P. B. Moyle. 2001. Historical and present distribution of Chinook salmon in the Central Valley drainage of California. Pages 71-176 *in* Contributions to the biology of Central Valley salmonids, Volume 1 of 2. R. L. Brown, Department of Water Resources, editor. California Department of Fish and Game, Fish Bulletin 179. Sacramento, CA.

Appendix A

Please find Volume 24, Number 3 of the IEP Newsletter, Summer of 2011 issue here. On page 23 of this issue is the "Using Harvest Rate and Harvest to Estimate White Sturgeon Abundance" article, which is Appendix A.

Appendix B

Salvage to SALMGEN fork length distribution comparison.

Table B-1. Sample sizes (N) of genetically tested (DNA) and salvaged fish, and results from Kolmogorov-Smirnov (KS) and Anderson-Darling K-sample (ADK) equality of distribution tests comparing fork lengths of genetically tested and salvaged juveniles. Tests were performed only for months where there were five or greater genetic samples. Months in which the fork lengths of genetically tested fish were not a representative sample of salvaged fish fork length distributions are in bold. Shading denotes migration years.

		N				*P-value*			
		CVP		*SWP*		*KS*		*ADK*	
Month	***Year***	***DNA***	***Salvage***	***DNA***	***Salvage***	***CVP***	***SWP***	***CVP***	***SWP***
9	1996								
10	1996								
11	1996			9	8		1.000		0.727
12	1996			8	8		1.000		0.749
1	1997								
2	1997								
3	1997	143	1347	74	588	**< 0.001**	0.224	**< 0.001**	0.265
4	1997	244	1527	204	1135	**0.007**	0.092	**0.008**	0.152
5	1997	141	791	89	440	0.140	0.698	0.245	0.603
6	1997	54	272	16	60	0.824	0.569	0.584	0.262
7	1997								
8	1997								
9	1997								
10	1997								
11	1997								
12	1997	12	17	28	28	0.968	1.000	0.609	0.723
1	1998	312	3677			**< 0.001**		**< 0.001**	
2	1998	152	3113			**< 0.001**		**< 0.001**	
3	1998	212	921			**< 0.001**		**< 0.001**	

Table B-1, continued

		N				*P-value*			
		CVP		*SWP*		*KS*		*ADK*	
Month	*Year*	*DNA*	*Salvage*	*DNA*	*Salvage*	*CVP*	*SWP*	*CVP*	*SWP*
4	1998	315	944			**< 0.001**		**< 0.001**	
5	1998	25	3146			**< 0.001**		**< 0.001**	
6	1998	10	1048			0.318		0.143	
7	1998								
8	1998								
9	1998								
10	1998								
11	1998								
12	1998								
1	1999	16	180			0.202		0.107	
2	1999	66	3180	26	187	**< 0.001**	0.596	**< 0.001**	0.421
3	1999	282	1020	139	250	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
4	1999	292	2789	167	3382	0.335	**< 0.001**	0.430	**< 0.001**
5	1999	310	3067	246	2770	0.353	0.278	0.402	0.344
6	1999	144	982	14	72	0.110	0.701	0.184	0.478
7	1999								
8	1999								
9	1999			9	9		1.000		0.745
10	1999								
11	1999	8	8			1.000		0.749	
12	1999								
1	2000	59	67	11	67	1.000	**0.047**	0.709	**0.037**
2	2000	69	2257	29	1202	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
3	2000	79	608	189	648	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
4	2000	300	2496	273	3946	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
5	2000	74	690	70	1237	**0.014**	**0.012**	**0.012**	**0.037**
6	2000	9	145			0.298		0.116	

Table B-1, continued

		N				*P-value*			
		CVP		*SWP*		*KS*		*ADK*	
Month	*Year*	*DNA*	*Salvage*	*DNA*	*Salvage*	*CVP*	*SWP*	*CVP*	*SWP*
7	2000								
8	2000								
9	2000	11	14	45	55	0.998	0.999	0.571	0.657
10	2000			18	22		1.000		0.660
11	2000			5	5		1.000		0.771
12	2000	1	12	16	24	1.000	0.998	0.726	0.662
1	2001	9	11	14	18	1.000	1.000	0.685	0.696
2	2001	87	91	163	189	1.000	0.688	0.712	0.407
3	2001	119	430	649	966	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
4	2001	248	1816	230	2070	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
5	2001								
6	2001								
7	2001								
8	2001								
9	2001								
10	2001								
11	2001								
12	2001	9	16	43	45	0.627	1.000	0.221	0.702
1	2002	6	29	11	15	**0.007**	1.000	**0.004**	0.646
2	2002			8	9		1.000		0.735
3	2002	112	177	61	71	0.672	1.000	0.364	0.682
4	2002	148	801	62	219	**< 0.001**	**0.007**	**< 0.001**	**0.005**
5	2002			5	225		**0.005**		**< 0.001**
6	2002	7	63			**< 0.001**		**< 0.001**	
7	2002								
8	2002								
9	2002								

Table B-1, continued

		N				*P-value*			
		CVP		*SWP*		*KS*		*ADK*	
Month	*Year*	*DNA*	*Salvage*	*DNA*	*Salvage*	*CVP*	*SWP*	*CVP*	*SWP*
10	2002								
11	2002								
12	2002	10	10	25	27	1.000	1.000	0.742	0.681
1	2003	16	18	47	54	1.000	1.000	0.712	0.639
2	2003	130	132	63	81	1.000	0.176	0.704	**0.044**
3	2003	179	293	185	659	0.172	**< 0.001**	0.295	**< 0.001**
4	2003	46	726	100	1345	0.203	**< 0.001**	0.143	**< 0.001**
5	2003								
6	2003								
7	2003								
8	2003								
9	2003								
10	2003								
11	2003	7	9			1.000		0.732	
12	2003	6	6			1.000		0.680	
1	2004	14	14	16	18	1.000	1.000	0.733	0.701
2	2004	63	67	90	91	1.000	1.000	0.704	0.575
3	2004	830	1310	722	751	**0.002**	1.000	**< 0.001**	0.687
4	2004	200	243	390	421	0.979	1.000	0.627	0.680
5	2004	90	157	97	128	0.973	0.997	0.619	0.670
6	2004								
7	2004								
8	2004								
9	2004								
10	2004								
11	2004								
12	2004								

Table B-1, continued

		N				*P-value*			
		CVP		*SWP*		*KS*		*ADK*	
Month	*Year*	*DNA*	*Salvage*	*DNA*	*Salvage*	*CVP*	*SWP*	*CVP*	*SWP*
1	2005	6	23			0.948		0.569	
2	2005	12	209			**0.031**		**0.040**	
3	2005								
4	2005			5	704		0.993		0.630
5	2005								
6	2005			240	330		0.999		0.636
7	2005								
8	2005								
9	2005								
10	2005								
11	2005								
12	2005	6	8	9	9	0.983	1.000	0.598	0.745
1	2006	70	87	9	43	1.000	**< 0.001**	0.680	**0.001**
2	2006	34	36	31	31	1.000	1.000	0.660	0.722
3	2006	58	59	106	110	1.000	1.000	0.703	0.668
4	2006	42	56	391	388	0.848	1.000	0.506	0.712
5	2006	462	504	71	71	1.000	1.000	0.689	0.716
6	2006	1029	1152	596	595	0.998	1.000	0.664	0.712
7	2006	55	55	11	11	1.000	1.000	0.718	0.739
8	2006								
9	2006								
10	2006								
11	2006								
12	2006	6	6			1.000		0.761	
1	2007	13	13			1.000		0.735	
2	2007	54	56	27	35	1.000	1.000	0.689	0.692
3	2007	129	130	96	99	1.000	1.000	0.713	0.692

Table B-1, continued

		N				*P-value*			
		CVP		*SWP*		*KS*		*ADK*	
Month	*Year*	*DNA*	*Salvage*	*DNA*	*Salvage*	*CVP*	*SWP*	*CVP*	*SWP*
4	2007	260	272	333	335	1.000	1.000	0.695	0.711
5	2007	39	39	76	83	1.000	1.000	0.720	0.665
6	2007	30	31			1.000		0.720	
7	2007								
8	2007								
9	2007								
10	2007								
11	2007								
12	2007								
1	2008	14	14	12	12	1.000	1.000	0.733	0.737
2	2008	58	60	31	31	1.000	1.000	0.712	0.722
3	2008	113	117	46	46	1.000	1.000	0.713	0.719
4	2008	529	564	463	470	1.000	1.000	0.705	0.710
5	2008	851	915	691	709	1.000	1.000	0.705	0.709
6	2008	29	30	46	48	1.000	1.000	0.714	0.715
7	2008								
8	2008								
9	2008								
10	2008								
11	2008								
12	2008								
1	2009								
2	2009	9	10	5	5	1.000	1.000	0.701	0.732
3	2009	151	158	77	80	1.000	1.000	0.698	0.713
4	2009	579	629	335	340	1.000	1.000	0.671	0.711
5	2009	354	378	214	244	1.000	0.895	0.707	0.548
6	2009	7	8			1.000		0.743	

Table B-1, continued

		N				*P-value*			
		CVP		*SWP*		*KS*		*ADK*	
Month	*Year*	*DNA*	*Salvage*	*DNA*	*Salvage*	*CVP*	*SWP*	*CVP*	*SWP*
7	2009								
8	2009								
9	2009								
10	2009								
11	2009								
12	2009								
1	2010	9	9	11	11	1.000	1.000	0.745	0.739
2	2010	79	81	19	19	1.000	1.000	0.711	0.728
3	2010	169	173	80	80	1.000	1.000	0.703	0.709
4	2010	444	464	89	89	1.000	1.000	0.698	0.715
5	2010	646	836	198	228	0.528	1.000	0.339	0.654
6	2010	48	97	45	60	0.533	1.000	0.214	0.690
7	2010								
8	2010								

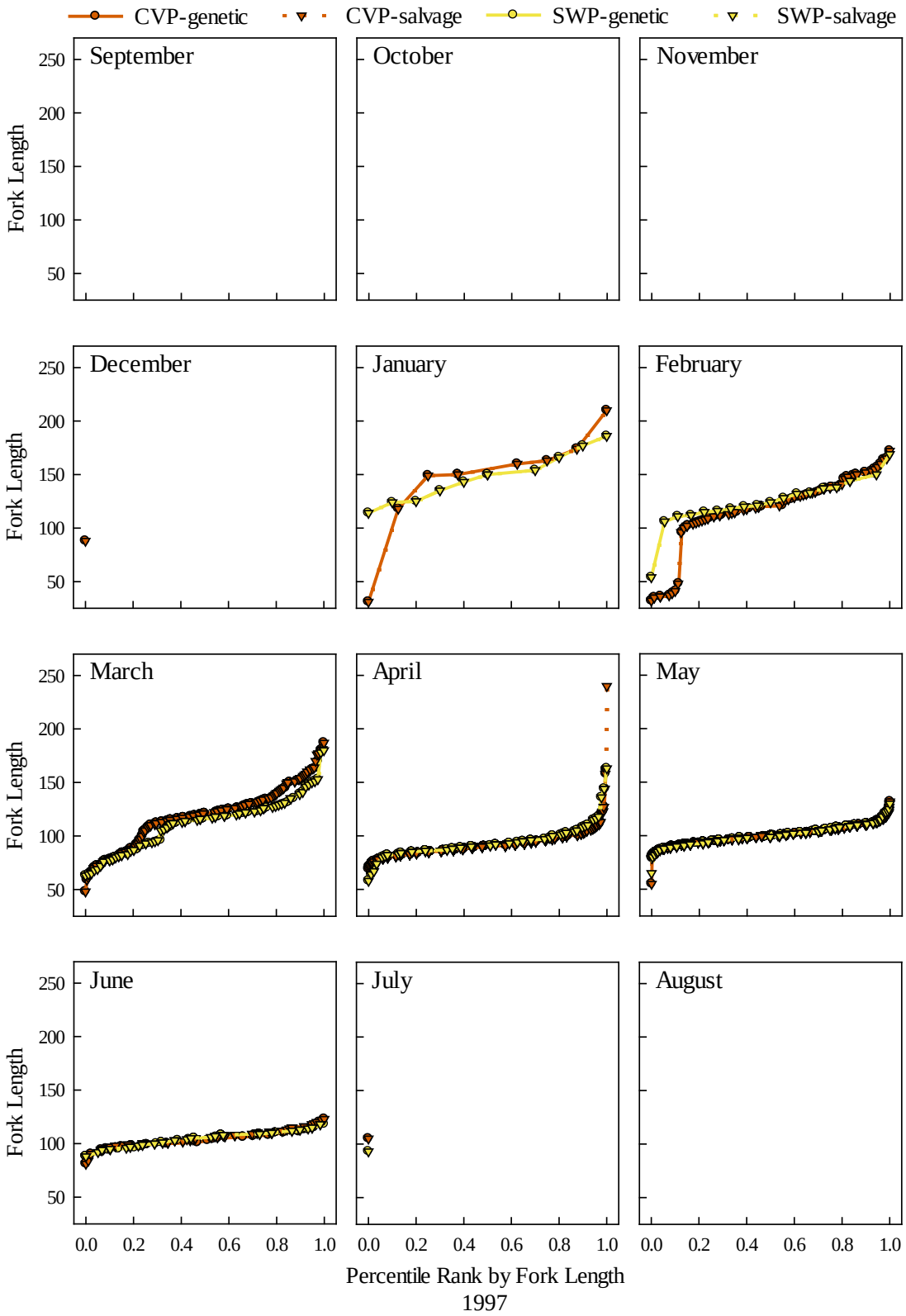

Figure B-1a. Fork length distributions of genetically tested (circle) and salvaged (triangle) Chinook Salmon from CVP (orange) and SWP (yellow) facilities, Sept 1, 1996 to Aug 31, 1997

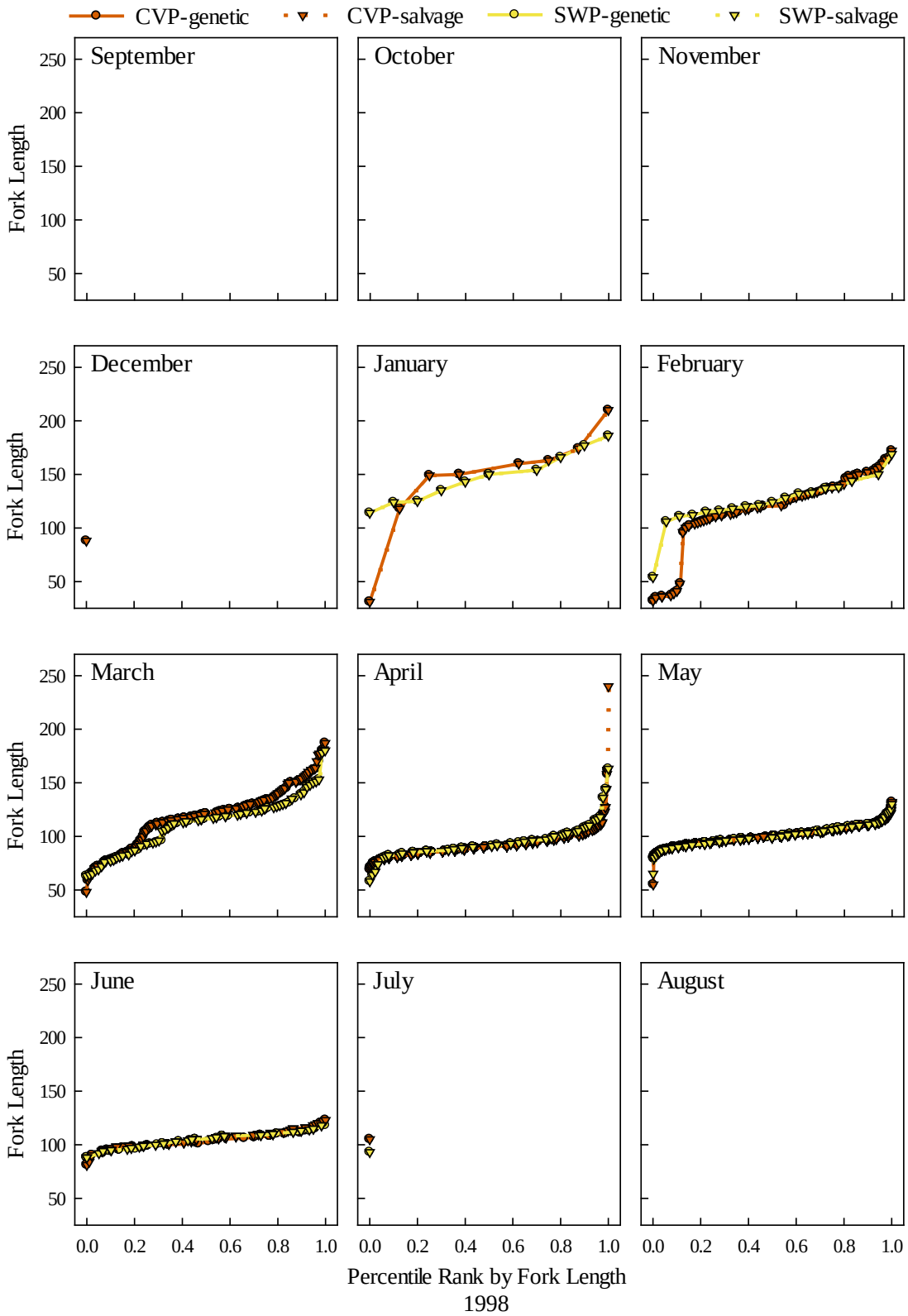

Figure B-1b Fork length distributions of genetically tested (circle) and salvaged (triangle) Chinook Salmon from CVP (orange) and SWP (yellow) facilities, Sept 1, 1997 to Aug 31, 1998

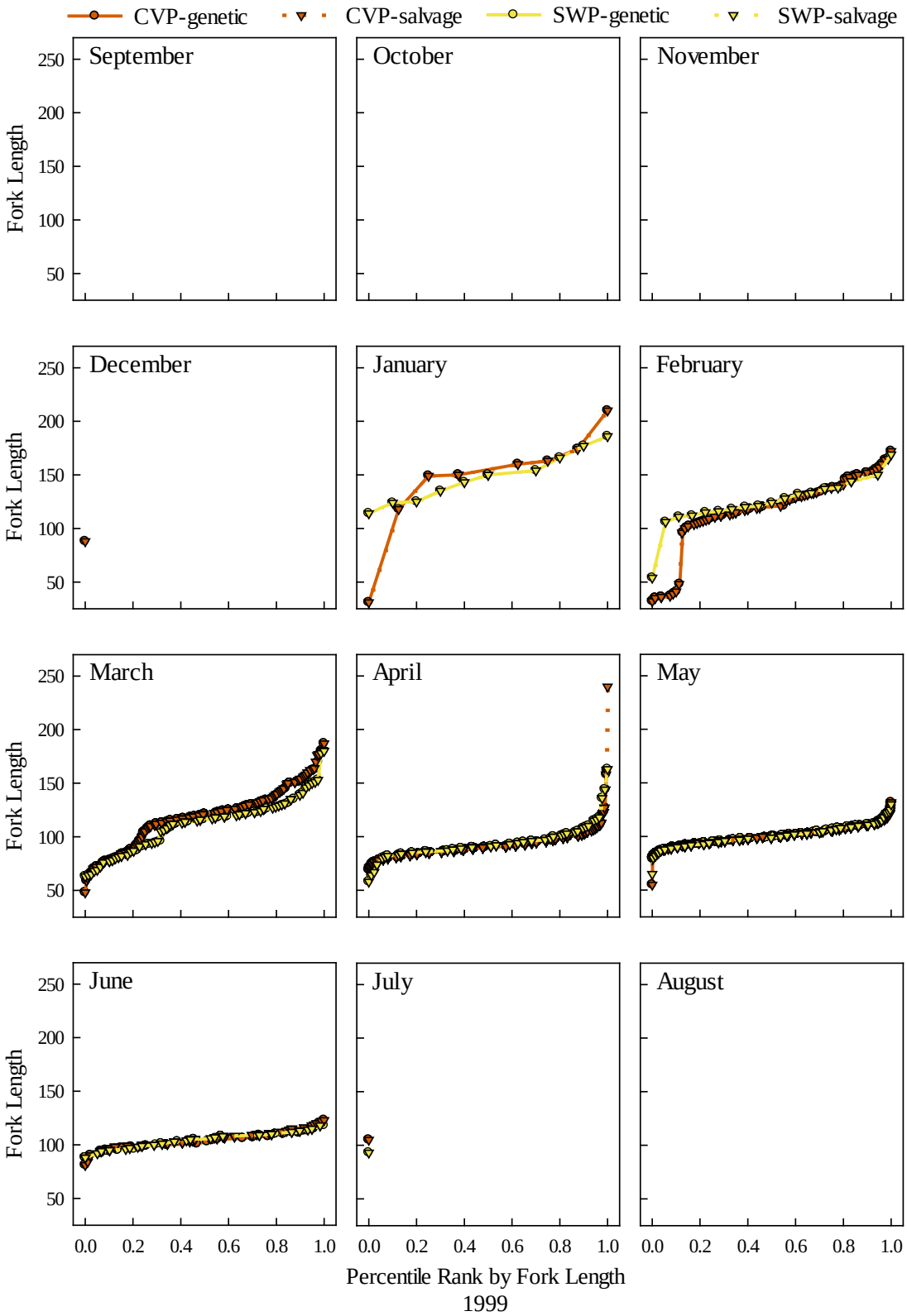

Figure B-1c Fork length distributions of genetically tested (circle) and salvaged (triangle) Chinook Salmon from CVP (orange) and SWP (yellow) facilities, Sept 1, 1998 to Aug 31, 1999

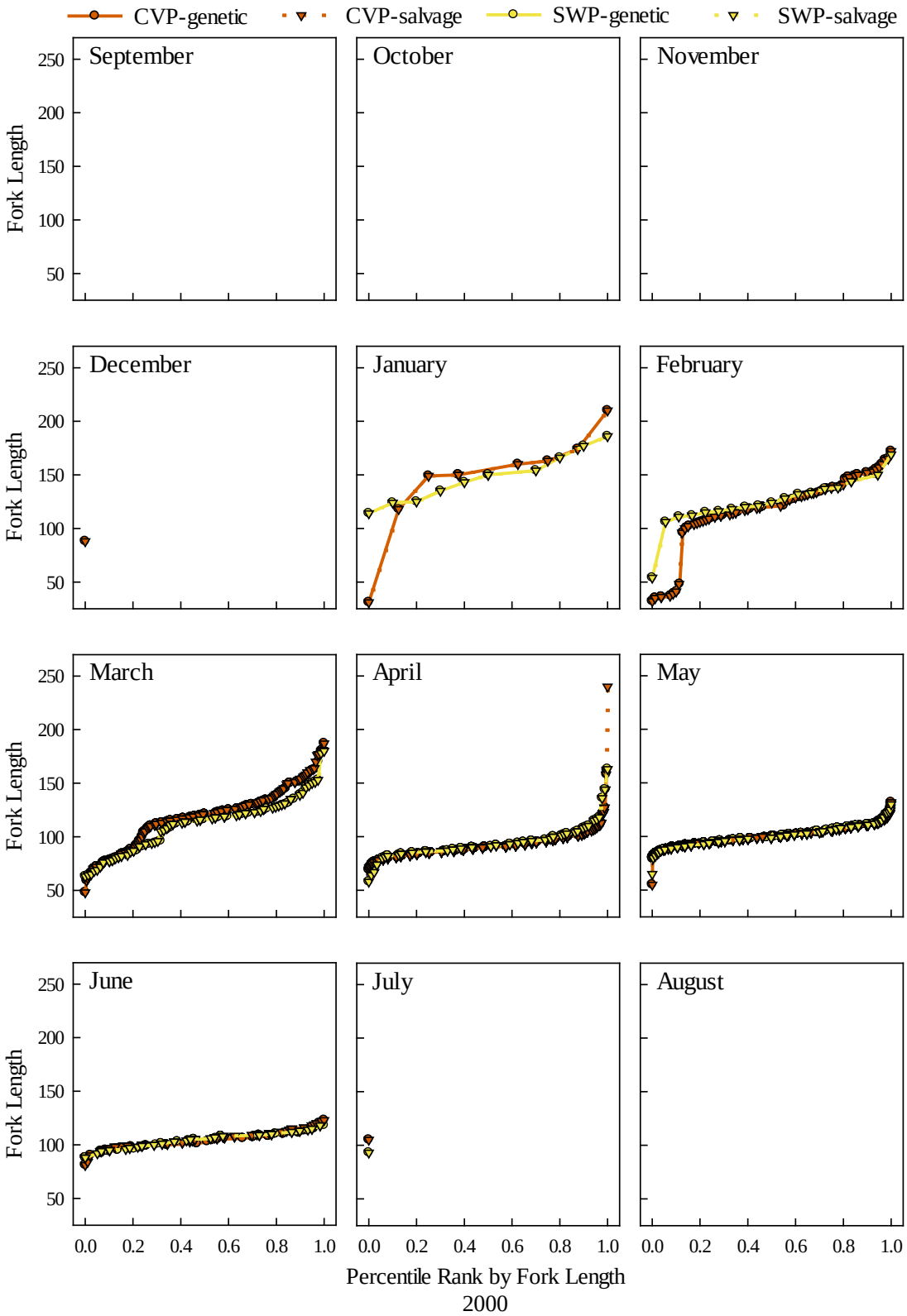

Figure B-1d Fork length distributions of genetically tested (circle) and salvaged (triangle) Chinook Salmon from CVP (orange) and SWP (yellow) facilities, Sept 1, 1999 to Aug 31, 2000

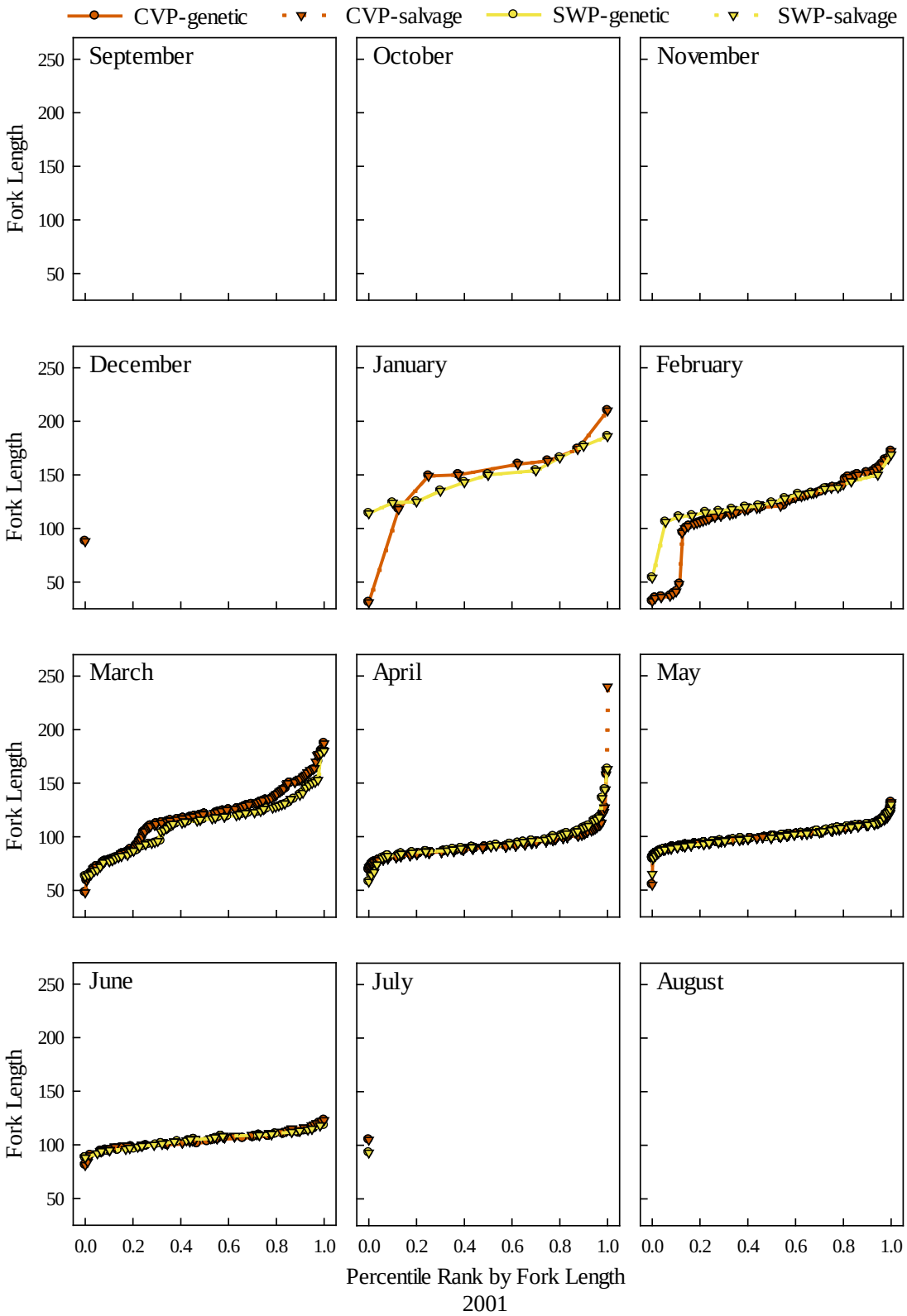

Figure B-1e Fork length distributions of genetically tested (circle) and salvaged (triangle) Chinook Salmon from CVP (orange) and SWP (yellow) facilities, Sept 1, 2000 to Aug 31, 2001

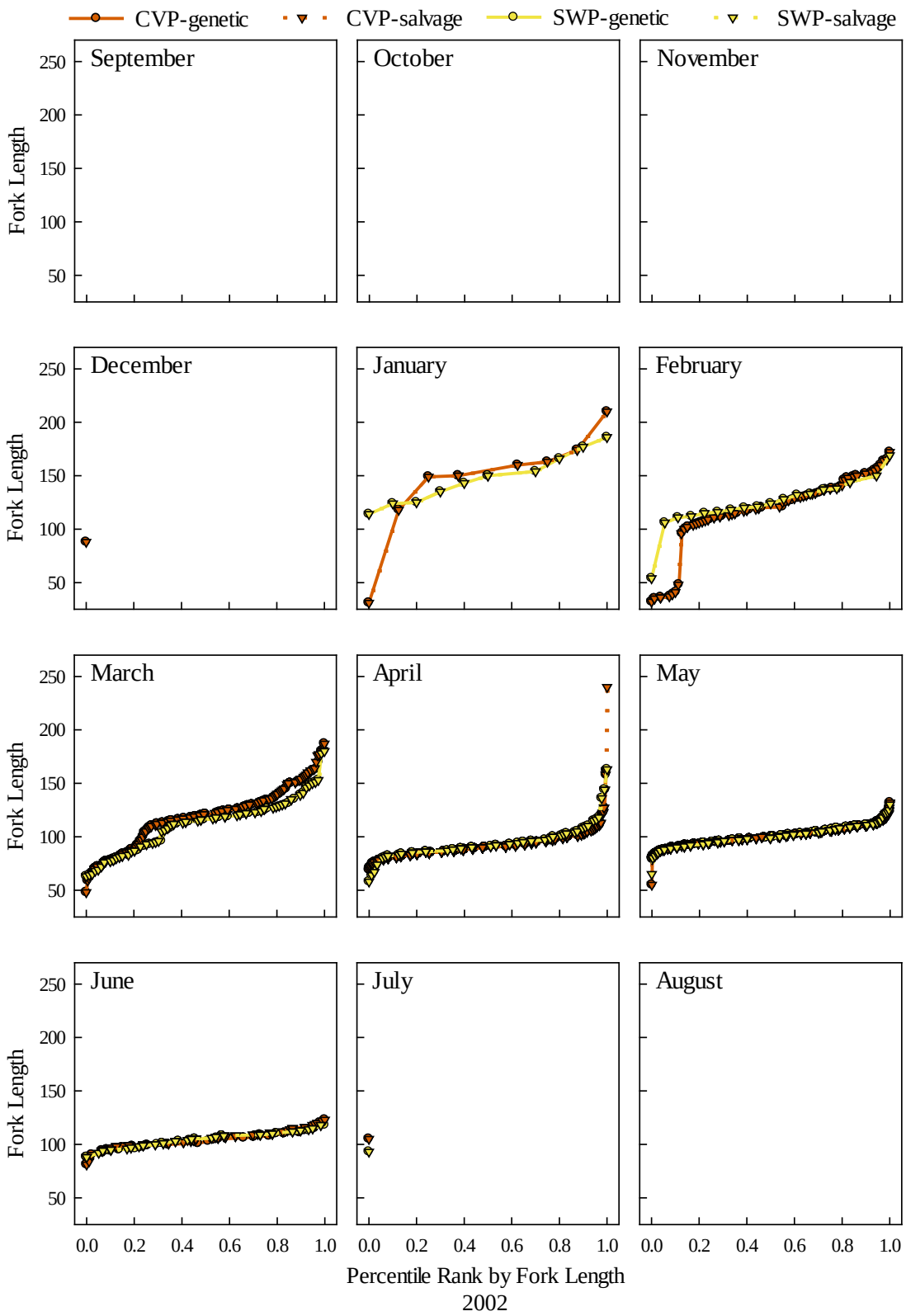

Figure B-1f Fork length distributions of genetically tested (circle) and salvaged (triangle) Chinook Salmon from CVP (orange) and SWP (yellow) facilities, Sept 1, 2001 to Aug 31, 2002

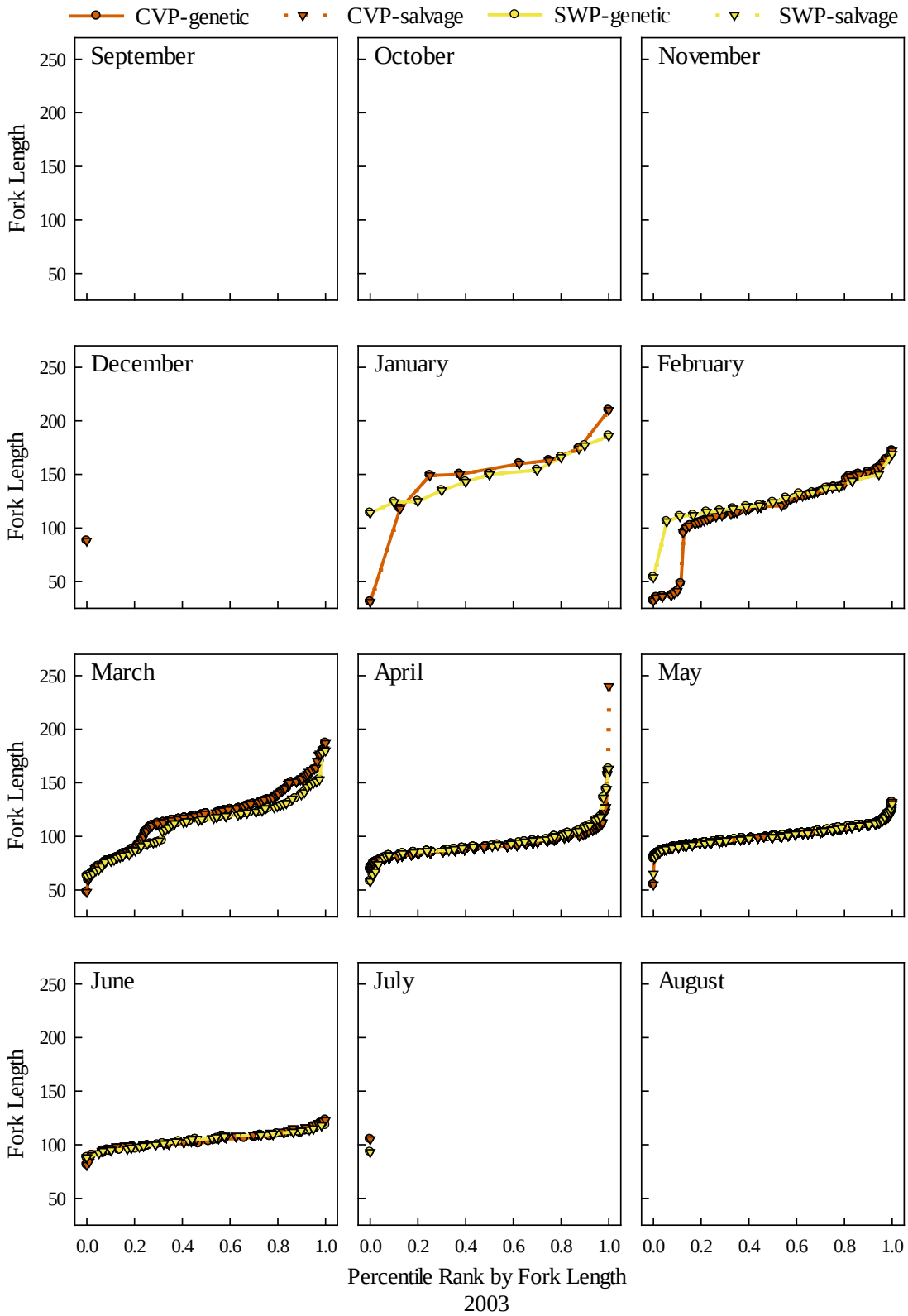

Figure B-1g Fork length distributions of genetically tested (circle) and salvaged (triangle) Chinook Salmon from CVP (orange) and SWP (yellow) facilities, Sept 1, 2002 to Aug 31, 2003

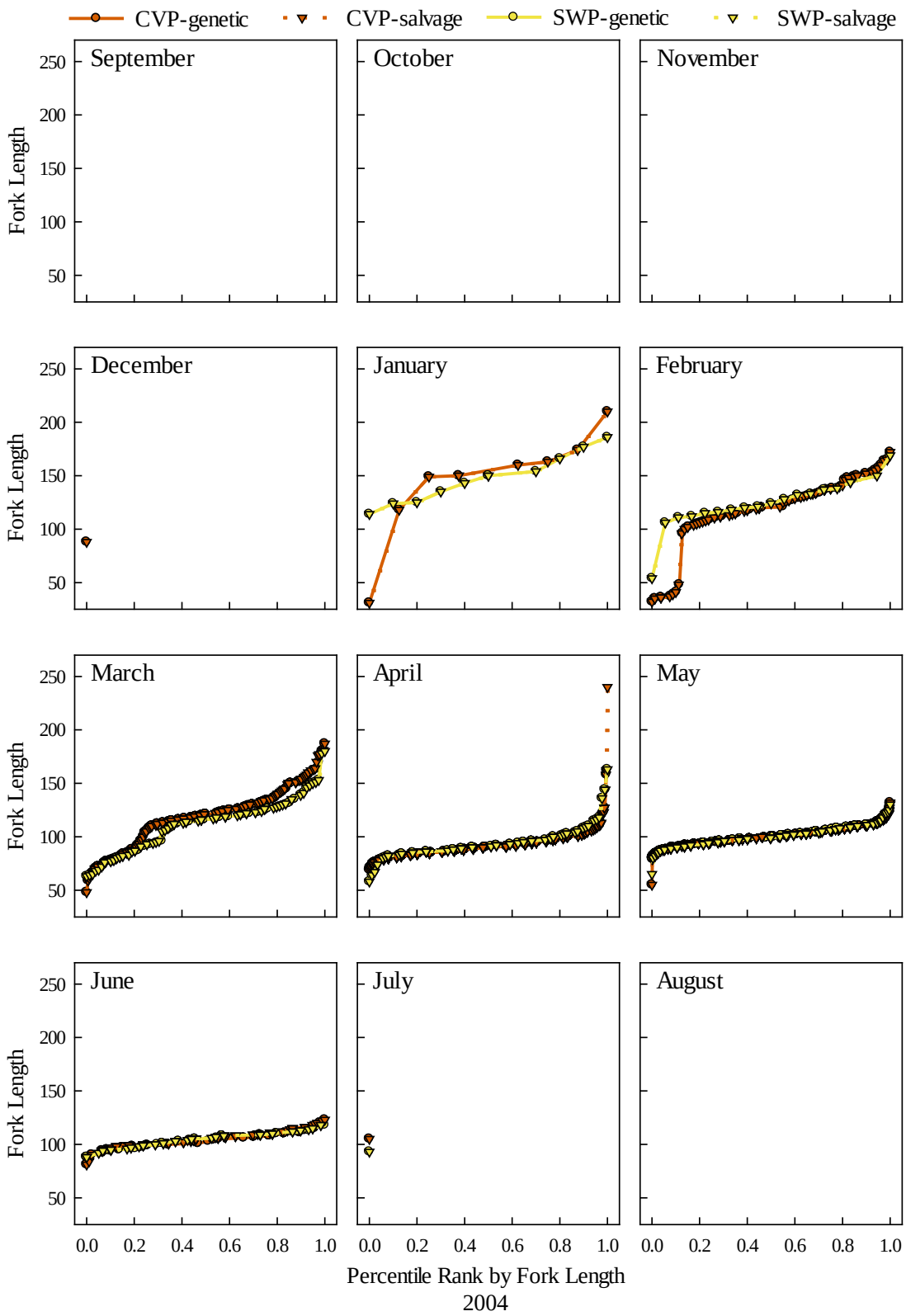

Figure B-1h Fork length distributions of genetically tested (circle) and salvaged (triangle) Chinook Salmon from CVP (orange) and SWP (yellow) facilities, Sept 1, 2003 to Aug 31, 2004

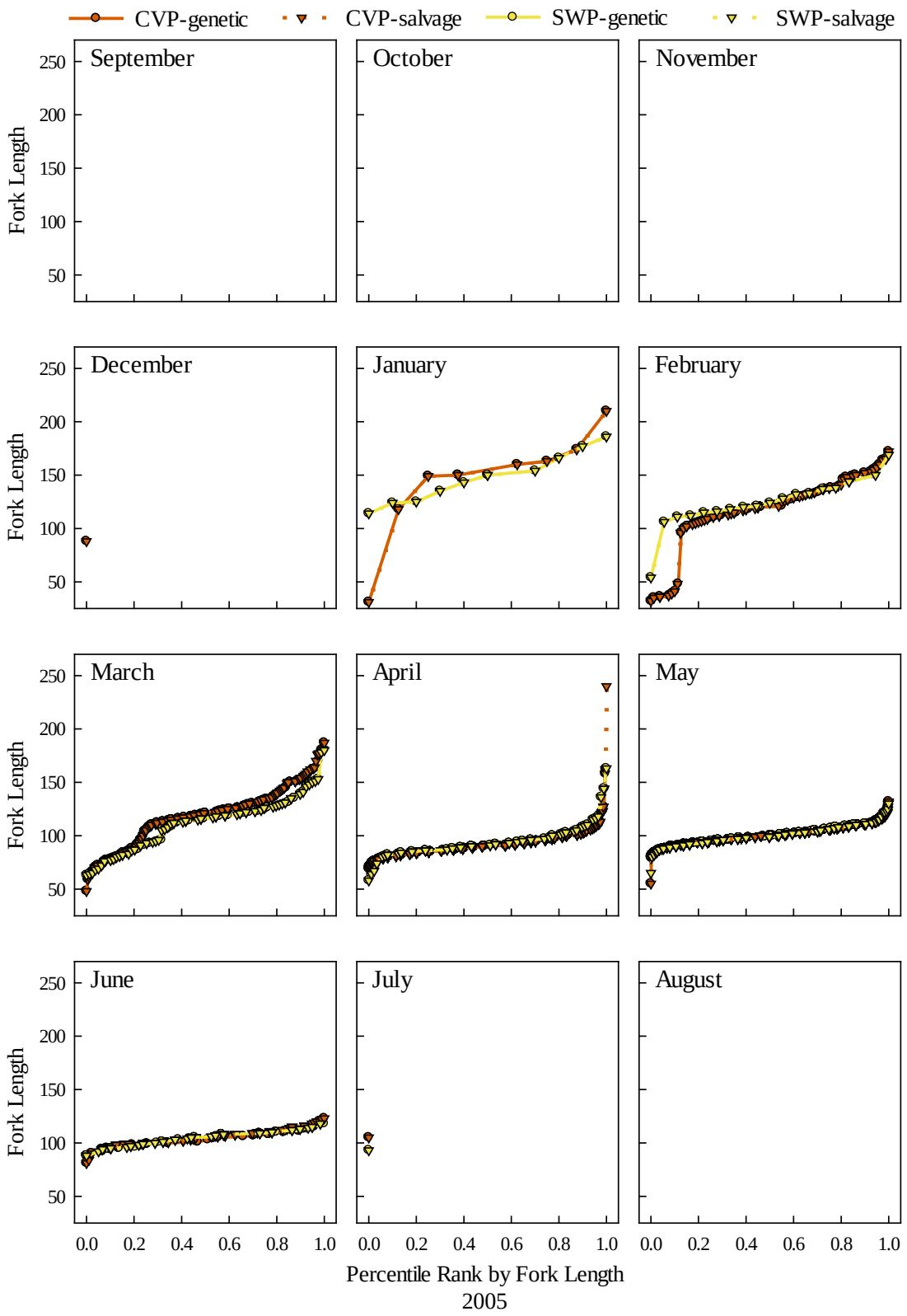

Figure B-1i Fork length distributions of genetically tested (circle) and salvaged (triangle) Chinook Salmon from CVP (orange) and SWP (yellow) facilities, Sept 1, 2004 to Aug 31, 2005

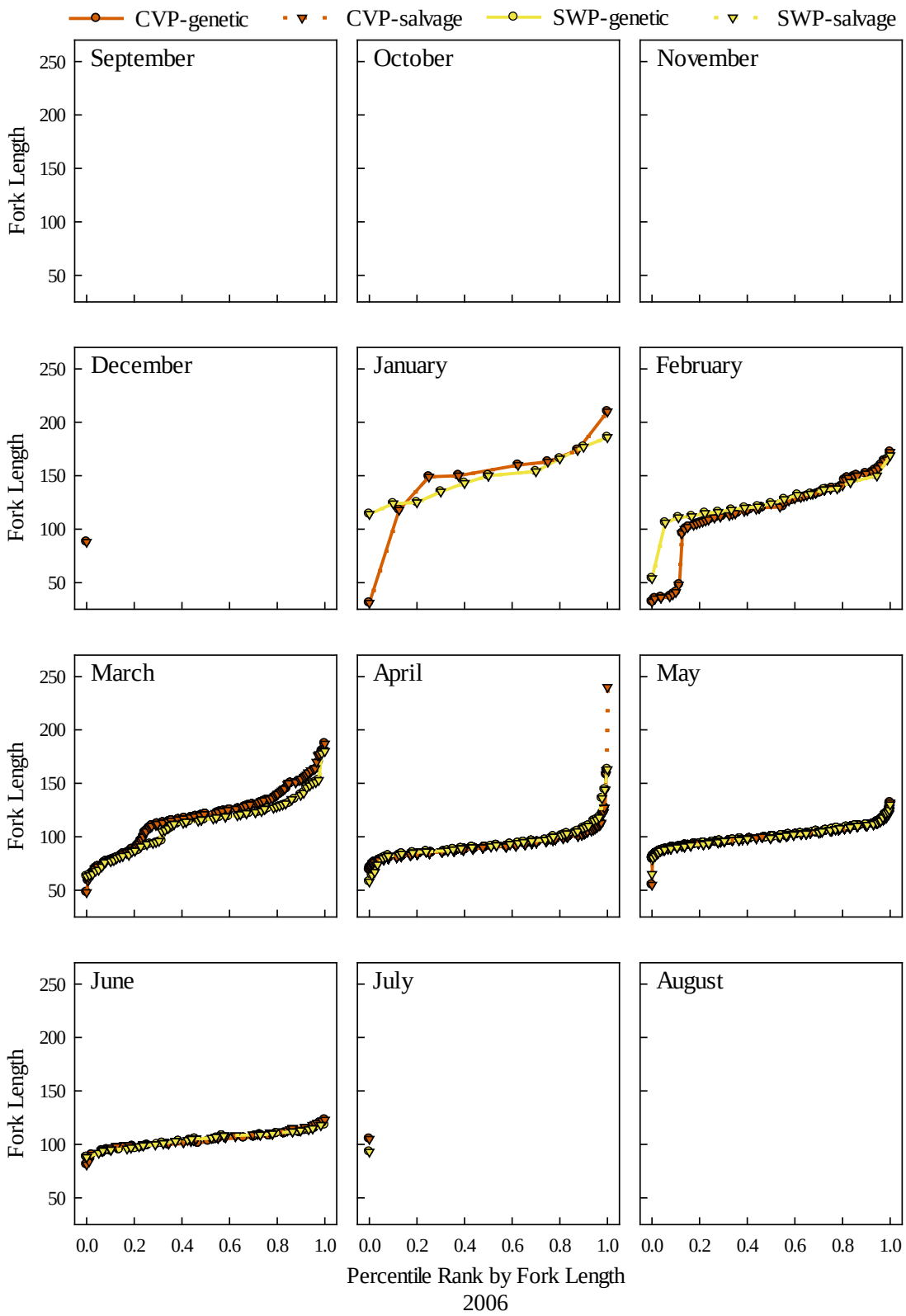

Figure B-1j Fork length distributions of genetically tested (circle) and salvaged (triangle) Chinook Salmon from CVP (orange) and SWP (yellow) facilities, Sept 1, 2005 to Aug 31, 2006

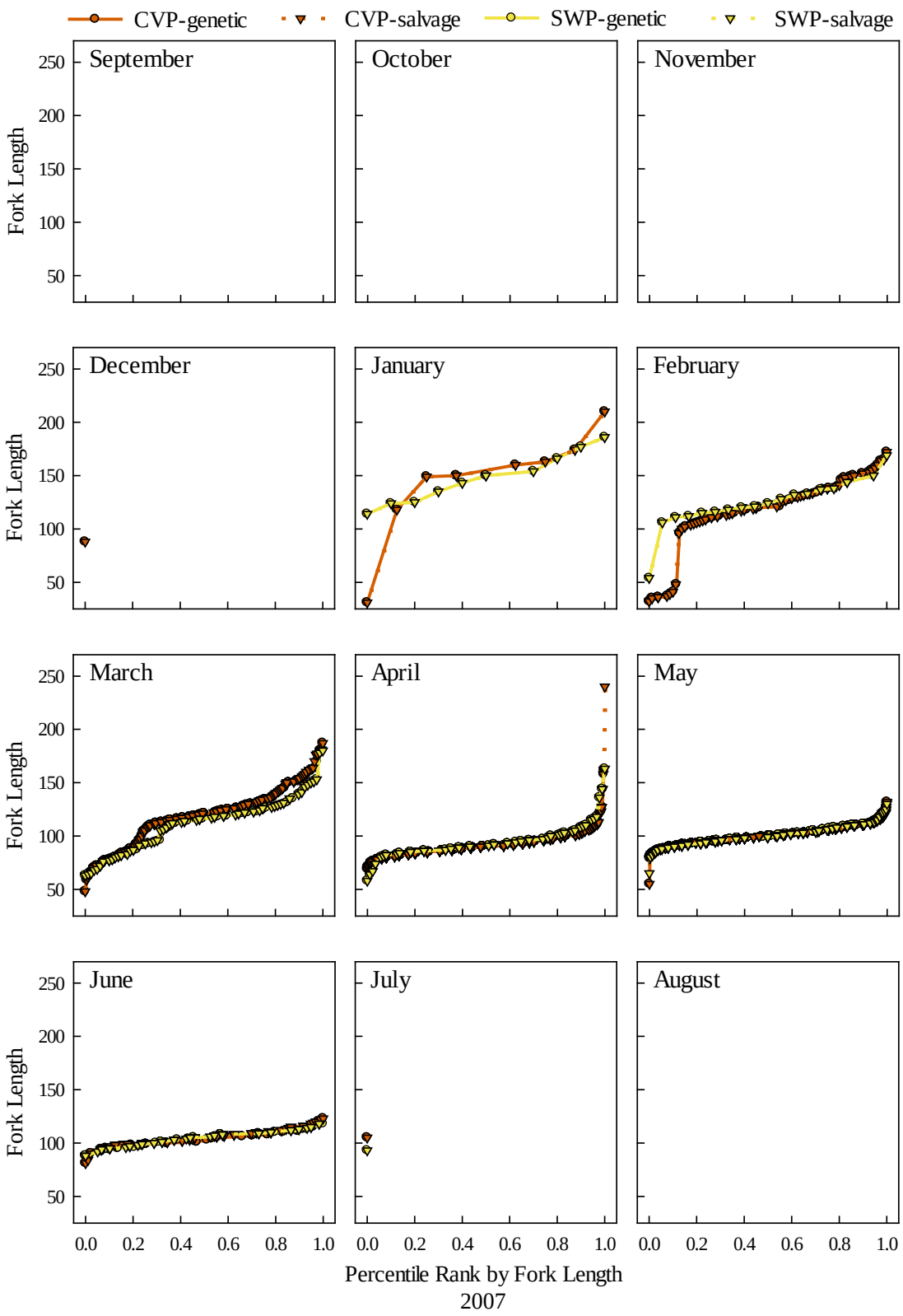

Figure B-1k Fork length distributions of genetically tested (circle) and salvaged (triangle) Chinook Salmon from CVP (orange) and SWP (yellow) facilities, Sept 1, 2006 to Aug 31, 2007

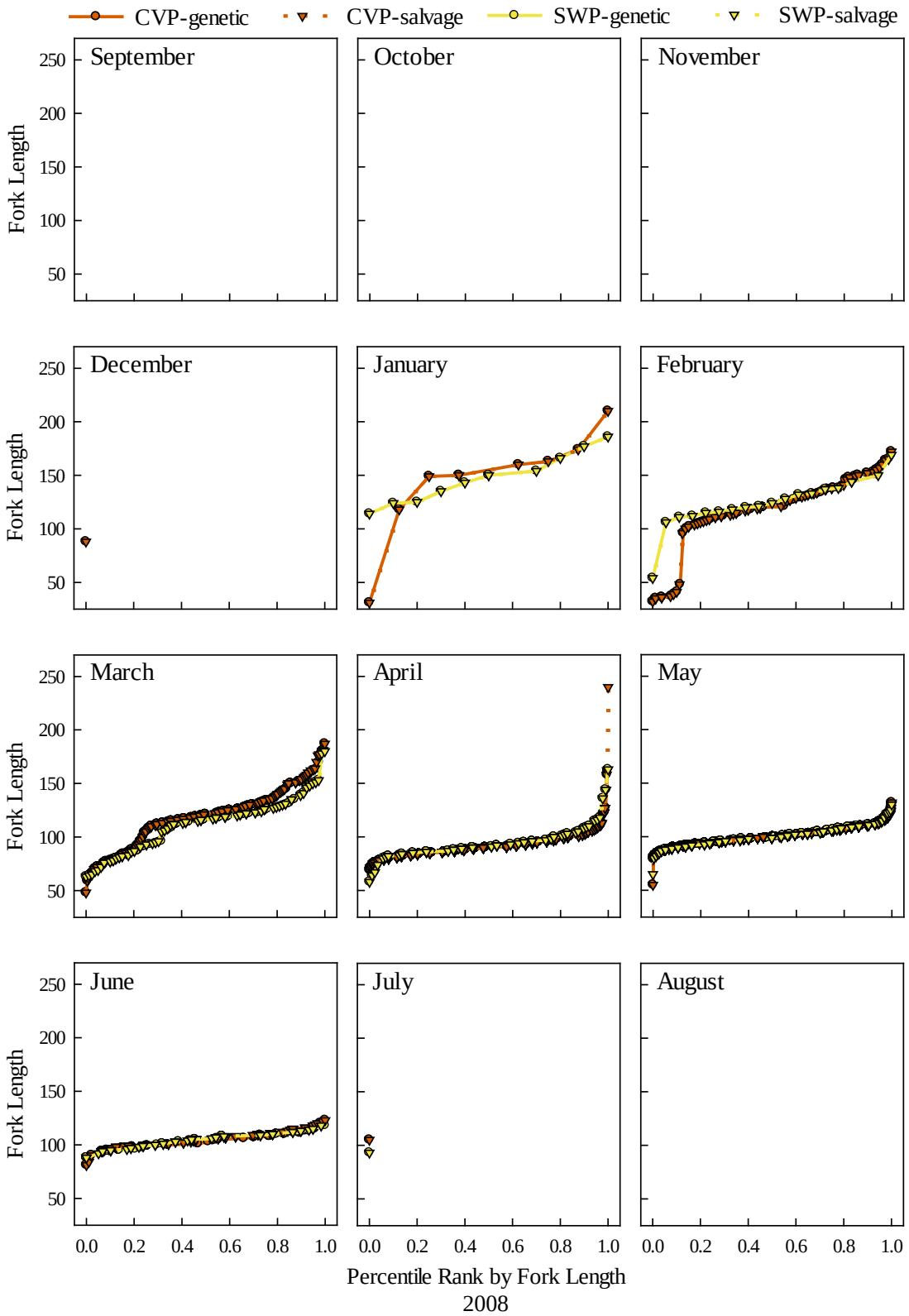

Figure B-1l Fork length distributions of genetically tested (circle) and salvaged (triangle) Chinook Salmon from CVP (orange) and SWP (yellow) facilities, Sept 1, 2007 to Aug 31, 2008

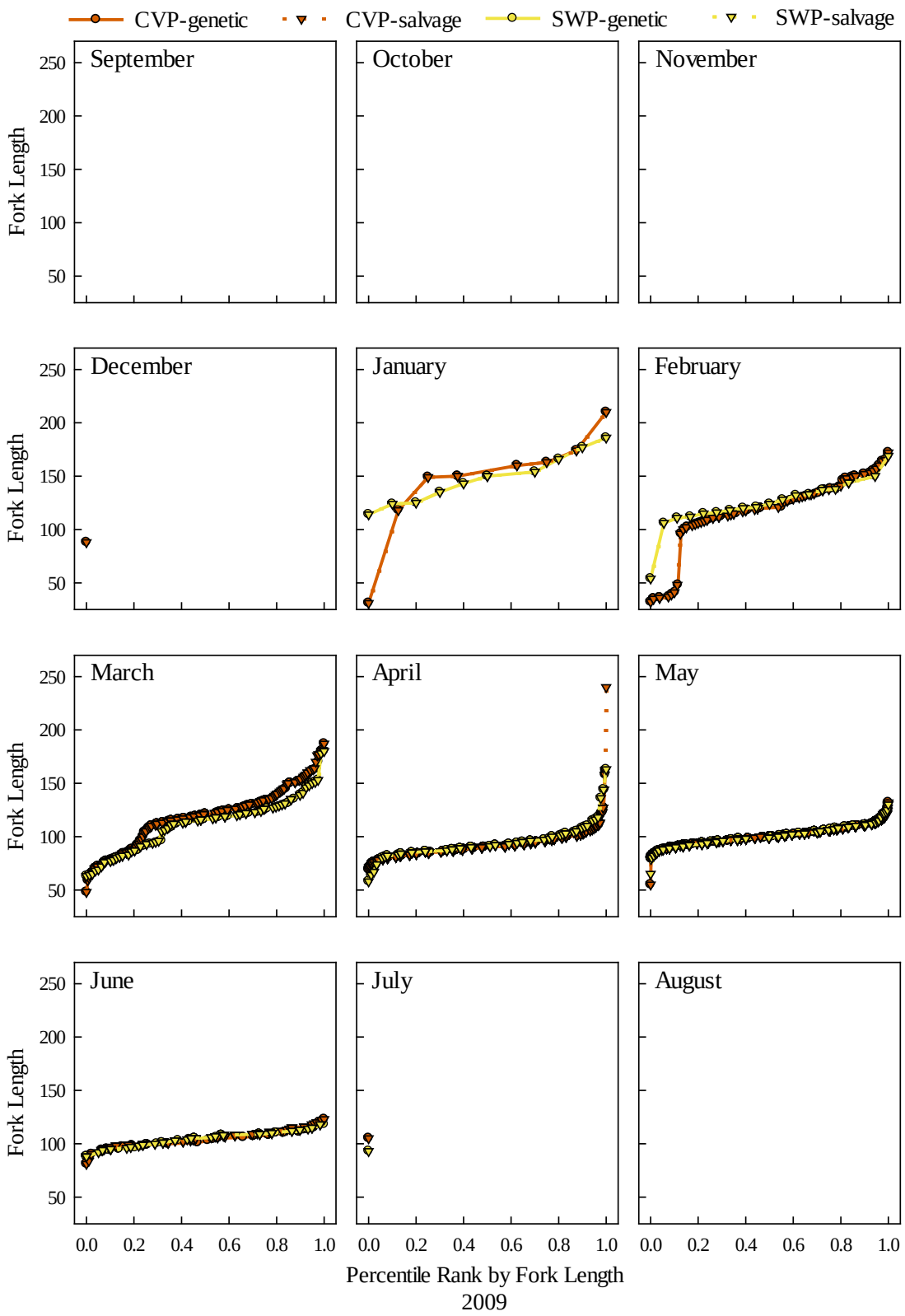

Figure B-1m Fork length distributions of genetically tested (circle) and salvaged (triangle) Chinook Salmon from CVP (orange) and SWP (yellow) facilities, Sept 1, 2008 to Aug 31, 2009

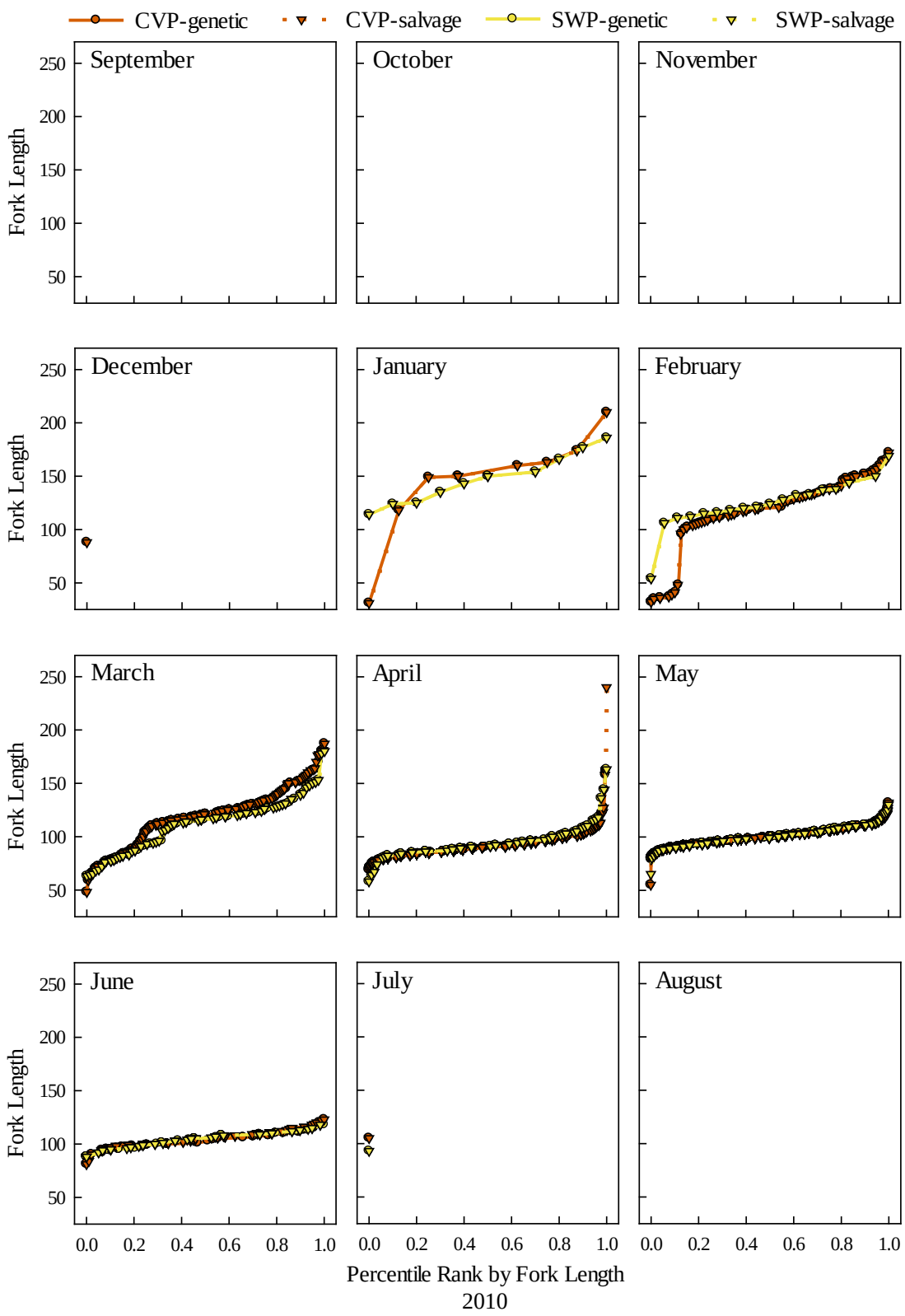

Figure B-1n Fork length distributions of genetically tested (circle) and salvaged (triangle) Chinook Salmon from CVP (orange) and SWP (yellow) facilities, Sept 1, 2009 to Aug 31, 2010

Appendix C. Regression results of ln (fork length) against time in days for migration years 2004 and 2006-2010

Table C-1 Early migrating older winter run

Year	*slope*	*P*	*intercept*	R^2	*N*
2004	-0.00137	0.035	3364.190	0.0323	137
2006	0.00213	0.008	-5216.367	0.0955	73
2007	0.0000152	0.982	-32.457	0.00000275	189
2008	-0.000798	0.058	1962.625	0.0286	126
2009	-0.00285	0.007	7003.029	0.0620	117
2010	0.00182	< 0.001	-4468.220	0.107	167

Table C-2 Spring run regression results

C-2a Early migrating older juveniles

Year	*slope*	*P*	*intercept*	R^2	*N*
2004	-0.00107	0.772	2631.907	0.0128	9
2006	–	–	–	–	2
2007	–	–	–	–	0
2008	-0.00436	0.166	10706.215	0.293	8
2009	0.00826	0.932	-20272.663	0.0114	3
2010	0.00644	0.511	-15795.863	0.482	3

C-2b Early migrating younger juveniles

Year	*slope*	*P*	*intercept*	R^2	*N*
2004	–	–	–	–	0
2006	–	–	–	–	1
2007	–	–	–	–	0
2008	–	–	–	–	1
2009	–	–	–	–	0
2010	–	–	–	–	0

C-2c Transition early-young to late-older juveniles

Year	*slope*	*P*	*intercept*	R^2	*N*
2004	0.0235	< 0.001	-57571.293	0.658	37
2006	0.0150	0.006	-36918.329	1.000	3
2007	–	–	–	–	0
2008	0.0117	0.068	-28709.261	0.724	5
2009	–	–	–	–	1
2010	–	–	–	–	0

C-2d Late migrating older juveniles

Year	*slope*	*P*	*intercept*	R^2	*N*
2004	0.0996	0.546	-234593.064	0.429	3
2006	-0.000760	0.448	1870.404	0.587	12
2007	0.00168	0.455	-4112.923	0.0716	10
2008	-0.00219	0.160	5379.849	0.119	18
2009	-0.000575	0.535	1416.190	0.0186	23
2010	0.00482	0.003	-11826.457	0.527	14

Table C-3 Fall run regression results

C-3a Early migrating older juveniles

Year	*slope*	*P*	*intercept*	R^2	*N*
2004	0.000756	0.348	-1848.956	0.0294	32
2006	0.00134	0.117	-3279.924	0.0885	29
2007	0.00137	0.011	-3367.445	0.0788	81
2008	0.0000208	0.974	-46.002	0.0000176	65
2009	-0.00288	0.066	7075.590	0.298	12
2010	0.000700	0.308	-1713.857	0.0168	64

C-3b Early migrating younger juveniles

Year	*slope*	*P*	*intercept*	R^2	*N*
2004	0.00154	0.219	-3782.093	.0169	91
2006	0.00854	< 0.001	-20948.430	0.586	79
2007	–	–	–	–	2
2008	0.00250	0.388	-6127.532	0.0394	21
2009	–	–	–	–	0
2010	0.0173	0.017	-42403.147	0.485	11

C-3c Transition early-young to late-older juveniles

Year	*slope*	*P*	*intercept*	R^2	*N*
2004	0.0210	< 0.001	-51403.354	0.334	401
2006	0.0314	< 0.001	-77105.236	0.501	84
2007	0.0302	< 0.001	-74011.087	0.784	16
2008	0.0220	< 0.001	-53966.935	0.394	30
2009	0.0137	< 0.001	-33590.105	0.150	92
2010	0.0102	0.002	-24946.978	0.144	64

C-3d Late migrating older juveniles

Year	*slope*	*P*	*intercept*	R^2	*N*
2004	0.00747	0.012	-18313.519	0.137	45
2006	-0.000109	0.191	271.576	0.000669	2553
2007	0.000217	0.332	-527.117	0.00143	685
2008	-0.000749	< 0.001	1841.908	0.0144	2544
2009	0.00233	< 0.001	-5703.799	0.126	1382
2010	0.00309	< 0.001	-7577.452	0.300	1423

Table C-4 Late-fall run regression results

C-4a Early migrating older juveniles

Year	*slope*	*P*	*intercept*	R^2	*N*
2004	-0.000736	0.513	1809.790	0.0181	26
2006	-0.000627	0.273	1544.190	0.0261	48
2007	0.000999	0.117	-2446.631	0.0392	64
2008	-0.00201	0.241	4946.967	0.0905	17
2009	-0.000415	0.734	1022.638	0.00696	19
2010	-0.000650	0.428	1601.995	0.0103	63

C-4b Early migrating younger juveniles

Year	*slope*	*P*	*intercept*	R^2	*N*
2004	0.0210	0.007	-51623.118	0.128	55
2006	0.0153	0.003	-37510.040	0.906	6
2007	–	–	–	–	0
2008	–	–	–	–	1
2009	–	–	–	–	1
2010	–	–	–	–	1

C-4c Transition early-young to late-older juveniles

Year	*slope*	*P*	*intercept*	R^2	*N*
2004	0.0374	< 0.001	-91799.112	0.479	61
2006	–	–	–	–	2
2007	–	–	–	–	0
2008	–	–	–	–	1
2009	–	–	–	–	2
2010	–	–	–	–	1

C-4d Late migrating older juveniles

Year	*slope*	*P*	*intercept*	R^2	*N*
2004	–	–	–	–	2
2006	0.000206	0.767	-501.109	0.00228	41
2007	0.00722	0.377	-17725.424	0.158	7
2008	-0.00143	0.45	3512.101	0.179	23
2009	-0.00106	0.221	2605.266	0.0202	76
2010	0.00102	0.224	-2492.065	0.0767	21