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# SPRING-RUN CHINOOK SALMON GENETIC MANAGEMENT PLAN - San Joaquin River Restoration Program

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## **Selected Abbreviations and Acronyms**

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CDFG	California Department of Fish and Game
Conservation Facility	San Joaquin River Salmon Conservation Facility
CV	Central Valley
CVP	Central Valley Project
CWT	Coded Wire Tag
Delta	Sacramento-San Joaquin Delta
DWR	California Department of Water Resources
EPA	U.S. Environmental Protection Agency
ESU	Evolutionarily Significant Unit
FESA	Federal Endangered Species Act
FMP	Fisheries Management Plan
FMWG	Fisheries Management Work Group
FR	Federal Register
FRH	Feather River Hatchery
GMP	Genetic Management Plan
GSI	Genetic Stock Identification
HGMP	Hatchery and Genetic Management Plan
HSRG	Hatchery Scientific Review Group
IUCN	International Union for Conservation of Nature
NMFS	National Marine Fisheries Service
NRDC	Natural Resources Defense Council
PBT	Parentage Based Tagging
PIT	Passive Integrated Transponder
Program	San Joaquin River Salmon Conservation and Research Program
Reclamation	U.S. Department of the Interior, Bureau of Reclamation
Restoration Area	San Joaquin River from Friant Dam to confluence with Merced River (~153 miles long)
Settlement	Stipulations of the Settlement Agreement
SJRRP	San Joaquin River Restoration Program
SWFSC	Southwest Fisheries Science Center
SWP	State Water Project
TAC	Technical Advisory Committee
UC Davis	University of California, Davis
USBR	U.S. Bureau of Reclamation

## SECTION 1 INTRODUCTION

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### 1.1 Background

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This Genetic Management Plan (GMP) provides guidance to the San Joaquin River Salmon Conservation and Research Program (Program) for the reintroduction of spring-run Chinook salmon to the San Joaquin River.

With the completion of Friant Dam in 1942, the upper San Joaquin River ran dry in several sections. It no longer supported consistent runs of spring-run Chinook salmon; the last observed run occurred in 1950 (Warner 1991). The Natural Resources Defense Council (NRDC) and other environmental groups undertook litigation in 1998, *NRDC et al. v. Kirk Rodgers et al.*, to restore water flows to the upper San Joaquin River, and the litigation was settled in 2006. The settlement agreement (Settlement) required restoration of flowing water to the system and began the San Joaquin River Restoration Program (SJRRP). The Settlement also established the goal:

*To restore and maintain fish populations in “good condition” in the mainstem San Joaquin River below Friant Dam to the confluence with the Merced River, including naturally reproducing and self-sustaining populations of salmon and other fish.*

Pursuant to this goal, the Settlement mandates the reintroduction of spring-run Chinook salmon beginning in 2012. The SJRRP is run by the agencies party to the Settlement, termed the implementing agencies. The Program differs from the SJRRP in that the Program is tasked with the on the ground process of reintroducing fish to the river, while the SJRRP is mandated by the settlement and oversees implementation of the entire Settlement, in an advisory role.

### 1.2 Reintroduction overview

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The implementing agencies, consisting of the Bureau of Reclamation (Reclamation), U.S. Fish and Wildlife Service (USFWS), National Marine Fisheries Service (NMFS), California Department of Fish and Game (CDFG), and California Department of Water Resources (DWR) organized a Program Management Team (PMT) and associated Work Groups to begin work implementing the Settlement. These groups have produced several documents providing support for the ESA section 10(a)(1)(A) permit application submitted by USFWS to NMFS on September 30, 2010.

- The Technical Advisory Committee (TAC) published Recommendations on Restoring Spring-run Chinook Salmon to the Upper San Joaquin River (Meade 2007). The TAC identified four periods to the reintroduction, referenced throughout this document, and the basis for these time periods is spelled out in the Recommendations document. The Reintroduction Period runs from January 1, 2012, to December 31, 2019; the Interim Period from January 1, 2020, to December 31, 2024; the Growth Population Period, from January 1, 2025, to December 31, 2040; and the Long-term Period, Beyond January 1, 2041. Based

on historic run size, and habitat capacity, the TAC developed a short-term goal of maintaining a minimum of 500 spawning fish and a long term goal of maintaining escapement at a minimum running average of 2,500 fish.

- The Fisheries Management Work Group (FMWG) published a Fisheries Management Plan: A Framework for Adaptive Management in the San Joaquin River Restoration Program (FMP, SJRRP 2010b) to describe the Program's approach to Restoration.
- The Genetics Subgroup, under the FMWG, produced the Stock Selection Strategy: Spring-run Chinook salmon document (Stock Selection Strategy; SJRRP 2010c) and the Reintroduction Strategy for Spring Run Chinook Salmon document (Reintroduction Strategy, SJRRP 2011). The general reintroduction strategy is described in the Reintroduction Strategy document, and a discussion of the strategies and sources to be used in the reintroduction can be found there.
- The California Department of Fish and Game contracted with UC Davis for the completion of the spring-run Chinook salmon Hatchery and Genetic Management Plan (HGMP; Bork and Adelizi 2010). The HGMP describes the details of hatchery management.

These documents and the underlying settlement agreement establish many of the baseline aspects of the reintroduction process. For example, the Settlement Agreement currently requires reintroduction of spring-run Chinook salmon to the San Joaquin by 2012, and the TAC and FMWG have adopted a timeline with population goals for the reintroduced population. Further, the Stock Selection Strategy constrains the choices for source populations that can be mined for reintroduction, and it has focused on the Feather River, Butte Creek, Deer Creek, and Mill Creek, although opportunistic collection may occur from other Central Valley tributaries (e.g., spring-run from Battle Creek, Clear Creek, Yuba River, potential strays into the San Joaquin basin, and fish salvaged from the Delta pumping facilities) (SJRRP 2011). Those documents should be reviewed in conjunction with this document in order to understand both the overall approach to reintroduction and the constraints placed on the decisions in this document. Figure 1 also provides a general timeline for the reintroduction. Nevertheless, the following executive summary from the HGMP provides an overview of hatchery operations that will assist the reader in following the GMP:

*In Fall 2010, a small-scale Interim Facility (Interim Facility) at the existing State operated San Joaquin Fish Hatchery began operation using fall-run Chinook salmon as a surrogate to provide the Program with practical experience captive rearing juvenile Chinook in the Conservation Facility on the same site. The interim facility will also allow the program to implement hatchery operations during the construction of the Conservation Facility, which is planned for completion in 2014. The Program Timeline in Figure 1 describes the roll-out of interim and full-scale facilities and their relationship to reintroduction strategies.*



*The CV spring-run Chinook salmon are listed as threatened under both the Federal Endangered Species Act (FESA) and the California Endangered Species Act (CESA). Spring-run hatchery production cannot commence until the appropriate permits have been issued. Collection of fish from this Evolutionarily Significant Unit (ESU) for broodstock will be governed by a FESA 10(a)1(A) enhancement of population permit. The reintroduced population will be designated an experimental population under FESA section 10(j), and associated 4(d) rules will be promulgated to allow for hatchery and monitoring operations. Preparation and review of the 10(a)1(A) federal permit and the 10(j) federal designation will be ongoing from 2010 to 2012. In keeping with the settlement agreement, the U.S. Fish and Wildlife Service (FWS) will submit a completed permit application to the National Marine Fisheries Service (NMFS) for the reintroduction of spring-run Chinook salmon as soon as practical, but no later than September 30, 2010. To facilitate reintroduction under the California Endangered Species Act (CESA), new State legislation (SB 1349) has been introduced to allow activities that may grant take of spring-run Chinook salmon to move forward without needing CESA coverage if the activities have obtained or have been provided take authorization by NOAA through an enhancement of survival permit or 4d regulation. This will effectively result in no State action for "take" for any activities that are covered under the federal authorizations.*

*Once approval to construct a new facility has been secured, construction of the full-scale Conservation Facility is scheduled to begin, ideally in 2011. However, delays in the State budget process and/or delays in allocation of funding may delay construction. In 2011, the permit to work with listed spring-run Chinook salmon will still be under review, and the Interim Facility will continue work with fall-run Chinook salmon.*

*Under the settlement agreement, NMFS will complete the review of the permit applications by April 30, 2012. If the applications are approved, broodstock collection from up to three main source populations (Feather River, Butte Creek, and the Deer/Mill Creek Complex spring-run Chinook salmon) will begin in 2012. Broodstock will be gathered primarily as eggs or juveniles, in order to minimize the impact on source populations while allowing for collection of enough fish to establish a successful broodstock. Broodstock gathered in 2012 will be reared in the Interim Facility and, upon reaching sexual maturity, will be spawned or be released to the river to spawn naturally.*

*Before completion of the full-scale facility, yearly broodstock collections should gather enough fish or eggs, roughly 300-500 total,*

*across all population, to produce 50-100 total adult pairs. Collections will continue until the full scale facility is constructed, which is planned to be completed in 2014. Additional source population fish may be collected for direct in-river releases, and some fish may be taken from the San Joaquin and its tributaries, depending on their provenance. All broodstock will be genotyped for parentage-based tagging (PBT) and to prepare breeding matrices, per HGMP Section 8 and will be PIT tagged for tracking and identification. Planned Interim Facility operations in 2013 should repeat 2012 actions.*

*With planned full-scale Conservation Facility construction ending in 2014, hatchery operations should begin the same year, and the Interim Facility will be integrated into the full-scale facility. As the full-scale facility comes on line, broodstock collection can ramp up to the higher levels identified in HGMP Sections 1.11.1 and 6, as permitted. To capture the most genetic diversity while minimizing impacts to the source populations, broodstock collections will continue every year for at least 4 years and potentially up to 8 years, depending upon returns in the San Joaquin and source population Rivers, and on the number of fish taken from the source populations every year. Full-scale operations are anticipated to collect up to approximately 2,700 fish or eggs per year from the source populations to allow for infertility, mortality, and unequal sex ratios, which should produce up to 450 adult pairs.*

*In 2014, yearling broodstock females collected in 2012 should be available for spawning, although this will likely be a small percentage of the anticipated restoration broodstock. These fish will be mated as discussed in HGMP Section 8. Conservation Facility egg production from spawning practices in any year will probably not exceed 750,000 eggs, although more fish may be produced if required to meet the reintroduction goals. See HGMP Section 9 for details. Offspring will be reintroduced to the River as discussed in HGMP Section 10, depending on conditions in the San Joaquin River and escapement for the reintroduced population.*

*Any adult escapement returns from the direct, in-river releases would begin returning in 2015 and 2016. Depending on escapement numbers, these may be available for use as broodstock. Broodstock collection from returns generally should not exceed 10% of the estimated in-river escapement (as determined to maintain population viability) unless river conditions preclude successful spawning.*

*Anticipated fish available for production in the hatchery from the small number of broodstock spawned in 2014 could begin returning in 2016. These fish will be genotyped or otherwise identified to determine their parentage. Depending on escapement numbers,*

*these may be available for use as broodstock. Genetic analysis of these returns should provide information on what fish crosses and reintroduction strategies have been most successful, although the Conservation Facility should gather this data for several years before using it to guide reintroduction efforts.*

*The first potential large returns of fish, from the full-scale Conservation Facility production, will be in 2020, which should provide information to evaluate restoration success. Dec. 31, 2019, marks the conclusion of the “Reintroduction Period” as identified in the Technical Advisory Committee (TAC) recommendations. Following the TAC recommendations, the return target for 2019 will be 500 “wild” fish. If returns do not meet this target in 2019 or any year thereafter, monitoring data will be reviewed and restoration strategies and efforts will be assessed by the TAC, in consultation with the implementing agencies, to recommend refinements in management actions to improve returns.*

*January 1, 2020 marks the beginning of the “Interim Period” identified in the TAC Recommendations, which establishes a target minimum population size of 500 wild fish returning annually throughout the Interim Period, ending December 31, 2024. TAC recommendations establish a 5-year running average target of 2,500 during the interim period.*

*As per the FMP Population Objectives, “Ten years following reintroduction, less than 15% of the Chinook salmon population should be of hatchery origin.” The Settlement further states that a self-sustaining population should be established by 2024. If the population does not meet these targets, monitoring data will be reviewed and restoration strategies and efforts will be assessed by the TAC and the implementing agencies to recommend refinements in management actions to improve returns.*

*Under the Settlement Agreement, the hatchery should be phased out by 2025, unless required for years with abnormally low flows insufficient to support the salmon population. Hatchery use in the post 2025 period will be assessed annually by the Hatchery and Monitoring Technical Team.*

### 1.3 Source stock constraints

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Many biological constraints affect the reintroduction process. In particular, the population sizes of spring-run Chinook salmon in California are low (see GrandTab 2010). Low population numbers limit the collection of fish from the source stocks and limit the number of source stocks available for use (described in the Stock Selection Strategy and in Section 5.1, this document). The Southwest Fisheries Science Center,

NOAA Fisheries Service, is preparing a separate document that will provide a simple model on collection impacts and benefits to guide decisions regarding which populations may be used and how many fish may be collected from each population (C. Garza, pers. comm.). Given the constraints on naturally produced CV spring-run Chinook populations, the Feather River hatchery population is also considered as a potential source for the reintroduction.

Once the source stock constraints for a given year have been developed, the Hatchery and Monitoring Technical Team should develop monitoring protocols based on those constraints, including development of a flow chart explaining the stock collection options for a given year, for inclusion in an annual report as part of the Donor Stock Collection Process document.

#### 1.4 Adaptive management

The SJRRP and the Program use an adaptive management approach, as described in the FMP (SJRRP 2010b) and Williams et al. (2007). Adaptive management recognizes and plans for the uncertainty in the restoration process, the availability of source stock for reintroductions, the success of the reintroduction methods, and myriad other factors that will influence the success of the reintroduction. All plans for the Program are subject to revision based on this adaptive management approach. For example, the operation of the hatchery, covered by the HGMP, will be guided by a Hatchery and Monitoring Technical Team, meeting twice a year or more to guide the Program.

While many of the Program's broad guidelines are controlled by the documents and permits discussed above, this Hatchery and Monitoring Technical Team will require technical guidance on the genetic impacts of their decisions. More broadly, if the hatchery plans are not permitted by NMFS or are otherwise derailed, reintroduction may proceed with only direct transfers of fish, and managing such a reintroduction would require technical information on the genetic impacts of potential approaches. The supporting documents do not address monitoring in the "out years", after the putative hatchery is no longer functioning, and development of such a monitoring program will require technical guidance on genetic issues.

#### 1.5 Objectives of the GMP

This GMP addresses the need for technical genetic information to guide the reintroduction. The GMP discusses the genetic risks in reintroducing spring-run Chinook salmon to the San Joaquin River and provides recommendations to minimize these risks. The GMP provides the technical genetic guidance necessary for adaptive management of the reintroduction process to maximize genetic diversity and effective population size. The GMP is not a supporting document for the permitting process, and indeed is not associated with the permitting process at all. It is a repository of the best available science designed to help managers make the best possible decisions within the framework created by the permits and their supporting documents.

The genetic management goals for spring- and fall-run Chinook salmon in the San Joaquin River Restoration Program (SJRRP) are to promote and protect genetic diversity within the reestablishing populations while safeguarding against negative genetic effects to source and non-target populations. The meaning and monitoring of genetic diversity is discussed in detail in Sections 5 and 6, below. Although this GMP references impacts to and monitoring of broodstock source populations, it is not intended to be a GMP for the entire spring-run Chinook salmon ESU, only for the ESU as it is actually or potentially impacted by the SJRRP program. Additional detail can be found in status reviews and other documents regarding specific spring-run populations that are referenced, but not comprehensively covered, in this GMP. Regardless, these goals should benefit the three Viable Salmonid Population (VSP) characteristics (abundance, spatial structure, and diversity) of the reestablished populations, discussed in Section 3. The GMP supports these goals by providing background and recommendations regarding: conserving, promoting, and monitoring genetic diversity of four general “groups”: the source populations used in reintroduction, the hatchery population, the in-river (San Joaquin River) population, and the post-reintroduction period population.

The authors are aware that genetics is only one component among many determinants of a successful reintroduction. Genetic factors should not be considered in isolation. Logistical, ecological, and other constraints will certainly factor into a successful strategy. The goal of this document is to provide advice on issues of genetic importance.

Finally, some parts of the GMP will be very similar to other documents and will draw heavily from those sources, but inclusion of those materials is necessary for a freestanding GMP. Those sections with significant material from other sources are noted.

## 1.6 Assumptions

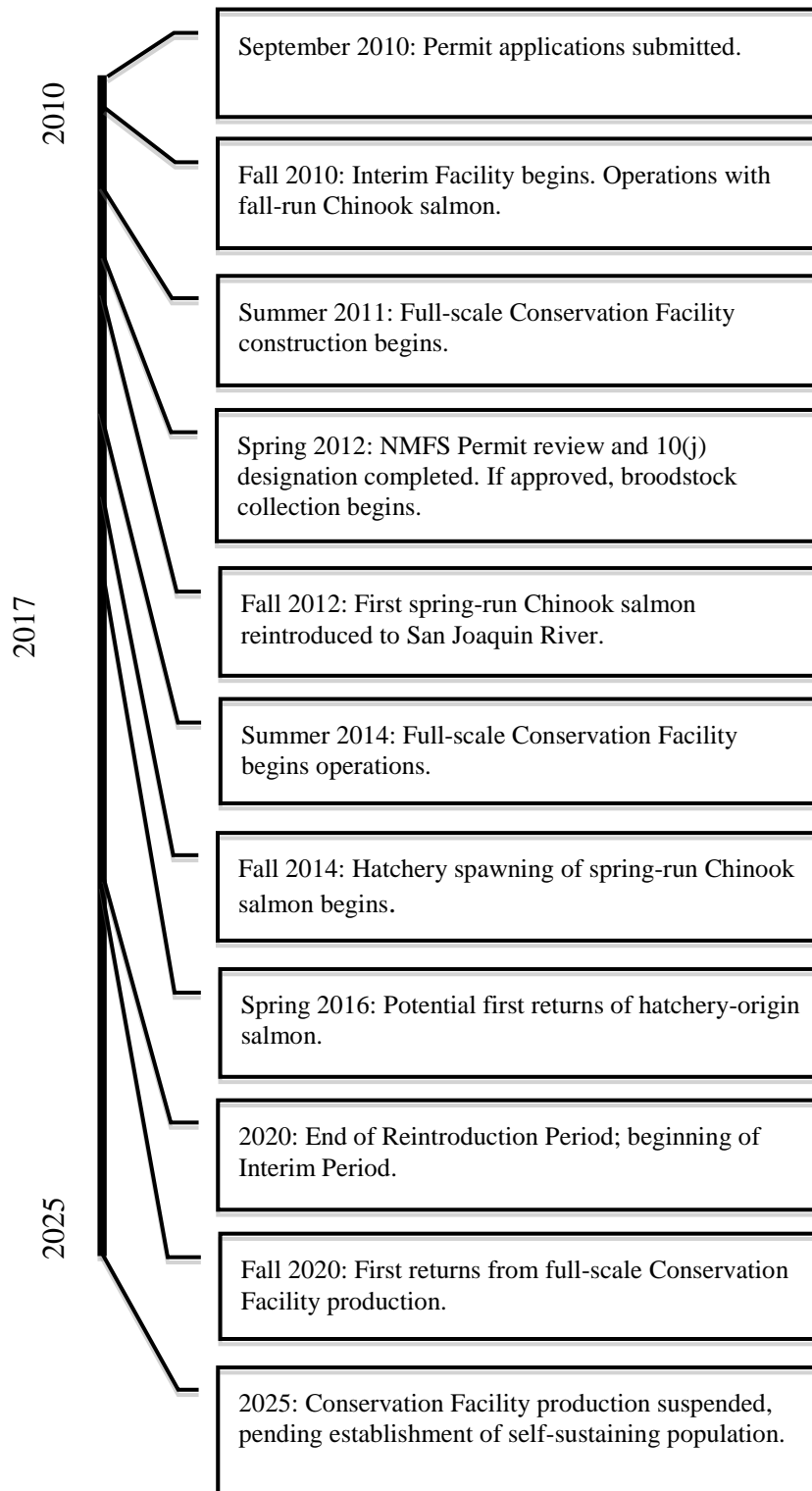
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By necessity, this document makes several assumptions about its readers. It assumes that readers have taken a college level genetics course and are at least familiar with basic concepts in population genetics. It assumes that readers are familiar with Chinook salmon populations in California and with their various life histories. Finally, it assumes that readers are familiar with the other documents involved in the reintroduction process: the TAC’s Recommendations on Restoring Spring-run Chinook Salmon to the Upper San Joaquin River ; the SJRRP Fisheries Management Plan: A Framework for Adaptive Management in the San Joaquin River Restoration (SJRRP 2010b); the SJRRP Stock Selection Strategy (SJRRP 2010c) and Reintroduction Strategy (SJRRP 2011), and the CDFG’s HGMP (Bork and Adelizi 2010). Without this background knowledge, understanding of this document may require extensive reference to other sources.

## 1.7 Organization of the GMP

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Section 2 introduces the reader to the current taxonomy and the known genetic diversity of the spring-run Chinook salmon, including the potential source stocks. Section 3 discusses the Viable Salmonid Population (VSP) concept and its relevance to upper San Joaquin River restoration goals. Section 4 discusses the potential biotic and abiotic factors that can affect the reintroduction process, providing some general background to the genetic concerns surrounding any reintroduction effort and some information specific to the Program's reintroduction. Section 5 provides guidelines for preserving and enhancing the genetic diversity of the source population, the hatchery population, the in-river population during supplementation, and the in-river population after supplementation has ended. Section 6 provides guidelines for monitoring the genetic diversity of the source populations, the hatchery population, and the in-river population. Section 7 provides guidance on the hatchery phase-out, as envisioned by the Settlement Agreement, Section 8 discusses contingency plans for myriad aspects of the reintroduction, and Section 9 briefly concludes the GMP. The appendix provides additional details regarding methodology for genetic analyses related to the reintroduction program.



**Figure 1. Program Timeline, 2010 to 2025**

Projected dates are contingent upon funding availability.

## SECTION 2 TAXONOMY AND DIVERSITY OF CENTRAL VALLEY SPRING-RUN CHINOOK SALMON

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### 2.1 Life history and taxonomic overview of Central Valley spring-run Chinook salmon

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Central Valley Chinook salmon exhibit a wide range of life history patterns. Tables 1 and 2 summarize the timing of life-history events and required spawning habitat for the different runs of Chinook salmon in the Central Valley and Figures 2, 3, and 4 show the distribution of the spring, winter, and fall runs, respectively. Table 3 (taken from Bork and Adelizi 2010) outlines the differences in life history characteristics within the largest populations of spring-run Chinook in the Central Valley. There are three Chinook salmon ESUs listed under the Endangered Species Act within California: Sacramento River winter-run (Endangered), Central Valley spring-run (Threatened), and California Coastal (Threatened; NMFS 2005). Additionally Central Valley fall and late-fall are listed as a Species of Concern (NMFS 2005). Within spring-run Chinook in the Central Valley there are two life-history strategies for juvenile migration: 1) Stream-type and 2) Ocean-type. Stream-type individuals stay in freshwater habitat for over a year and then enter saltwater, while ocean-type individuals migrate to sea soon after emerging from the gravel (Moyle et al. 2008). In the naturally reproducing populations of Central Valley spring Chinook, most juveniles exhibit ocean-type life history patterns, however stream-type are present as well (Williams 2006). For further explanation of the factors that influence this life history variation, see Myers et al. (1998).

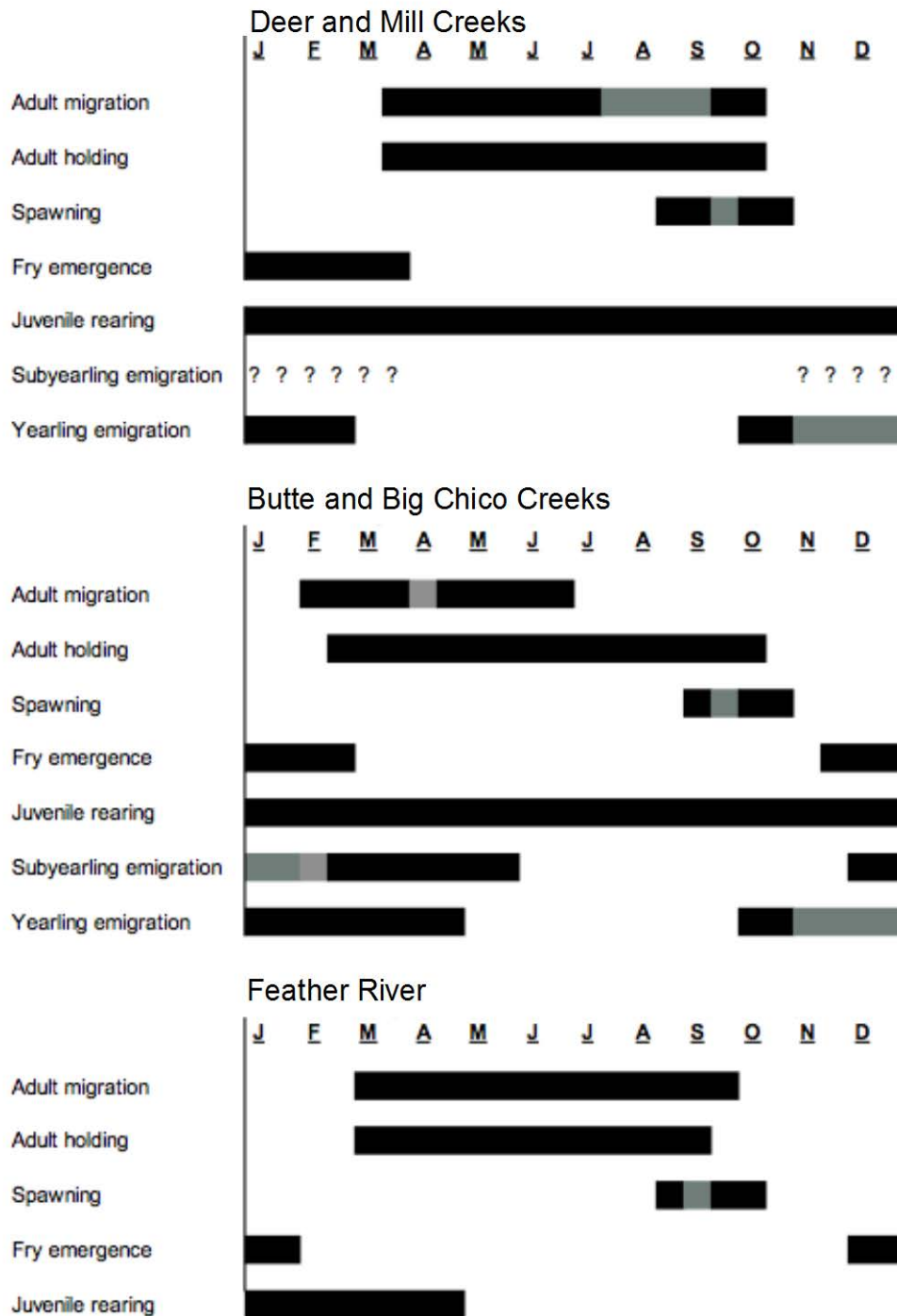
The Central Valley spring-run Chinook salmon ESU was originally listed as threatened on September 16, 1999 (NMFS 1999) and the listing was revised on June 18, 2005 (NMFS 2005). NMFS treats an ESU as constituting a Distinct Population Segment, and therefore is a “species,” under the Endangered Species Act. The original listing was based on several studies that investigated population differentiation in California and Oregon Chinook salmon (NMFS 1999). These studies found Central Valley Chinook are genetically distinct from Chinook in other areas (Utter et al. 1989, Gall et al. 1992, Myers et al. 1998).

Banks et al. (2000) used microsatellite molecular markers to investigate differentiation among winter-, spring-, fall-, and late fall-run Chinook salmon in the Central Valley. They found each run timing of Chinook was genetically distinct. They also found the populations of spring-run from Deer Creek and Mill Creek were similar to each other but distinct from spring-run in Butte Creek.



**Table 1. Timing of life history events for Chinook salmon in various Central Valley streams.**

Grey color denotes peak timing. Adapted from USBR (2008).



**Table 2. Spawning habitat and life history characteristics of Chinook salmon runs in the Central Valley.**From <http://www.swr.noaa.gov/hcd/cvschshd.htm>

<b>Upstream Migration Season (Run)</b>	<b>Typical Spawning Habitats</b>	<b>Spawning Habits</b>	<b>Approximate Elevation Salmon Reach</b>
Fall	Lower rivers and tributaries, valley floor and foothill reaches.	Spawn soon after entering natal streams, limited by deteriorated physical condition and ripeness of eggs.	To 1000 ft elevation, based on the McCloud River (H. Rectenwald and R. Yoshiyama, pers. comm.)
Late-Fall	Upper mainstem rivers, including the upper mainstem reaches of the Sac. R. and major tributaries (currently blocked by the Shasta Dam), and perhaps other valley streams such as the American R. and southern San Joaquin R.	Spawn upon entry into stream, limited by deteriorated physical condition and ripeness of eggs. Largest of the Sac. R salmon. Juveniles require summer cold water flows.	Unknown
Spring	Higher streams, with adequate spring fed run-off or snow melt runoff to keep summer water temperatures low. Historically abundant in the San Joaquin system.	Hold in the stream for several months before spawning. Smaller bodied fish enables access to higher reaches.	~1500 ft or ~2500 ft to 3000 ft if spawned earlier
Winter	Spring fed headwaters where cold constant summer flows exists.	Hold in the stream for several months before spawning. Smaller bodied fish enables access to higher reaches.	Headwater reaches

**Table 3. Timing of major life-history events for the most abundant stocks of Central Valley spring-run Chinook.**

Table 2.1 in Bork and Adelizi (2010).

Life History Characteristics	Feather River		Butte Creek		Deer/Mill Creeks	
Adult Run Timing	April - May		February – June, peaking in mid-April.		March – early July	
Spawning Timing	September		Late-September to early-November, peaking in early-October .		September	
Spawning adult age class structure*	Age 2	10.9%	Age 2	0%	Age 2	Unknown
	Age 3	46.9%	Age 3	53%	Age 3	Unknown
	Age 4	41.2%	Age 4	47%	Age 4	Unknown
	Age 5	0.68%	Age 5	0%	Age 5	Unknown
Sex Ratio (M:F)**	1.2:1		1:1.18		Unknown	
Size Range (FL)	Females**** - 782 mm Males**** - 829 mm		Females*** - 762 mm. Males*** - 793 mm.		410 mm to 1002 mm with the majority 600-800 mm.	
Outmigration Timing (all three population show two primary life histories for young, fry emigrating within weeks of emergence (ocean-type) and juveniles remaining in the river for roughly one year before emigrating (stream-type))	Emergence: Nov. – Apr., peaking in Jan. Outmigration of yearlings: Unk. Outmigration of fry: Dec. – June, peaking Feb. to Apr.		Emergence: Nov. – Apr., peaking in Jan. Outmigration of yearlings to the Delta: Nov. – Apr. Initial outmigration of fry to Sutter Bypass – Nov. to Feb. Final outmigration of fry from Sutter Bypass to the Sac. River and Delta – Feb. to May.		Emergence: Nov.- Apr. peaking around Feb. Outmigration of yearlings: Oct. – Apr. Outmigration of fry: Feb. – June	
Straying Rate	High		Unknown		Unknown	

\* Feather River data are average percent by age of spring-run and fall spawning run returning to hatchery, 2000-2004. Butte Creek data based on tag recoveries in 2007, although age varied widely in the Butte Creek population. Age 3 fish were a much higher percentage in 2002, '02, '04, and '05, and Age 4 were much higher in 2003 and '06. 2007 data based on scale aging for all fish, including untagged fish suggested a much higher percentage of age 3 returns for both the Feather River and Butte Creek, at 68% and 72%, respectively (Grover and Kormos 2007).

\*\* Feather River data are averaged from 1997 through 2007. Butte Creek data averaged 2001-2006, from carcass surveys.

\*\*\* 2001-2007 averages.

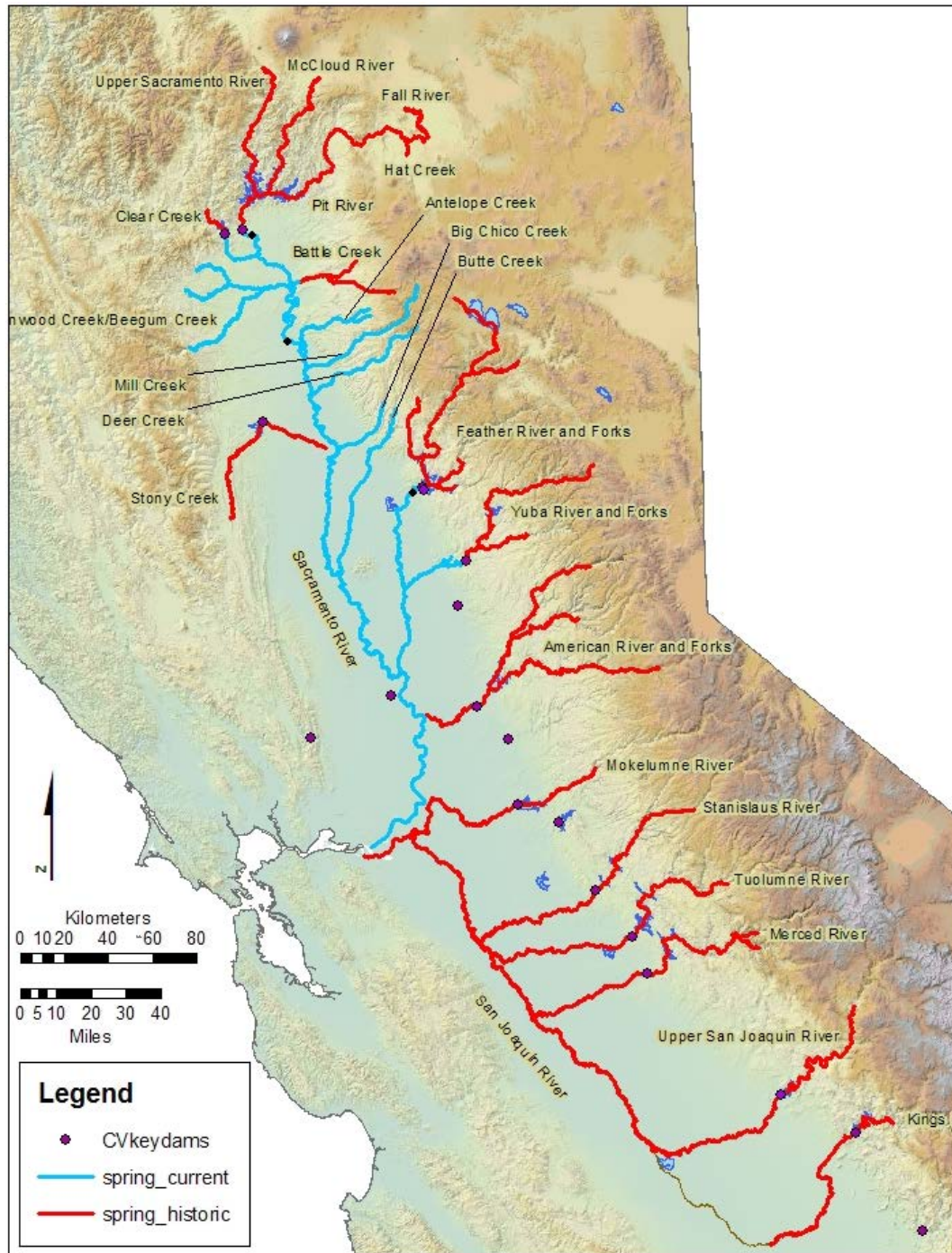
\*\*\*\* Based on 2006-2008 spring-run broodstock (pers. comm. Ryon Kurth, CA DWR).

Another study that contributed to the listing decision analyzed variation in the major histocompatibility complex class II exon in Sacramento River Chinook salmon runs (Kim et al. 1999). Kim et al. (1999) found winter-run Chinook salmon in the upper Sacramento River were the most genetically divergent run, the fall and late fall runs were closely related, while the spring-run was genetically intermediate between the winter- and fall/late fall-runs. They also sampled Butte Creek spring-run Chinook salmon

and found these salmon were genetically similar to Sacramento River mainstem spring-run samples. Separate investigations of mitochondrial DNA determined, however, that fall-, late fall-, spring-, and winter-runs of Central Valley Chinook salmon are significantly differentiated from each other (Nielsen et al. 1994, Nielsen 1995).

Based on these findings and low population abundances, NMFS declared Central Valley spring-run Chinook an ESU warranting protection under the ESA (NMFS 1999). This original Central Valley spring-run Chinook ESU listing included all naturally spawned populations of spring-run Chinook salmon in the Sacramento River and its tributaries in California, including the Feather River. However, this original listing did not include the Feather River Hatchery spring-run population.

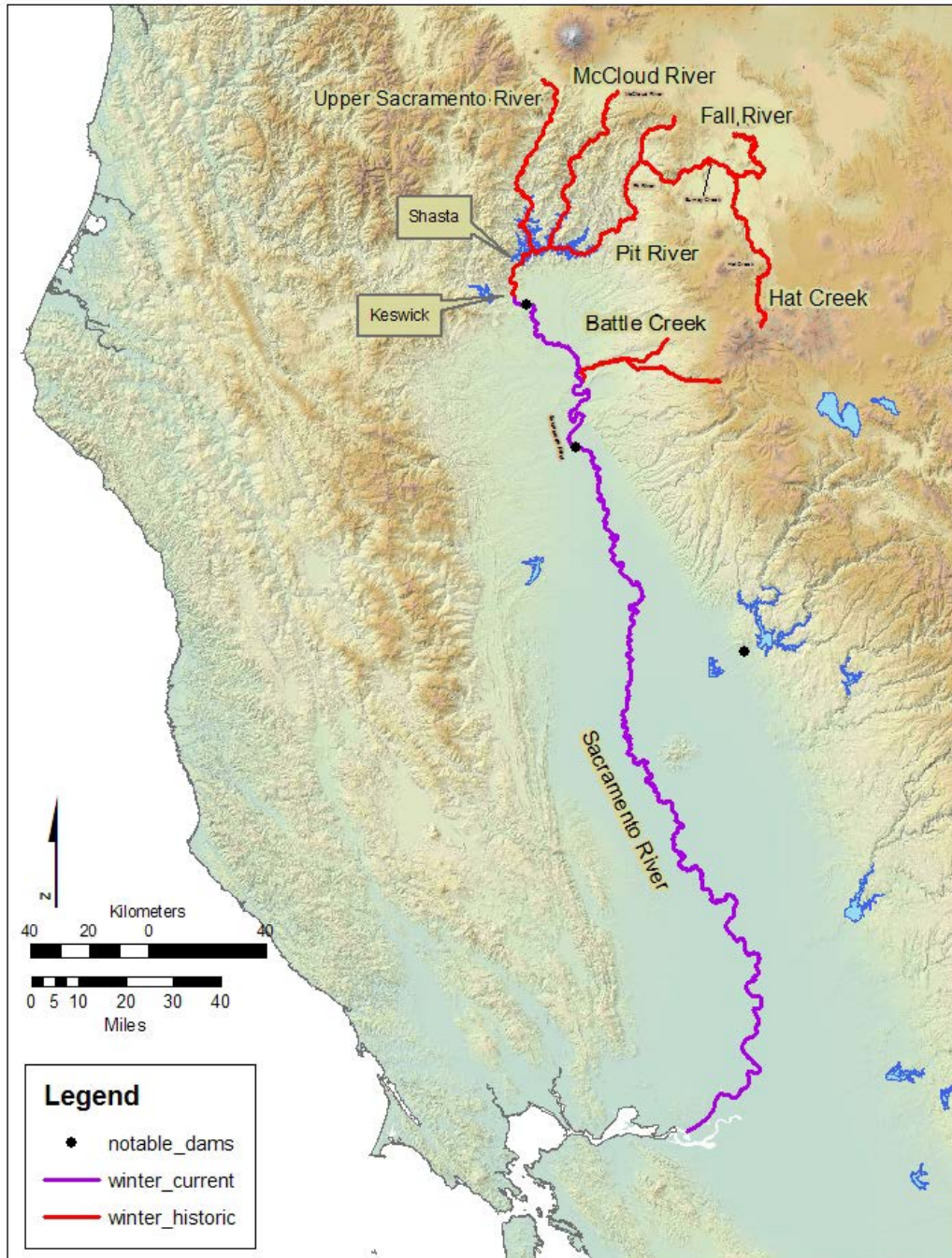
Following the original 1999 listing, subsequent studies further elucidated the genetic relationships among Central Valley Chinook salmon. Hedgecock (2002) used 12 microsatellite loci to evaluate Chinook salmon in the Central Valley. He found the putative spring-run Feather River Chinook were genetically much more closely related to Central Valley fall-run fish ( $F_{ST}=0.008$ , sig. difference) than to spring-run Chinook from Butte ( $F_{ST} = 0.034$ ) and Mill and Deer Creeks ( $F_{ST} = 0.016$ ). Based on these outcomes, he combined Central Valley Chinook salmon into six major groupings (Figure 5).



**Figure 2. Map of current Central Valley spring-run Chinook distribution overlaid on historic distribution**

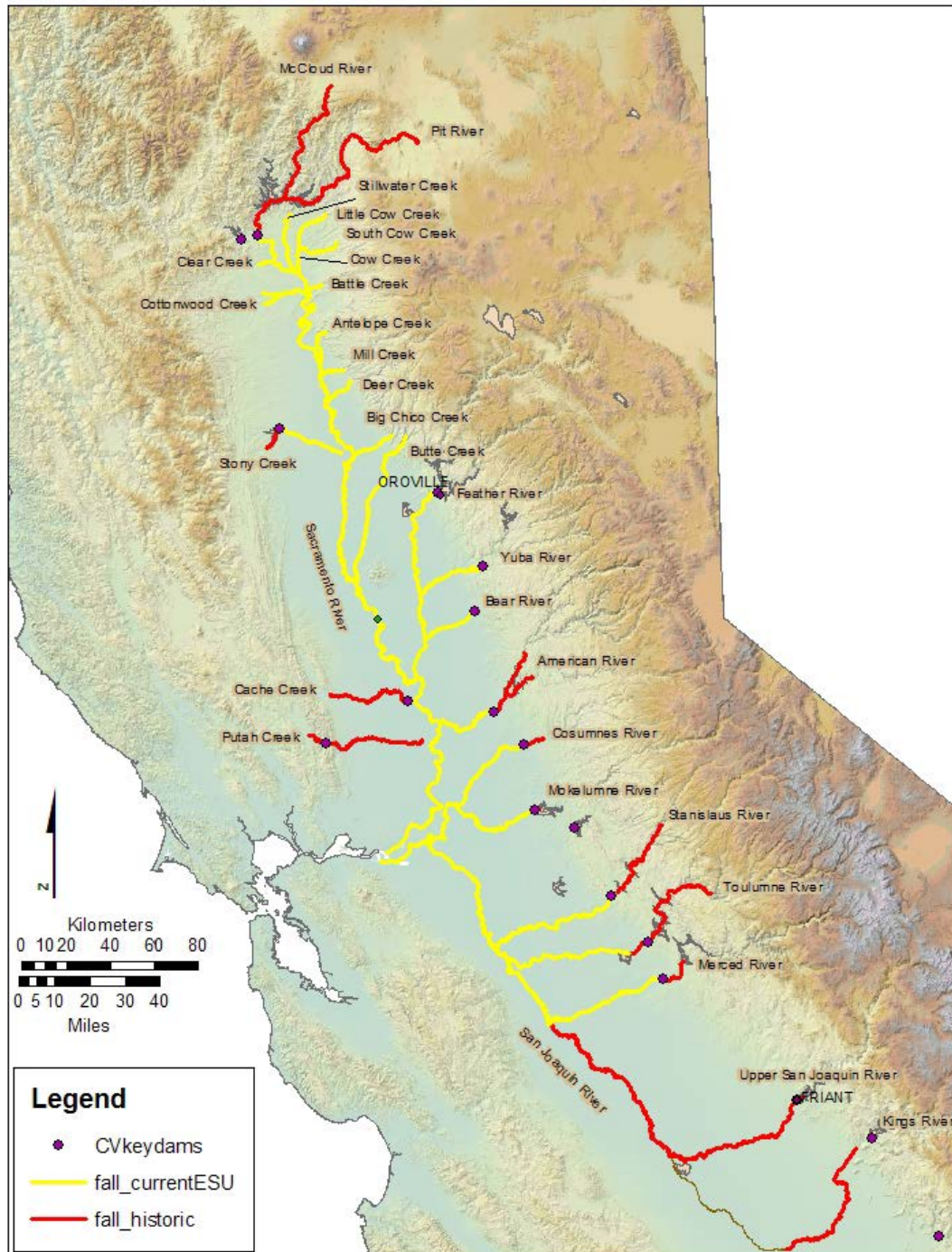
The current distribution (blue lines) is overlaid on the historic distribution (red lines). Although Battle Creek is not shown as part of the current distribution, spring-run Chinook have recently been found there (GrandTab 2010). Keystone dams, defined as the first major barrier to anadromy, are shown by "Cvkeydams" points. Map data derived from Schick et al. (2005).





**Figure 3. Map of current Central Valley winter-run Chinook distribution overlaid on historic distribution**

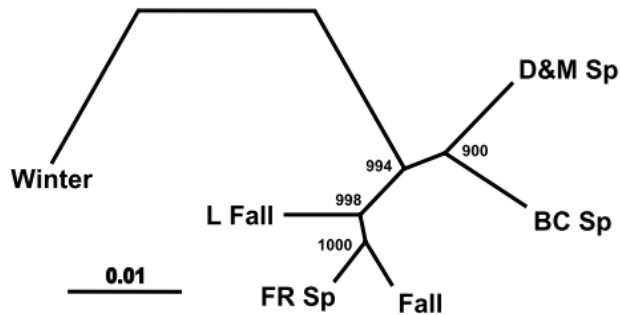
The current distribution (purple lines) is overlaid on the historic distribution (red lines). Location of Shasta and Keswick dams also given. Map data derived from Schick et al. (2005).



**Figure 4. Map of current Central Valley fall-run Chinook distribution area overlaid on historic distribution**

The current distribution (yellow lines) is overlaid on the historic distribution (red lines). Historical connection between Kings and upper San Joaquin River shown by brown line. Keystone dams, defined as the first major barrier to anadromy, are shown by “CVkeydams” points, with Friant and Oroville dams labeled. Map data derived from Schick et al. (2005).





**Figure 5. Neighbor joining tree of Central Valley Chinook populations**

Cavalli-Sforza and Edwards chord distances for D&M = Deer and Mill creeks; BC = Butte Creek; FR = Feather River; Sp = spring-run Chinook; L Fall = late fall-run Chinook from upper Sacramento R; Winter = winter-run Chinook from the Sacramento R, Fall sampled from throughout the Central Valley, including the Feather River. The numbers at the branch points indicate the bootstrap values based on 1000 iterations. From Hedgecock (2002).

In 2005, NMFS reevaluated their listing determination and concluded that hatchery stocks should be included in the ESU if they are not reproductively isolated from populations in the ESU (NMFS 2005). The NMFS' Central Valley Technical Recovery Team determined that:

*(1) The naturally spawning population of spring-run Chinook in the Feather River represents the level of reproductive isolation and the evolutionary legacy of the ESU, and thus warrants inclusion in the ESU; and (2) the Feather River Hatchery spring-run Chinook stock is no more divergent relative to this local natural population than would be expected between two closely related populations in the ESU, and thus it also warrants inclusion in the ESU.*

Therefore, based on this finding, the Central Valley spring-run Chinook ESU was revised to include all naturally spawning populations of spring-run Chinook in the Sacramento River and its tributaries, as well as the Feather River Hatchery population. NMFS policy states that a salmonid population is "considered 'distinct' for purposes of the ESA if it represents an evolutionarily significant unit (ESU) of the biological species. An ESU is defined as a population that 1) is substantially reproductively isolated from conspecific populations and 2) represents an important component of the evolutionary legacy of the species" (Myers et al. 1998). This designation as an ESU requires protection of the genetic integrity of the population and its evolutionary legacy. Inbreeding with fall-run Chinook salmon is listed as a risk to the spring-run ESU (Myers et al. 1998). Therefore, it is important to manage the spring-run Chinook population to minimize introgression with other runs outside the ESU.

Following the 2005 ruling, Garza et al. (2008) used 20 microsatellite markers to further evaluate relationships among Central Valley Chinook salmon. Similar to other studies, they found little differentiation among fall-run Chinook in the Central Valley.

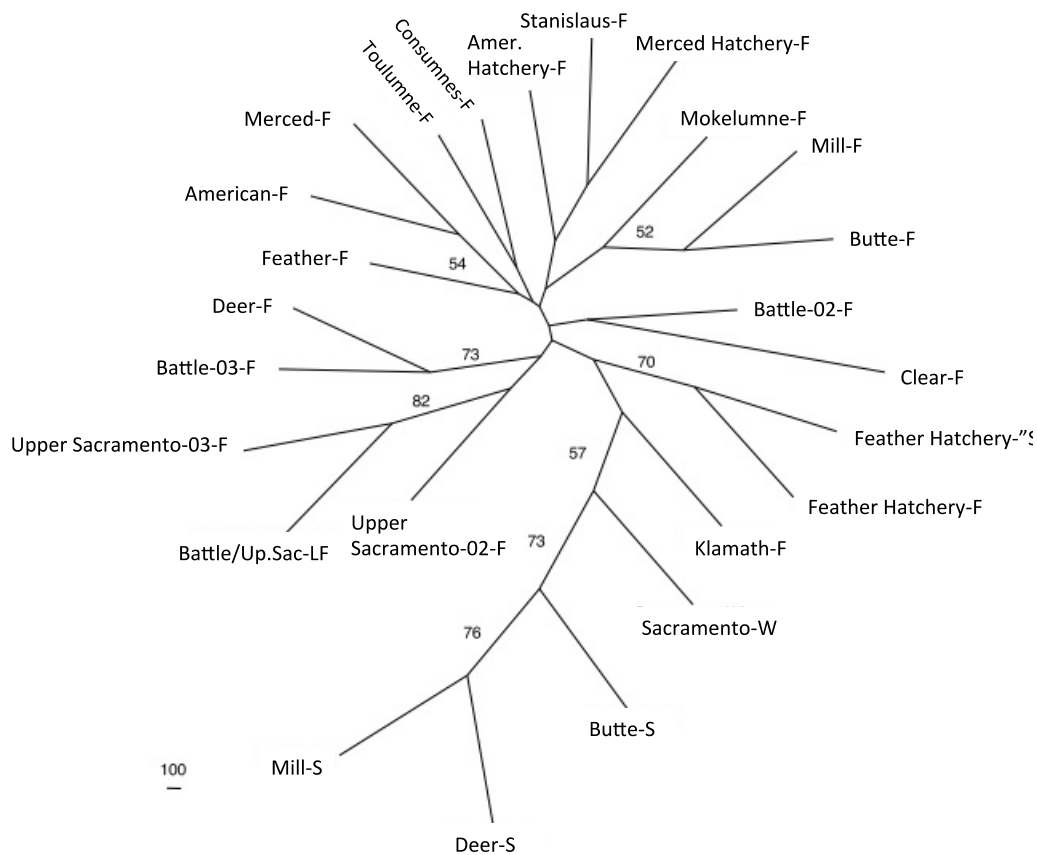


However there is evidence of slight but significant differentiation between fall- and late fall-run in the upper Sacramento River and Battle Creek. See Figure 6 for a tree topology they found and Table 4 for  $F_{ST}$  values from their study. They found the Merced River Hatchery fall-run is the most divergent of any fall-run population. Their study shows that in Mill, Deer, and Butte Creeks, the naturally spawning fall- and spring-runs from the same creek are not closely related, suggesting reproductive isolation between runs within creeks. The three naturally spawning spring-run populations (Mill, Deer, and Butte creeks) are monophyletic and distinct from one another, based on significant pairwise  $F_{ST}$  values. Similar to Banks et al. (2000), they determined winter-run Chinook is more closely related to spring-run than fall-run. They found slight but significant differences between the Feather River Hatchery “fall-“ and “spring-“ stocks, but they found that the Feather River Hatchery “spring” run is genetically “fall” run. Based on linkage disequilibrium they found only in the Feather River Hatchery “spring-“ run, they conclude that Feather River Hatchery “spring” run retains remnants of genetic ancestry with the naturally spawning Feather River spring-run that existed prior to the dam and hatchery, but that the hatchery stock has been heavily introgressed by fall-run fish.

**Table 4. Pairwise  $F_{ST}$  comparisons between Central Valley spring-run and fall-run Chinook salmon**

$F_{ST}$  values are above the diagonal. Values in bold are not significantly different from zero. Results below the diagonal are exact test of genic differentiation (Raymond and Rousset 1995). Plus sign (+) denotes significant at  $p < 0.05$ , NS= non-significant. From Garza et al. (2008).

	Merced	Merced-Hatchery	Toulumne	Stanislaus	Mokelumne	Consumnes	American	American-Hatchery	Feather	Feather-Hatchery	Feather-HatcherySP	Butte	Butte-Sp	Deer	Deer-Sp	Mill	Mill-Sp	Battle-02	Battle-03	Battle/Up. Sac-LF	Up. Sacramento-02	Up. Sacramento-03	Up. Sacramento-W	Clear	Klamath
Merced	---	0.025	<b>-0.001</b>	<b>0.003</b>	<b>0.001</b>	<b>0.001</b>	<b>-0.001</b>	0.003	<b>-0.002</b>	<b>0.002</b>	0.006	<b>0.003</b>	0.027	<b>-0.003</b>	0.013	<b>-0.001</b>	0.012	0.004	<b>0.002</b>	0.009	<b>0.002</b>	0.003	0.156	0.007	0.082
Merced-Hatchery	+	---	0.030	0.020	0.023	0.031	0.031	0.020	0.023	0.025	0.033	0.033	0.055	0.025	0.041	0.025	0.042	0.024	0.031	0.030	0.033	0.030	0.176	0.039	0.100
Toulumne	NS	+	---	0.004	<b>0.001</b>	<b>0.002</b>	0.003	0.005	<b>0.001</b>	0.002	0.008	0.007	0.032	<b>0.002</b>	0.016	0.005	0.017	0.009	<b>0.001</b>	0.007	0.004	0.003	0.159	0.012	0.080
Stanislaus	+	+	+	---	0.003	0.006	0.008	0.005	0.004	0.003	0.009	0.008	0.029	0.003	0.016	0.007	0.016	0.007	0.005	0.012	0.007	0.006	0.147	0.014	0.077
Mokelumne	+	+	+	+	---	0.002	0.005	0.002	<b>0.001</b>	0.002	0.007	0.003	0.030	<b>0.001</b>	0.015	0.002	0.016	0.008	0.003	0.009	0.004	0.002	0.150	0.011	0.080
Consumnes	+	+	+	+	+	---	0.007	0.006	<b>0.003</b>	0.004	0.009	0.006	0.032	<b>0.002</b>	0.017	0.005	0.019	0.010	0.005	0.009	<b>0.002</b>	0.003	0.161	0.012	0.089
American	NS	+	+	+	+	+	---	0.006	0.004	0.007	0.010	0.011	0.032	0.006	0.018	0.009	0.019	0.011	0.004	0.011	0.009	0.008	0.156	0.016	0.085
American-Hatchery	+	+	+	+	+	+	+	---	0.003	0.004	0.008	0.009	0.028	0.005	0.015	0.007	0.017	0.009	0.005	0.012	0.006	0.005	0.141	0.012	0.081
Feather	NS	+	+	+	+	+	+	+	---	0.002	0.006	<b>0.001</b>	0.028	<b>-0.002</b>	0.013	0.003	0.017	0.006	0.004	0.007	0.004	0.003	0.162	0.010	0.086
Feather-Hatchery	+	+	+	+	NS	+	+	+	+	---	0.003	0.007	0.026	<b>0.000</b>	0.011	0.005	0.013	0.006	0.002	0.009	0.002	0.003	0.141	0.011	0.077
Feather-HatcherySP	+	+	+	+	+	+	+	+	+	+	---	0.011	0.026	0.006	0.013	0.009	0.012	0.012	0.006	0.014	0.005	0.007	0.130	0.014	0.077
Butte	+	+	+	+	+	+	+	+	+	+	+	---	0.034	<b>0.005</b>	0.020	<b>0.001</b>	0.019	0.013	0.010	0.014	0.007	0.008	0.163	0.008	0.085
Butte-Sp	+	+	+	+	+	+	+	+	+	+	+	+	---	0.027	0.021	0.032	0.020	0.032	0.023	0.035	0.025	0.023	0.138	0.035	0.107
Deer	NS	+	+	+	NS	+	+	+	NS	+	+	+	+	---	0.009	<b>0.001</b>	0.015	<b>0.002</b>	<b>-0.002</b>	0.010	<b>0.003</b>	<b>0.001</b>	0.163	0.012	0.087
Deer-Sp	+	+	+	+	+	+	+	+	+	+	+	+	+	+	---	0.019	0.005	0.021	0.012	0.022	0.015	0.012	0.142	0.024	0.094
Mill	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	---	0.018	0.005	0.005	0.011	0.003	0.005	0.162	0.009	0.089
Mill-Sp	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	---	0.022	0.014	0.024	0.015	0.015	0.139	0.021	0.086
Battle-02	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	---	0.006	0.014	0.006	0.008	0.156	0.015	0.096
Battle-03	+	+	+	+	+	+	+	+	+	+	+	+	+	NS	+	+	+	+	---	0.009	0.002	<b>0.002</b>	0.142	0.013	0.082
Battle/Up. Sac-LF	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	---	0.007	0.004	0.163	0.018	0.092
Up. Sacramento-02	+	+	+	+	+	+	+	+	+	+	+	+	+	NS	+	+	+	+	+	+	---	0.002	0.142	0.011	0.088
Up. Sacramento-03	+	+	+	+	+	+	+	+	+	+	+	+	+	NS	+	+	+	+	+	+	+	---	0.143	0.011	0.090
Up. Sacramento-W	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	---	0.146	0.208
Clear	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	---	0.087
Klamath	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	---



**Figure 6. Maximum likelihood bootstrap consensus tree**

Letters following sampled waterway denote run timing: F-fall, S-spring, LF-late fall, W-winter. Samples were collected in 2002 and 2003, with the exception of winter-run samples which were collected between 1995-2004. The numbers following several of the waterway names indicate the year when there was significant differentiation among years sampled (see Table 4 for  $F_{ST}$  values). From Garza et al. (2008).

The most recent status reviews of Central Valley spring-run Chinook can be found in NMFS (2005), Lindley et al. (2007), and Moyle et al. (2008).

## 2.2 Potential source stock genetic diversity overview

The TAC spring-run recommendations (Meade 2007), the Stock Selection Strategy document (SJRRP 2010c), and the HGMP (Bork and Adelizi 2010) all provide overviews of the genetic diversity in the three primary populations of spring-run Chinook salmon under consideration for broodstock use. Adult escapement in 2010 for Mill, Deer, Butte, and Feather River Hatchery was 482, 262, 1160, and 1661 (GrandTab 2010). Other Central Valley spring-run populations were deemed too small or ephemeral for serious consideration for use except on an opportunistic basis, per the Stock Selection Strategy document. Rather than review previously completed work, the HGMP summation is presented here:

*The three potential source populations are genetically distinct, and the Mill Creek and Deer Creek populations are treated as one stock for purposes of stock selection (Banks et al. 2000, Garza et al. 2008). While the Mill and Deer Creek stocks are genetically distinct, Banks et al. (2000) and Garza et al. (2008) concluded that the two stocks should be treated as a single complex due to the high degree of gene flow and similar phenotypes between the two stocks. These two stocks do have a higher degree of genetic differentiation than that found between the Feather River fall and “spring-run” fish. However, the phenotypic differences between the Feather River spring-run and fall-run warrant their treatment as two separate populations for reintroduction purposes.*

*Three studies [referenced above] have evaluated the relative genetic diversity of the three potential spring-run source populations. Banks et al. (2000) conducted a microsatellite study of the Mill/Deer Creek and Butte Creek stocks, excluding fish from the Feather River spring-run stock. Their study found that the observed heterozygosity was essentially identical in the two stocks – 0.61 vs. 0.62 in the Mill/Deer and Butte Creek stocks, respectively. They found that the allelic diversity, as measured by the average number of alleles observed per locus, was about 6% higher in the Mill/Deer Creek stock than in the Butte Creek stock (6.60 vs. 6.18 respectively), although the difference did not appear to be statistically significant.*

*Garza et al. (2008) supplies a second dataset, consisting of data for 20 microsatellite loci from Chinook salmon sampled in 2002 & 2003. These data are discussed [...] in HGMP Section 6.2.3. To recap the salient results, the observed heterozygosities were 0.77, 0.77, 0.74 and 0.78 for Mill Creek, Deer Creek, Butte Creek and Feather River [Hatchery] stocks, respectively. The mean allelic richness per locus of the Mill Creek, Deer Creek, Butte Creek and Feather River stocks were 11.09, 10.85, 9.76 and 11.25, respectively. The statistical significance of these differences was not reported (...).*

*Finally, the third dataset consists of recent unpublished data from 169 single nucleotide polymorphism (SNP) loci developed by the Genetic Analysis of Pacific Salmonids (GAPS) consortium and by the Molecular Ecology and Genetic Analysis Team of the Southwest Fisheries Science Center (Garza unpublished). In this study, Deer and Mill Creeks were considered as one population. Data were available for the Deer/Mill Creek (N=71), Butte Creek (N=54) and Feather River (N=94) spring-run stocks. The SNP dataset found the observed heterozygosity was 0.29, 0.26 and 0.31 in the Mill/Deer Creek, Butte Creek and Feather River stocks, respectively. The mean number of alleles was 1.91, 1.88 and 1.91 in the Mill/Deer*

*Creek, Butte Creek and Feather River stocks, respectively. Again, the statistical significance of any differences in these means was not reported.*

*While the significance of the observed differences is not reported for these three studies, the measures of genetic diversity in all of the datasets were the lowest for Butte Creek, intermediate for Mill/Deer Creek and the highest for Feather River spring-run fish. The biological significance of these data in terms of spring-run are unclear, given the known introgression of fall-run genes in the spring-run fish in the Feather River population (tagging studies have found that some offspring from Feather River spring-run mating return as fall-run fish, and vice versa (CDFG 1998)). The higher allele number and higher heterozygosity in the Feather River are likely due, at least in part, to this observed introgression (...).*

*The genetic diversity of the salmon returning to the San Joaquin tributaries and the fish that have begun returning to the dewatered section of the upper San Joaquin has not been documented. These fish, of unknown provenance, form a small population that may be ephemeral. Additional research is necessary to establish the origin of these fish and to document the population size and diversity so their impact on the reintroduction program can be assessed.*

While this last group, fish returning to the San Joaquin tributaries, may or may not be spring-run, they are a likely source of strays into the reintroduced population and therefore are important to consider in the reintroduction effort. The 10(a)(1)(A) permit application discusses additional small spring-run groups in the Central Valley; please see that document for details.

## SECTION 3 THE VIABLE SALMONID POPULATION CONCEPT

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Considerable debate exists regarding the prioritization of salmon stocks or populations for conservation, with early discussion focused on the appropriate scale of conservation (Allendorf et al. 1997, Currens et al. 1998, Wainwright and Waples 1998). McElheny et al. (2000) introduced the Viable Salmonid Population (VSP) concept to aid in assessing the conservation status of Pacific salmonid populations and ESUs. They define a VSP as “an independent population of any Pacific salmonid (genus *Oncorhynchus*) that has a negligible risk of extinction due to threats from demographic variation, local environmental variation, and genetic diversity changes over a 100-year time frame.” An “independent population,” in turn, is defined as “...any collection of one or more local breeding units whose population dynamics or extinction risk over a 100-year time period are not substantially altered by exchanges of individuals with other populations.” Other researchers have subsequently expanded on how to best characterize independent units of conservation (reviewed in Waples and Gaggiotti 2006), however, the VSP criteria continue to be an important framework for species status reviews (e.g., Good et al. 2005). Below we describe the VSP concept, its applications to the SJRRP, and genetic measures appropriate for making inferences relevant to particular VSP criteria.

### 3.1 The VSP concept and the SJRRP spring-run Chinook population

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The in-river population may be subject to VSP criteria (see below) for evaluating Central Valley spring-run Chinook ESU recovery, per the forthcoming proposed 10(j) rule. The VSP criteria provide useful guidelines for evaluating the conservation status of the San Joaquin River population, which has the potential to influence the overall genetic health and diversity of the Central Valley spring-run Chinook ESU. Ensuring long-term population viability will rely heavily upon the feedback obtained during and following Reintroduction Period genetic monitoring activities, described in Section 6.3 and the appendix of this document. The reintroduced SJRRP spring-run Chinook population, once established, is intended to be an independent population, not sustained by stocking of hatchery or other broodstock sources and exhibiting local breeding dynamics.

### 3.2 Genetic considerations associated with VSP criteria

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There are four criteria for describing a VSP (McElhany et al. 2000): abundance, growth rate/productivity, spatial structure/connectivity, and diversity. Of these criteria, selected guidelines from this document, summarized below, involve genetic measures or will benefit from genetic data for characterizing and/or monitoring population status (see Section 6 for genetic monitoring recommendations).

#### 3.2.1 Abundance

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Abundance is a key factor in population viability. Lande (1988) asserted that demographic factors are more likely to drive threatened populations to extinction before genetic factors even come into consideration. Others, however, suggest that genetic factors do, in fact, drive populations to extinction (Spielman et al. 2004), and perhaps the two can be seen as acting in concert. A population should be sufficiently large to 1) maintain genetic diversity over the long term and 2) have a high probability of surviving natural and anthropogenic perturbation. Furthermore, the VSP guidelines for abundance provide guidance at two levels: “viable” and “critical.” Critical describes a population at higher risk of extinction in the short term. A population would be critically low if it is reduced below replacement or is at risk from inbreeding depression or fixation of deleterious mutations. Estimation of effective and census population size, genetic diversity, inbreeding estimates for hatchery and in-river populations, and heterozygosity (and other diversity measures) can aid in assuring that sufficient genetic diversity is retained over time to allow the population to persist above critical levels and in the face of natural and anthropogenic perturbation. A review of techniques to estimate these genetic variables and the uncertainty and sensitivity surrounding such estimations is given in the appendix.

### 3.2.2 Growth rate/productivity

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Closely related to estimates of abundance, estimates of population growth rate should indicate that a population is consistently replacing itself and maintaining abundance at viable levels. This includes both whole life cycle and stage-specific productivity measures. Additionally, according to McElhany et al. (2000) a VSP (that includes naturally spawning hatchery fish):

- 1) should exhibit sufficient productivity from naturally-produced spawners to maintain population abundance at or above viability thresholds in the absence of hatchery subsidy.
- 2) should exhibit sufficient productivity during freshwater life history stages to maintain its abundance at or above viable thresholds, even during poor ocean conditions.
- 3) should not exhibit sustained declines in abundance that span multiple generations and affect multiple broodyear-cycles.
- 4) should not exhibit trends or shifts in traits that portend declines in population growth rate.

Genetic and non-genetic methodologies may be employed independently and in concert to provide estimates of census and effective population size for use in productivity estimates; parentage based tagging (see full description in appendix) will be useful in parsing hatchery versus wild-spawned progeny productivity. Additionally, it can be used to assess stage-specific and family-specific survivorship, which may further aid in establishing causes of observed declines in productivity based on multi-year averages and trends.

Criterion 3 (above) states that a viable population should not exhibit sustained decline. Salmon populations are expected to have natural cycles of sustained declines

related to environment cycles such as the Pacific Decadal Oscillation, though populations clearly do not respond uniformly to such oscillations (Tolimieri and Levin 2004, Crozier et al. 2008). Modeling studies may be useful in differentiating the impact of decadal oscillation from unnatural population decline or decline related to anthropogenically induced climate change.

### 3.2.3 Spatial structure and connectivity

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Genetic data, specifically neutral molecular genetic markers such as single nucleotide polymorphisms (SNPs) or microsatellite DNA, are particularly useful in evaluating spatial structure and connectivity (e.g., gene flow, migration rates, straying rates) among individuals and among populations at multiple spatial scales. However, the lag time between changes in ESU- or population-level spatial structure and the ability to detect such changes genetically provides a challenge to timely adaptive management. Furthermore, the complex life-cycles of spring-run Chinook, with a three- or four-year generation time and potential metapopulation structure, may further complicate attempts to manage populations. Annual monitoring and actions trends over several years (as opposed to single-year population dynamics) should aid in preserving the natural evolutionary processes that generate and shape salmonid diversity.

### 3.2.4 Diversity

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Diversity occurs within and among populations and is not limited to genetic diversity, though genetic diversity is certainly an important aspect of overall diversity. It is recommended that anthropogenic influences not substantially alter natural genetic variation in traits such as run timing, cohort structure/composition, genetic diversity, and natural gene flow (levels and direction); many of these traits may be measured using neutral markers and PBT (see appendix) to monitor population diversity levels and distribution over time.

Population status evaluations should take into account uncertainty in sampling and in estimating parameters for all of the VSP criteria. Uncertainty surrounding genetic estimates of effective population size, errors in estimation of variables such as gene flow and migration rates, and uncertainty regarding the level of diversity needed for genetically healthy populations should be made explicit and accounted for in management recommendations, or where possible, modeled through power analysis to determine sensitivity of measures to detect changes or differences.



## SECTION 4 POTENTIAL BIOTIC AND ABIOTIC FACTORS

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This section describes some of the primary genetic-related factors that are important to consider when implementing a successful Chinook salmon restoration program. The purpose of this section is to introduce these factors, explain why they may impact restoration efforts, and provide some general recommendations for ameliorating negative consequences. Any factors that are particularly relevant to consider for specific activities and populations (e.g., conserving genetic diversity of source populations) are directly discussed in relation to these topics in later sections.

### 4.1 Effective population size

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The effective population size ( $N_e$ ) is the size of an idealized (i.e., theoretical) population affected by inbreeding or genetic drift at the same rate per generation as the population under study (Wright 1931, Kimura and Crow 1963).  $N_e$  can be considerably smaller than the census population size ( $N_c$ ) due to fluctuations in population size, overlapping generations, unequal sex ratios, and family size variation that exceeds a Poisson distribution. A recent comprehensive estimate for 56 Pacific Northwest Chinook salmon populations reported  $N_e/N_c$  of  $\sim 0.04 - 0.32$  for the stream-type life history and  $\sim 0.05 - 0.36$  for the ocean-type life history (Waples *et al.* 2010). To use an example, this means that a stream-type population of 100 spawners per generation has the same rate of genetic drift as an idealized population of only 4 – 32 spawners per generation. The estimated  $N_e$  of a generation can be related to the effective number of breeders ( $N_b$ ) in a single year by the following equation:  $N_e = g \cdot N_b$ ; where  $g$  is the mean age of adults at reproduction (i.e., generation time) (Waples 1990). Therefore, with a mean 3.5 year generation time, a hatchery program spawning 300 individuals per year will have an estimated  $N_e$  of approximately 1050 individuals. This estimation assumes equalization of progeny amongst the spawners and that multiple brood years contribute to spawning in a single year (Campton 2004).

The genetic consequences of a low  $N_e$  (e.g., genetic drift, allele frequency changes, reduced genetic variation, and increased inbreeding) can lead to further reductions in reproductive success, population growth, and adaptive potential, along with increased vulnerability to stochastic events (reviewed by Earnhardt 1999, Hare *et al.* 2011).  $N_e$  and selection have a reciprocal relationship (reviewed by Hare *et al.* 2011). As  $N_e$  declines, genetic drift can overcome selection of fitness-related traits (Willi *et al.* 2006) and, if sustained, can lead to a “mutational meltdown” by the accumulation of deleterious mutations (Lynch and Gabriel 1990). Conversely, if selective pressures on life history traits are strongly directional, overall genetic diversity may be lost, along with a considerable reduction in  $N_e$  (Santiago and Caballero 1995).

Most demographic parameters evaluated to assess a population's viability under Viable Salmonid Population criteria (i.e., population size, population growth rate, and diversity; see Section 3) are tightly linked with  $N_e$ . Therefore,  $N_e$  estimates, which

combine genetic effects with the life history of a species, can enable predictions regarding current and future viability. There is no standard in the literature regarding the optimal number of founders for successful reintroductions (Fraser 2008), though the general consensus is the more, the better. A recent review across taxa cited evidence to support that several thousand individuals are typically needed to obtain a Minimal Viable Population (MVP) for long-term population persistence (Traill *et al.* 2010). Allendorf *et al.* (1997) set extinction risk criteria specifically for Pacific salmonids and suggested that minimally  $N_c > 2,500$  or  $N_e > 500$  is needed to avoid a high risk of extinction. In the context of reintroductions, reaching a MVP estimate of several thousand individuals for the in-river population should be progressive, with a goal of the population increasing over multiple generations.

Considerable effort should be made to equalize sex ratios and family size contributions prior to reintroduction. This will minimize the reduction in  $N_e$  that can occur if a small number of breeders produce the majority of the next generation. Differential family survival is common, particularly in captivity (Allendorf 1993), and can have large impacts for highly fecund species such as Chinook salmon. For instance, efforts to equalize family contributions for winter-run Chinook salmon returning to the Sacramento River likely aided in producing high  $N_e/N_c$  ratios of  $\sim 0.5$  and greater than unity in two examined years (Hedrick *et al.* 2000). In the Conservation Facility, it is recommended to equalize sex ratios at spawning and minimize family size variance at the egg stage to maintain high  $N_e/N_c$  and  $N_b/N_c$  ratios and minimize selection to captivity (Allendorf 1993). Even greater gains in  $N_e/N_c$  ratios and reduced captive selection may occur if family size variance is minimized when offspring mature, just prior to release. Additionally, precocious males (“jacks”) should not be entirely excluded from hatchery matings since they increase connectivity among year classes, thus increasing  $N_e$ , and are a natural salmonid life history variant. Therefore, it is recommended that jacks are used in hatchery matings in proportion to the relative number of offspring that they typically contribute to a wild population (see sections 5.2 and 6.2 for additional details).

A particular concern with hatchery supplementation is the Ryman-Laikre effect (Ryman and Laikre 1991), which occurs when hatchery supplementation decreases the  $N_e$  of the population being supplemented. It comes about because of increased family sizes, skewed sex ratios, or variance in population size over generations for the hatchery propagated or reared segment of the population in comparison to the segment reproducing in the wild. The consequence is an overall reduction in  $N_e$  for the total population. For the first generation of reintroductions, the Ryman-Laikre effect will not be a concern, since a wild population does not currently exist in the Restoration Area. During hatchery propagation and rearing, efforts should be made to equalize family sizes and use 1:1 sex ratios to maintain large  $N_e/N_c$  ratios (see Section 5.2 for details). If hatchery supplementation continues through several generations, however, the Ryman-Laikre effect could hinder reintroduction success by raising the  $N_{eh}$  to the potential detriment of the  $N_{ew}$ . The negative impacts to the population will depend on the number of generations that the hatchery supplements (i.e., more generations may lead to greater negative effects). There is a tradeoff that occurs because increasing the overall population census size to meet restoration goals may actually reduce the  $N_e$ , thereby causing a loss of genetic variability that could affect the long-term reintroduction

success. Waples and Do (1994) conducted simulations for salmonid populations to show that if population size stays high after supplementation ceases, the risk of inbreeding negatively affecting the population is marginal.

The initial naturalized population will be quite small and fluctuate in population size. These fluctuations in size can substantially reduce  $N_e$  for a semelparous species with variable age structure, such as Chinook salmon (Waples 2002, Waples et al. 2010). Continuing gene flow into the population, through translocation, hatchery propagation, or hatchery rearing of source populations may increase  $N_e$  and total genetic diversity of the in-river population. These benefits, however, need to be weighed against: 1) potential detrimental effects to the source populations; 2) artificial selection of maladapted traits during hatchery propagation; 3) potential decrease in  $N_e$  due to the Ryman-Laikre effect; and 4) increased time it may take to establish locally adapted genotypes. Finally, although  $N_e$  is one of the standard measures used to assess long-term population viability, decline in heterozygosity and loss of allelic diversity can occur at very different rates (Allendorf and Luikart 2007). Therefore, to accurately assess the genetic diversity of a population over time,  $N_e$  estimates should not be used to the exclusion of other standard genetic diversity indices (e.g., allelic richness).

#### Summary of recommendations for effective population size

- *Equalize family size contributions and the sex ratio of breeders during hatchery propagation.*
- *Include precocious males during hatchery propagation to obtain the same relative number of offspring as they typically produce in the wild.*
- *Avoid dramatic fluctuations in population size between generations. This can be partially controlled during hatchery supplementation.*
- *For all populations affected by the Program, frequent monitoring of  $N_e$  and other standard genetic diversity indices is recommended (see Section 5 and the appendix for specific monitoring and methodology recommendations).*

## 4.2 Gene flow, inbreeding/outbreeding depression, and potential population effects

### 4.2.1 Gene flow

Gene flow among spring-run populations and introgression between fall- and spring-run ESUs may occur, either intentionally or unintentionally, during the restoration process. While many studies have been conducted on this topic, the genetic consequences of inter-population or inter-ESU mating are currently impossible to predict ahead of time. Hybrids can show reduced, equal, or higher fitness than “pure” populations depending on the interaction between the environment and their genetic makeup (Arnold and Hodges 1995). The primary advantage of population interbreeding is an increase in genetic variation and subsequent increased ability to adapt to new environments along with reduced risk of inbreeding depression. Disadvantages include

an increased risk of outbreeding depression and homogenization of genetically distinct populations.

For the purposes of this restoration effort, we define “hatchery” fish as spring-run Chinook that were propagated in a hatchery setting and “natural” fish as spring-run Chinook that have recolonized or returned to the upper San Joaquin River and were not propagated in a hatchery during any life stage. Gene flow between the following distinct fish groups/populations/ESUs is possible during the course of spring-run Chinook restoration to the upper San Joaquin River:

- 1) Strays and natal homing fish
- 2) Hatchery-propagated and natural fish
- 3) Different source populations
  - a. During hatchery propagation
  - b. After in-river outplanting
- 4) Spring-run and fall-run

As suggested by Ryman (1991), acceptable levels of gene flow are related to naturally occurring levels, i.e., those levels occurring without human interference. However, given that this spring-run reintroduction effort is not truly “natural”, an acceptable level of hybridization for each potential hybridization scenario is difficult to ascertain ahead of time. A modeling study may be done in the future to clarify potential genetic consequences given varying hybridization scenarios.

#### 4.2.2 Straying and homing

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Straying of Chinook salmon occurs when individuals spawn at a location other than their natal origin; homing is defined as the behavior of returning to a formerly-occupied location as opposed to a novel location (Gerking 1959). Since a large portion of the existing salmon range was glaciated 10,000 – 15,000 years ago, most extant populations originated through the process of straying since that time (Quinn 2005). Therefore, straying should not be viewed as “unnatural” and complete prevention of straying would eliminate the species’ ability to (re)colonize suitable unoccupied habitat. Further, the beneficial exchange of genetic variation between populations can lower the risk of inbreeding. On the other hand, homing to natal sites may reinforce local adaptation and increase reproductive fitness. Transplant experiments have typically shown higher performance of local populations versus transplanted populations (Reisenbichler 1988). High levels of straying can lead to genetic homogenization between populations and the spread of maladaptive alleles. Even low levels of persistent one-way migration will lead to the loss of a local population’s unique advantageous alleles, if the proportion of migrants is greater than the allele’s selection coefficient (Grant 1997). The original source of the strays will experience depletion in adult escapement, which may consequently lower that population’s abundance and genetic diversity, thereby negatively affecting their VSP potential. A central debate among salmonid geneticists is how much straying should be allowed for a given population to promote genetic exchange while not hindering local adaptation and reproductive fitness (Grant 1997).

Straying is anticipated to occur both into and out of the Restoration Area. Straying combined with reproduction will lead to gene flow and may alter the genetic diversity of the restored population and other populations experiencing strays originating from the Restoration Area.

Chinook salmon that may stray into the upper San Joaquin River include fish:

- 1) of hatchery origin (e.g., from Feather River Hatchery)
- 2) spawned in other tributaries of the San Joaquin or Sacramento river basins

Strays out of the Restoration Area may include fish:

- 1) of hatchery origin (i.e., from SJRRP Conservation Facility)
- 2) that are directly transferred from source population(s) to the Restoration Area
- 3) that are naturally spawned in the upper San Joaquin River

As stated in the FMP (SJRRP 2010b), typical straying rates for Chinook salmon are not precisely known. Straying among wild populations can be quite variable across populations, but most likely is considerably below 5% for non-local populations to maintain both neutral and adaptive alleles (Grant 1997). While there are a small number of reported stray rates for fall-run, there are no reported estimates for naturally occurring spring-run populations. In the Feather River HGMP, Cavallo et al. (2009) reported straying rates of 3.13% for hatchery reared spring-run fish from the FRH released into San Pablo Bay, and only 0.02% for fish released in-river. FRH fall-run experienced approximately 10% straying out of the system at this time (Cavallo et al. 2009). Several previous genetic studies have not detected genetic differentiation among Central Valley fall-run populations, with the exception of the Merced River Hatchery and late fall-run (Banks et al. 2000, Garza et al. 2008), so it is unclear how extensive straying of non-hatchery fall-run Chinook salmon is throughout the Central Valley. Based on available reported straying rates, we infer that spring-run Chinook are less likely to stray into the upper San Joaquin River than fall-run Chinook salmon. However, small numbers of spring-run Chinook with Feather River Hatchery CWTs have been found straying into San Joaquin basin tributaries annually along with other unmarked spring-returning Chinook salmon. Fall-run Chinook from all Central Valley hatcheries are found in San Joaquin tributaries annually. It is possible that natural fish differ in straying rates compared to hatchery fish, and Quinn (2005) states that ~ 95 – 99% of all salmonids accurately home.

Another factor affecting straying rates is the amount of water flowing from a river into the ocean. Returning adult salmon depend on olfactory cues to guide them back to their natal waters (Dittman and Quinn 1996). With increased water exports for use outside the river system, there are reduced in-river flows. A study outplanted fall-run Chinook salmon from the Merced River Hatchery into several San Joaquin basin tributaries and found that straying was positively correlated with water export rates at the CVP and SWP export pumps, and therefore with lower in-river flows. High export

rates were associated with 11 – 17% straying rates while low export rates were associated with < 3% straying rates (Mesick 2001). Since export rates are not controlled by the Program, high export rates may sometimes occur; however these data point to the importance of maintaining adequate water flows in the San Joaquin River.

Another major human induced cause of straying is trucking of smolts from hatcheries to downstream areas. This is done to reduce outmigrating smolt mortality rates. For example, since 2002 50% of spring-run Chinook from the Feather River Hatchery have been trucked and released into the San Francisco or San Pablo bays and this trucking has resulted in higher overall survivorship of these fish as returning adults (Cavallo et al. 2009). Trucking smolts to increase survivorship, however, almost certainly comes at the cost of increased rates in straying. For instance, the hatchery component of the Central Valley fall-run Chinook ESU is frequently trucked and released off-site closer to the ocean. This ESU, with spawning locations in both the Sacramento and San Joaquin river basins, is considered a single panmictic population and one of the primary factors likely responsible for the ESU's genetic homogenization is increased straying due to trucking (Williamson and May 2005). Therefore, to allow the reintroduced population to successfully home to the Restoration Area in high proportions, we do not recommend trucking to distant locations. However, localized trucking is a viable option for particular high mortality “hotspots” and we recommend that studies be conducted to identify these locations.

Straying and gene flow rates may not be 100% correlated due to higher fitness of the local population, assortative mating strategies, or heritable straying. For example, a chum salmon (*Oncorhynchus keta*) study on Vancouver Island found that gene flow between straying and homing fish was substantially lower than expected based on stray presence in spawning areas (Tallman and Healey 1994). The reproductive success and interbreeding of the in-river target population and strays should be assessed to determine the true genetic consequences of straying. If all emigrating juveniles and immigrating adults are genetically screened using a SNP panel, as discussed in Section 6 and the appendix, individual identification will be possible and an estimate of gene flow rates between straying and homing salmon may occur.

Salmon sometimes stray into an area but leave prior to spawning, thereby “testing the waters” (Quinn 2005). These salmon can sometimes become trapped during their explorations by hatcheries or physical barriers (e.g., weir) and not be allowed to accurately home. It is recommended that spring-run Chinook be allowed to volitionally leave the Restoration Area until the appropriate seasonal time that a weir must be in place to prevent spring- and fall-run introgression, determined based on life history timing for Central Valley Chinook salmon (See Table 1). Operation of a weir is outside the scope of this GMP but the timing of weir placement will have substantial consequences for introgression.

Straying can result in introgression between wild and hatchery stocks. This is a concern for conservation and management, potentially lowering overall fitness in the wild (Hindar et al. 1991). Long-term straying will cause neutral alleles, and likely all but the most reproductively advantageous alleles, to be lost in the local population (Grant

1997). Short-term (1 – 2 generations) or inconsistent straying will likely not have as great an effect on recipient populations since natural selection may remove maladapted alleles (Grant 1997). Some salmon studies have found that hatchery fish tend to stray more frequently than natural origin fish (McIsaac 1990, Labelle 1992), while others have not detected a significant difference in straying rates (Jonsson et al. 1991, Potter and Russell 1994). If Feather River Hatchery spring-run fish are used as a restoration source population, then unintended straying from or into the Feather River will be impossible to determine without the use of genetic PBT (see appendix) or distinct physical tags. A physical mark that distinguishes San Joaquin River hatchery produced fish from other hatcheries would be ideal for identifying and removing hatchery strays. Naturally spawned outmigrating smolts will not be physically marked; however we recommend obtaining a genetic sample of all emigrating smolts to enable identification of these fish as returning adults through PBT. The genetic homogenization of the Central Valley fall-run ESU also necessitates genetic PBT or otolith microchemistry to distinguish straying and homing fish in the Restoration Area. All unmarked fish in the upper San Joaquin River should be assumed to be from non-target populations, unless genetic or otolith analysis indicates the origin of the fish is from the in-river target population.

#### 4.2.3 Inbreeding and outbreeding depression

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Both inbreeding and outbreeding can cause deleterious changes in fitness. Inbreeding depression is defined as a decrease in fitness due to the mating of related individuals (Wright 1977, Charlesworth and Charlesworth 1987). In the context of reintroduction, factors that can increase inbreeding are small numbers of breeding individuals, unbalanced sex ratios, and genetically homogenous source populations (Fave et al. 2008). Inbreeding is especially problematic in small isolated populations, which may historically have relied on some degree of gene flow from neighboring populations (Sato and Harada 2008). In fact, it has been shown that a small number of immigrant individuals can greatly increase the fitness of an inbred population, so called “genetic rescue” (Tallmon et al. 2004). Annual supplementation of Chinook salmon to the in-river population during the Reintroduction and Interim periods could be of benefit in the absence of natural straying. It is recommended that levels of natural straying be closely monitored through genetic means. In addition, the level of inbreeding should be assessed both during the initial period of reintroduction during which supplementation from source populations will occur annually and after supplementation ceases. An estimate of relatedness— $F_{IS}$ —should be calculated from microsatellite markers and/or SNPs and used to estimate inbreeding levels for individuals collected from source populations for reintroduction purposes as these individuals will have unknown parentage. Pedigrees give a more accurate estimate of inbreeding levels than  $F_{IS}$  so  $F$  should be estimated using known family relationships whenever possible (Pemberton 2004). Therefore, for subsequent generations reproducing in the river or at the Conservation Facility, in addition to  $F_{IS}$ , PBT should be used to reconstruct pedigrees and obtain a more accurate measure of the inbreeding coefficient ( $F$ ). Inbreeding should be considered a problem if  $F$  rises above 0.25 (Margan et al. 1998). If inbreeding levels are significant, additional supplementation should be considered as a strategy to reduce the likelihood of inbreeding depression. For a hatchery population, starting with a



diverse founder stock is an important factor in reducing inbreeding. The founder stock refers to the individuals taken from source stocks and placed into the conservation hatchery to be used for breeding or transferred directly into the reintroduction area. The goal is for this founder stock to have comparable diversity to the source populations, as measured by population parameters such as allelic richness,  $F_{IS}$ ,  $F_{ST}$ , heterozygosity, etc. Equalizing sex ratios in the founder stock is another factor to consider for preventing inbreeding. Simulations of fish populations with similar breeding structure (high fecundity, overlapping generations, etc.) to Chinook have demonstrated that skewed sex ratios in a reintroduced population can increase inbreeding (Fave et al. 2008). In addition, the results of the Fave study indicate that supplementation over several years can also greatly reduce inbreeding as opposed to a single reintroduction event.

Outbreeding depression is the loss of fitness and productivity due to hybridization between members of genetically dissimilar populations of a species. Outbreeding depression is thought to occur through disruption of local adaptation to a particular environment or the break-up of co-adapted gene complexes. Outbreeding depression is increasingly recognized as a cause of reduced fitness in reintroduced vertebrate populations, especially those which have been assembled using individuals from different donor populations (Leberg 1993, Marshall and Spalton 2000, Edmands 2007). For example, crosses between pink salmon (*Oncorhynchus gorbuscha*) from spatially distant populations in Alaska produced reduced return rates in the  $F_1$  generation (Gilk et al. 2004). Additionally, the negative fitness consequences for outbreeding are often not realized until two or more generations later (Edmands 1999). While outbreeding depression can have serious fitness consequences, outbreeding does not always lead to outbreeding depression (Edmands 2007). A meta-analysis of outbreeding experiments in fish demonstrated variable responses to outbreeding and concluded that it is not currently possible to predict with any certainty cases in which outbreeding depression will result (McClelland and Naish 2007). For the San Joaquin River system, there is a potential risk that outcrossing (occurring either in the hatchery or in the river) could lead to maladapted individuals that perform poorly in the new environment of the Restoration Area; however, it is impossible to determine beforehand if outcrossing will result in reduced fitness for these fish.

Theory (Barton 2001, Edmands 2002) suggests that in cases of hybridization that produce changes in fitness, the magnitude of these fitness consequences can possibly be predicted by the extent of divergence between the original populations (i.e., less divergence will lead to less negative fitness consequences). Ideally, divergence comparisons should include life history, environmental, and genetic information. When comparing genetic differentiation levels ( $F_{ST}$ ) among Central Valley fall- and spring-run populations, Garza et al. (2008) report that differentiation is quite small (see Table 4 of this GMP) in comparison to other studied salmonid populations, with a mean pairwise  $F_{ST} = 0.021$ . This may be encouraging for lower risk of reduced fitness related to hybridization from restoration efforts. However, lack of substantial genetic differentiation at neutral loci does not indicate that local adaptation and selection is not occurring within populations. Specific alleles that are selectively advantageous can quickly



become established, although this introgression may be difficult to detect (Barton 2001, Fitzpatrick et al. 2010). Hundreds of markers spanning the genome may be necessary to detect signatures of selection in natural populations. Advantageous alleles can have large impacts on fitness, with their effect varying across environments.

If independent source populations are propagated in a hatchery, they may be intentionally interbred to increase genetic diversity and reduce relatedness between mated pairs. The decision to intentionally conduct inter-population hatchery propagation is currently being debated. Ideally the hatchery would have equal numbers of fish from the different donor stock populations in sufficient numbers to allow all possible crosses between source populations, along with pure crosses within the sources, to be made. This would increase population genetic diversity while allowing an assessment of reproductive fitness of returning fish from the various mating schemes. However, due to the likely small numbers of individuals from certain donor stock populations and the probable inclusion of FRH fish, it is best to keep donor population stocks separate to avoid possible outbreeding depression and the possibility of overwhelming the underrepresented genotypes from source populations contributing relatively fewer individuals. Given the FRH spring-run's genetic similarity to fall-run, limiting crosses between FRH fish and other source populations is recommended in order to preserve the genetic integrity of spring-run. It is recommended that small-scale breeding studies or examination of populations already hybridizing in the wild be conducted prior to large-scale intentional hybridization to examine the risks of outbreeding depression for these populations. Since intrinsic outbreeding depression does not become evident until the F<sub>2</sub> or later generations, these studies should be conducted for multiple generations.

The risk of outbreeding depression needs to be weighed against the potential benefits of outcrossing. In some situations, hybrids actually outperform their parental types (so-called heterosis or "hybrid vigor") or allow novel combinations of genes that confer some sort of adaptive advantage in the new environment (Rieseberg et al. 2003). While heterosis may occur in the F<sub>1</sub>, outbreeding depression may emerge in the F<sub>2</sub> or beyond. Outcrossing could inject needed genetic diversity into broodstock populations and mitigate for low genetic diversity captured in the early mining of source populations, which have been shown to have low to moderate levels of genetic diversity (though no strong indications of extensive inbreeding; Banks et al. 2000, Garza et al. 2008). Intentional outcrossing should be considered if significant inbreeding is observed in the hatchery or in the natural in-river population.

According to Frankham (2010) "predicting the risk of outbreeding depression is the most important unmet scientific challenge in the field [of conservation genetics]". Until outbreeding depression can be accurately predicted, reintroduction efforts will need to continue to weigh this risk with that of lower genetic diversity and inbreeding depression. Currently, we recommend not intentionally interbreeding genetically differentiated populations. We agree with Edmands (2007) that intentional hybridization between populations should not be conducted without evidence of inbreeding depression, except on an experimental basis. Even if intentional inter-population crosses are not conducted, there is still a high likelihood that spring-run populations will hybridize in the Restoration Area. Additionally, spring-run straying into and out of the

Restoration Area may breed with spring-run Chinook originally from other tributaries in the Central Valley.

#### 4.2.4 Summary of potential genetic consequences of gene flow to specific populations

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##### 4.2.4.A Source populations

It is a high priority that source populations not be compromised by the collection of individuals for reintroduction (see Section 5.1). Inbreeding depression could impact the source populations if sampling is biased (e.g., takes from limited area of spawning grounds or unintentionally skews sex ratios) or take levels are too high. Ways to collect samples in a non-biased manner are discussed in Section 5.1 and sustainable take guidelines will be established by federal fisheries agencies via the permitting process and guidance from NMFS. Outbreeding depression is not likely to occur in the source populations as they are only donating individuals to the restoration and are not receiving any new individuals other than potential strays, which will hopefully be kept to a minimum by following best hatchery practices.

Depending on the reintroduction method(s) employed, fish transplanted from source populations may experience greater straying and potentially even return to their ancestral location (McIsaac and Quinn 1988, Pascual and Quinn 1994). During early phases of reintroduction, returns to ancestral locations are not likely to have negative genetic impacts on either the source population(s) or the restoration population unless a large proportion of spring-run Chinook stray out of the Restoration Area, thus lowering the effective population size of the reintroduced population. Low levels of continued exchange will not have a negative genetic effect, although diseases may be transmitted from one population to another. During later reintroduction phases, as local adaptation occurs, increased rates of straying may become more genetically unfavorable for the donor populations. In general, straying of spring-run Chinook salmon with FRH ancestors may also be more genetically unfavorable due to increased risks from hatchery straying mentioned previously. Additionally, due to the partial heritability of run timing observed in salmonids (Smoker et al. 1998), introgression with fall-run Chinook in the FRH is a concern for maintaining a consistent spring-run phenotype for any spring-run fish straying from the Restoration Area into the Feather River. Straying out of the Restoration Area will most likely occur in highest proportion into nearby tributaries (e.g., Merced, Stanislaus, Tuolumne). This stepping stone model is the most common circumstance observed in other regions (Grant 1997). It is possible that habitat conditions in these other tributaries will result in high mortality of straying fish, less chance of spring-run mating due to low density, and increased risk of introgression with fall-run Chinook salmon. Alternatively, straying into these tributaries may enable recolonization of these tributaries by spring-run, if biotic and abiotic conditions are favorable.

##### 4.2.4.B Hatchery population

For the hatchery population, inbreeding and outbreeding depression are both potential concerns. If inbreeding occurs in the hatchery population, there is a risk of losing source population genetic diversity. For this reason, it is recommended that matings between closely related individuals be avoided, by estimating relatedness ( $F_{IS}$ ) between individuals prior to spawning them. This requires that the hatchery have adequate holding facilities such that breeding-age individuals can be held for a period of days while samples are analyzed for genetic determination of relatedness. When the parentage of breeding individuals is known (via PBT), crosses between closely related individuals (e.g., sibs, half sibs) is easily avoided. When one or more of the breeding individuals is of unknown parentage, estimates of relatedness can be determined using molecular markers, such as microsatellites or SNPs (see appendix for methodology). As discussed above, outbreeding may have a deleterious effect on hatchery outcomes and is only recommended on an experimental basis or if inbreeding is detected in the hatchery population.

Mounting evidence has demonstrated that traditional hatchery origin fish and their progeny often have considerably reduced fitness in the wild (McGinnity et al. 2003, Araki et al. 2007b). Though natural recolonization of the Restoration Area may be possible, it is extremely improbable that a sufficiently diverse, self-sustaining population would colonize the Restoration Area in the foreseeable future. Therefore, all or almost all reintroductions will occur from either hatchery supplementation or direct transfer from chosen source populations. Because the direct transfer of source populations may include use of fish from the Feather River, both approaches—independently or collaboratively—may intentionally reintroduce hatchery origin fish during the Reintroduction Period and potentially the Interim Period of the restoration process.

#### *4.2.4.C In-river population*

Inbreeding and/or outbreeding may affect the in-river population. As discussed above, small isolated populations are susceptible to inbreeding. As part of continued genetic monitoring, inbreeding coefficients should be estimated annually through PBT-reconstructed pedigrees or calculations of  $F_{IS}$ . If a trend of increasing inbreeding coefficients becomes apparent, managers may want to consider additional supplementation from the source populations. Outbreeding depression may have an effect on the in-river population, especially if multiple source populations are used for reintroduction. See Section 5.3 of this document for further discussion of the in-river population.

Straying of hatchery origin fish from other tributaries into the Restoration Area and subsequent interbreeding with natural origin fish is a concern. From a genetic standpoint, long-term hatchery supplementation and gene flow between hatchery and natural fish may threaten the fitness of the reintroduced population by limiting adaptation to the natural environment. If conservation hatchery methods are properly implemented, however, the benefits of short-term hatchery supplementation may exceed the potential risks. It is encouraging that a steelhead supplementation study of the Hood River in Oregon found that captive-bred steelhead (local origin, single generation) had identical reproductive fitness as wild steelhead, and increased

population size (Araki et al. 2007a). This study along with a recent meta-analysis of fitness consequences after hatchery stocking (Araki and Schmid 2010) emphasize the point that not all hatchery supplementation is likely to be equally harmful or beneficial and improved hatchery practices are critical for long-term success. Long-term effects of supplementation remain untested and are almost certainly case-specific so the intentional use of hatchery origin fish should proceed cautiously and with extensive monitoring of fitness consequences that can be directly attributed to hatchery influence. See sections 6.2 and 6.3 for specific recommendations regarding genetic monitoring of the hatchery and in-river populations. Specific genetic concerns that may be associated with hybridization between hatchery and natural fish include: reduced effective population size, loss of intrinsic/extrinsic genetic adaptations, and increased genetic load due to domestication selection. These negative consequences may be ameliorated by minimizing gene flow with hatchery origin fish, if at all possible, once the reintroduced population has become established and no longer requires annual supplementation. This will provide the reintroduced population the opportunity to undergo natural selection to the local environment.

It is quite likely that some degree of hybridization will occur within the Restoration Area between source populations, unless positive assortative mating inhibits these matings. We recommend monitoring the degree of hybridization across all potential groups annually to inform both the adaptive management process and the scientific community. Detecting low levels of hybridization can be challenging but the robust monitoring recommended in Section 6.3 provides the best opportunity to do so. It will be particularly interesting to monitor reproductive fitness levels of between versus within source population matings using PBT since hybridization can sometimes increase fitness in particular environments.

Given the geographic distance between the Restoration Area and Butte, Mill, and Deer creeks, it is unlikely that substantial straying will occur between non-hatchery spring-run populations and the restored in-river population. Levels of straying comparable to those in natural populations may be beneficial to facilitate increased genetic diversity and reduced inbreeding risk and should therefore not be discouraged.

#### 4.2.4.C.1 Introgression between spring- and fall-run Chinook salmon

There is considerable overlap in spawning periods of fall-run (late September to December) and spring-run (late August to October) Chinook in the Sacramento River basin (see Tables 1 and 4, Section 2). This spawning overlap will likely also occur in the San Joaquin River basin after spring-run Chinook restoration, since fall-run in the Tuolumne River spawn from late October to January (Yoshiyama et al. 1998, Moyle 2002). Therefore, introgression between fall- and spring-run may occur in both the Sacramento and San Joaquin River basins. Historically, spring-run spawning grounds in the San Joaquin River were spatially distinct from fall-run spawning sites. Because the location of Friant Dam has eliminated access to historic spring-run spawning sites, spring- and fall-run spawning sites may overlap significantly. Specific genetic concerns associated with hybridization between spring- and fall-run ESUs include: loss of ESU-

specific intrinsic/extrinsic genetic adaptations, and most importantly, loss or reduction in the spring-run ESU's ability to consistently maintain a spring-running phenotype.

Genetic differentiation among spring- and fall-run ESUs: Spring-run populations differentiation from all sampled fall-run pairwise comparisons is in the range of  $F_{ST} \sim 0.008 - 0.030$ . As discussed in Section 2, of the Central Valley spring-run populations, Butte Creek Chinook are the most genetically differentiated from fall-run Chinook salmon. Of all fall-run sampled sites, Merced River Hatchery fall-run samples are the most genetically differentiated from all spring-run populations. Therefore, unintentional hybridization between Butte Creek spring-run and any fall-run (particularly Merced River Hatchery fall-run) may pose the highest relative risk of outbreeding depression. Despite spatial and temporal overlap of Chinook salmon spawning runs in the Central Valley, no evidence for natural hybridization among runs has been documented (Banks et al. 2000, Garza et al. 2008), with the exception of the Feather River (Garza et al. 2008) which is almost certainly due to Feather River Hatchery operations (Sommer et al. 2001). Lack of documentation, however, is not evidence that hybridization is not occurring between these ESUs in non-hatchery influenced environments, since low levels of introgression can be quite difficult to detect. Although the Feather River Hatchery is currently taking steps to create a more segregated spring-run population, outplanting FRH-origin fish into the Restoration Area will lead to the intentional introduction of some degree of fall-run genes into the restored spring-run population. To maintain phenotypic integrity, fall-run and spring-run should not hybridize at all or at only very low levels, preferably well below 5%. To facilitate continued segregation between the two run types, we recommend that hybridization between spring- and fall-run is prevented, or at least severely limited, in the Restoration Area through the use of segregation barriers.

### Summary of recommendations for gene flow, inbreeding/outbreeding depression and population effects

- *Ideally, conduct a limited number of crosses between source populations, spanning two or more generations, to assess the fitness consequences of hybridization. Intentional mating between source populations should be avoided except on an experimental basis.*
- *Limit gene flow between non-SJR hatchery and natural populations, once annual supplementation is no longer necessary through monitoring at weirs and use of best hatchery practices.*
- *Gene flow between populations attributed to restoration efforts should only occur at levels comparable to natural rates (<5%).*
  - *Intentional hybridization should only be conducted if populations are showing clear signs of inbreeding depression.*
  - *If intentional hybridization is conducted, only spring-run populations with low genetic and adaptive differentiation should be interbred.*
  - *Hybridization between spring- and fall-run should be prevented through the use of effective barriers spatially segregating ESUs.*
- *In-river monitoring of hybridization should be conducted annually, as part of standard genetic monitoring.*
- *All SJR hatchery fish should be physically marked if feasible.*
- *Use best biological practices to ensure proper imprinting and homing to natal river (e.g., release in upstream portion of Restoration Area, maintain proper flow rates, provide attractive habitat and migration routes. See HGMP for details).*
- *Do not truck outmigrating smolts to distant locations; though localized trucking past identified mortality hotspots is acceptable.*
- *Attempt to remove straying hatchery fish from system:*
  - *if Restoration Area hatchery fish can be visibly distinguished from other hatchery fish.*
  - *if the SJRRP does not use a hatchery for propagation.*
  - *once intentional Restoration Area hatchery propagation ceases and the final conservation hatchery generations have successfully returned.*
- *Obtain a genetic sample of all emigrating smolts and immigrating adults to estimate rates of straying and gene flow (see Section 6.3)*
- *During hatchery propagation, avoid intentionally mating closely related individuals to prevent inbreeding depression*
- *Genetic monitoring should include an annual estimate of inbreeding coefficients of the in-river population, determined through PBT-reconstructed pedigrees and estimates of  $F_{IS}$ .*
- *Trends of increasing inbreeding coefficients in the in-river population should be identified and addressed through supplementation from source populations ( $F$  should not be allowed to rise above 0.25).*



### 4.3 Disease, contaminants, habitat, and other environmental factors

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#### 4.3.1 Diseases in California salmonids

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There are several infectious diseases of California salmonids that can affect Chinook salmon. These include furunculosis caused by *Aeromonas salmonicida*, microsporeosis caused by *Loma salmonae*, bacterial kidney disease caused by *Renibacterium salmoninarum*, vibriosis caused by *Vibrio anguillarum* and *V. ordalii*, proliferative kidney disease (PKD) caused by *Tetracapsuloides bryosalmonae*, whirling disease caused by *Myxobolus cerebralis*, and infectious hematopoietic necrosis viral disease (IHNV). Due to the lack of accurate and non-invasive tests, it is not feasible to screen all fish that appear healthy for all of the above diseases. Sacrifice of a subset of individuals for pathology and testing is one option for disease monitoring, however the number of fish may be very small from some source populations and may be deemed too precious to sacrifice for this purpose. Thus, in order to prevent introduction of pathogens into the hatchery or Restoration Area, individuals should be selected from populations with no known disease issues. Quarantine is also a recommended precaution, as well as vaccination against furunculosis when feasible (Woodford 2000). Preventing the introduction of diseases to the Restoration Area—especially in the conservation hatchery—is of the utmost importance, as it is very difficult to eradicate a disease once it has become established in a wild population. Infectious diseases such as PKD have caused high mortality in California hatcheries in the past and could pose a threat to the San Joaquin River hatchery population (Hedrick et al. 1984). PKD is endemic to the Merced River, part of the San Joaquin River basin (Foott and Hedrick 1987). It is likely unavoidable that fish naturally recolonizing the San Joaquin will bring some infectious agents with them. However, selecting disease-free fish for the hatchery should be a top priority, as disease can be a large problem in dense rearing conditions.

#### 4.3.2 Disease and genetics

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There are several studies demonstrating the genetic basis of disease resistance in Chinook salmon. There is variable disease resistance both within and between populations (Chevassus and Dorson 1990). Resistance to vibriosis and whirling disease both have genetic components, as demonstrated by differential familial susceptibility to the diseases (Beacham and Evelyn 1992, Schisler et al. 2006). Major histocompatibility complex (MHC) diversity has been identified as one genetic factor that influences disease resistance in salmonids. While there are no published studies correlating spring-run MHC diversity with disease resistance, winter-run Chinook heterozygotes for MHC class II loci had lower disease susceptibility to *V. anguillarum*, IHNV, and *M. cerebralis* than did homozygotes (Arkush et al. 2002). If MHC genetic diversity is lost, it is likely that disease susceptibility will increase. The reintroduction of individuals with high MHC diversity will likely increase the new San Joaquin River population's ability to withstand challenges from infectious agents. MHC studies of California Chinook salmon have focused on comparisons of allele frequencies between the four runs and not between different populations within the spring-run ESU. Of the three major source

populations being considered for reintroduction—Butte Creek, Mill/Deer creeks, and the Feather River Hatchery (see Stock Selection Strategy document; SJRRP 2010c)—there is published data on MHC heterozygosity for Butte Creek. Examination of 13 Butte Creek spring-run individuals indicated that MHC class II heterozygosity was comparable to other Sacramento River Chinook ESUs (Kim et al. 1999). Further study comparing the MHC diversity among the potential source populations would be helpful in informing reintroduction. However, selection based on MHC loci is not recommended at this time. Instead it is a higher priority for the reintroduced population to have an overall high level of genetic diversity which will be determined via neutral markers. MHC diversity is just one genetic component of a host's ability to combat disease. The overall goal of obtaining and maintaining as much genetic diversity as possible will provide the reintroduced population with the best chance of withstanding the potential onslaught of a wide range of diseases.

#### *4.3.3 Pollutants and contaminants*

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Agricultural and urban runoff is an increasing problem for water quality and fish health worldwide. The San Joaquin River has significant levels of organic and inorganic contaminants due to runoff, which is especially of concern in the Sacramento-San Joaquin Delta, where many juvenile Chinook are thought to reside for considerable periods of time before outmigration to the ocean (Pereira et al. 1996). Fish raised in agricultural subsurface drainwater that is discharged into the lower San Joaquin River experience significant mortality (Saiki et al. 1992). Exposure to aromatic and chlorinated compounds that are commonly found in urban estuaries causes increased incidence of vibriosis in Chinook (Arkoosh et al. 2001). Juvenile salmonids have been found to bioaccumulate chlorinated and aromatic hydrocarbons which can lead to immunosuppression and increased disease susceptibility (Arkoosh et al. 1998). While contaminants are not a threat unique to the reintroduced population, as the Delta is a habitat shared by all Central Valley Chinook populations, the upper San Joaquin River may face increased contamination risk. The restoration area is flanked closely by agricultural lands and agricultural runoff could pose a significant challenge for the reintroduced population as it is struggling to take hold. Pyrethroid insecticides used in agriculture are extremely toxic to fish, causing high mortality in the larval and juvenile stages and reduced development and growth at sub-lethal levels (Amweg et al. 2005). In addition to direct effects on fish, pyrethroid insecticides may also indirectly affect juvenile fish by eliminating aquatic invertebrates which serve as a major food source (Haya 1989). Selenium contamination in particular is known to be high in the San Joaquin River system and can be a serious threat to wildlife, causing developmental defects in many species and local extinction in others (Luoma and Presser 2000). Because continuous water flows have just recently been reestablished for the upper San Joaquin River, there are only a few field seasons (fall 2009, spring and fall 2010) of data on contaminant load in the river. The Program has developed a Water Quality Monitoring Plan (SJRRP 2010a) and corresponding Quality Assurance Plan. Monitoring of contaminants in the river started during the interim flows of fall 2009, and has continued during all interim flow periods. Grab samples and real-time monitoring is conducted throughout the Restoration Area to determine if contaminant “hot spots” exist.



#### 4.3.4 Habitat

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The success of the reintroduced population is largely dependent on the presence of appropriate habitat. It is important that carrying capacity of the existing habitat for each life stage is evaluated and that reintroduction numbers fall below this capacity (Bjornn and Reiser 1991), see Table 5 of this GMP for predicted adult carrying capacity ranges. Lack of adequate habitat can cause high mortalities which would be detrimental to the genetic diversity of the introduced population. Spawning gravels are the main habitat type that is required in the upper San Joaquin River. The quality and distribution of spawning gravels should be evaluated to ensure desirable gravel/sediment size, as this can affect the quality of egg and fry survival rates, along with the total number of eggs spawned in an area (Reiser and White 1988). While spawning gravels have not been shown to be a limiting factor for other populations in the Central Valley, riffles have been created in other tributaries such as the Merced River, and gravel restoration is important if adequate habitat is found to be lacking in the upper San Joaquin River (Kondolf et al. 1996). Water flow is another abiotic factor that can affect the survival during spawning and nursery periods for Chinook. Increased water flows during these developmental periods correlate with higher annual abundance indices (Stevens and Miller 1983). The availability of food in the Delta for juvenile Chinook is another potential limiting factor for recruitment (Jassby and Cloern 2000). Habitat is a large topic which is more comprehensively addressed in the FMP (SJRRP 2010b).

#### 4.3.5 Environment and genetics

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There is evidence of significant local adaptation in salmonids. Factors influencing development, growth, physiology, behavior, and life history have been shown to have a genetic component (Taylor 1991). Several studies have found traits with significant genotype-by-environment interactions, such as the jacking phenotype (Heath et al. 1994). The unique environment of the San Joaquin River may interact in distinct, and perhaps unforeseeable, ways with individuals locally adapted to different areas. This is something to keep in mind when selecting individuals for reintroduction. For example, thermal tolerance is thought to be a heritable trait. If the water temperatures in the San Joaquin River increase, potentially as a response to global climate change or as a result of increased demand for limited water resources, this may create a selective pressure for thermally tolerant individuals (Beacham and Withler 1991). Spawning time is another heritable trait in salmonids. This trait makes a population especially vulnerable to selection in hatcheries (Quinn et al. 2002). While there are many examples such as these in the literature about how habitat can affect Chinook populations, it is impossible to predict exactly what conditions will be like in the San Joaquin River and how the reintroduced population will respond to these conditions. Therefore it is of the utmost importance to reintroduce a genetically diverse population of fish that will have the highest likelihood of being able to cope with environmental challenges.

**Summary of recommendations for disease, pollutants, habitat and other environmental factors**

- *For reintroduction, individuals should be selected from disease-free populations when possible, with particular effort made to keep the hatchery population free of infectious agents.*
- *Quarantine and vaccination against furunculosis is recommended for fish selected from each source population when feasible.*
- *Water quality and contaminant levels should be closely monitored throughout the Reintroduction Area. If a concern, effects on in-river population should be evaluated.*
- *Adequate spawning gravels should be available in-river. If these do not exist, spawning gravels should be created.*
- *The re-introduced population should be genetically diverse to provide the necessary genetic building blocks to respond to selective pressures, such as environmental change.*

## SECTION 5 CONSERVING GENETIC DIVERSITY

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The Convention on Biological Diversity lists gene diversity as one of three levels of diversity, along with ecosystems and species, to be conserved and sustainably used (reviewed by Laikre *et al.* 2010). Genetic diversity is the genetic variation present in a group (e.g., population, metapopulation, or species). High genetic diversity is associated with a reduced risk of inbreeding depression and increased adaptive potential in both captive and wild populations (Frankham 2005). An example of the importance of conserving high genetic diversity within a species is the observed biocomplexity of aggregate populations of sockeye salmon in Bristol Bay, Alaska. The considerable life history variation within the stock complex is believed to have maintained the high productivity of the local fishery for over two decades despite changing environmental conditions (Hilborn *et al.* 2003, Schindler *et al.* 2010). Maintaining and promoting genetic diversity is an essential component to the success of spring-run Chinook reintroduction to the upper San Joaquin River. Important factors to consider when conserving genetic diversity for each of the population types (source, hatchery, in-river) are detailed below.

### 5.1 Conserving genetic diversity of source populations

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As detailed in the Reintroduction Strategy document (SJRRP 2011), the primary strategies being considered for reintroduction purposes are:

- 1) Reintroduction of donor stocks of various life stages directly into the San Joaquin River Restoration Area (i.e., translocation)
  - a. life stages collected: eggs, juveniles, or adults
  - b. life stages released into Restoration Area: eggs, juveniles, or adults
- 2) Reintroduction of cultured fish originally collected from available donor stocks (i.e., captive rearing)
  - a. life stages collected: eggs or juveniles
  - b. life stages released into Restoration Area: juveniles
- 3) Reintroduction of offspring of cultured fish originally collected from available donor stocks and reared to broodstock age (i.e., captive propagation and rearing)
  - a. life stages collected from source: eggs or juveniles
  - b. life stages released into Restoration Area: eggs or juveniles

The non-genetic advantages/disadvantages of each strategy are discussed extensively in the Reintroduction Strategy document and will not be reviewed in this GMP. While adult collections and translocations are under consideration, the Reintroduction Strategy document largely favored egg and juvenile collection and release given the potentially large negative impact adult collections could have on the source populations, with the exception of the Feather River Hatchery (SJRRP 2011).

The primary source populations being considered for reintroduction are the three most abundant spring-run Chinook salmon populations in the Central Valley: Butte Creek, Deer and Mill creek complex, and the Feather River Hatchery (SJRRP 2010c). Additionally, opportunistic collection may occur from other Central Valley tributaries (e.g., spring-run from Battle Creek, Clear Creek, Yuba River, potential strays into the San Joaquin basin, and fish salvaged from the Delta pumping facilities) (SJRRP 2011). The within and among population patterns of genetic diversity for each of the three primary source populations under consideration are summarized in Section 2 of this GMP and further details can be found in the recent Garza et al. (2008) report. Additional details concerning the historic and extant conditions, life history, population size, hatchery influence, and inter-basin transfers for each population can be found in the Stock Selection Strategy document for spring-run Chinook (SJRRP 2010c).

The TAC spring-run recommendations (Meade 2007) list the following criteria for consideration when deciding the most appropriate stock for reintroduction:

*(1) stock should be of local or regional origin from the Central Valley; (2) stock should be genetically diverse; (3) stock should take into account the status of the source population; (4) stock should not jeopardize existing Chinook salmon stocks in the San Joaquin basin; (5) stock should have life-history characteristics that maximize probability of successful reintroduction into the San Joaquin River; (6) stock should have behavioral and physiological characteristics that fit conditions expected to occur on the San Joaquin River; and (7) stock should not be of hatchery origin, except under extreme circumstances.*

The establishment of a San Joaquin in-river population offers a significant benefit for the spring-run Chinook ESU; as noted by Lindley et al. (2007), “Central Valley spring-run Chinook salmon fail the representation and redundancy rule for ESU viability,” due to the low number of populations, their spatial arrangement, and the risks of regional fires or environmental stochasticity. Risks to the source populations from collection in years with low census numbers must be weighed against the representation and redundancy benefits to the ESU as a whole. The full benefit only accrues when the newly reintroduced population captures much of the existing diversity in the source populations. This improves the likelihood that sufficient diversity will be present upon which selection can act to improve the reintroduced population’s overall fitness.

It is of primary importance that the reintroduction of spring-run Chinook salmon to the Restoration Area has minimal deleterious consequences for the source populations. In fact, this reintroduction effort will hopefully lead to continued persistence of the Central Valley spring-run ESU by creating another VSP outside of the Sacramento River basin. ESU representation and redundancy in the San Joaquin River may protect the ESU from extinction if a localized factor causes extirpation in the Sacramento River drainage. The following broodstock collection factors may affect existing genetic diversity of source populations:

- 1) Number of individuals collected

- 2) Life stage(s) collected
- 3) Selection on phenotypes
- 4) Spatial and temporal sampling
- 5) Collection methods

#### 5.1.1 Number of individuals to be collected

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Typically, large populations have greater allelic diversity compared to small populations, and, therefore, have greater genetic potential to respond to changing environmental conditions. To protect the genetic integrity of the source populations, it is recommended that the minimum number of individuals be collected from each source population that captures a large proportion of the genetic diversity found in that population. Recommendations regarding number of individuals to collect for broodstock are found in Section 6 of the spring-run Chinook HGMP for the San Joaquin River (Bork and Adelizi 2010) and the Reintroduction Strategy document (SJRRP 2011), which details the recommended number of individuals to be collected for each life stage (egg, juvenile, adult) in the context of each reintroduction method (hatchery propagation and/or rearing, translocation) to obtain adult targets mentioned below. As stated in the HGMP:

*While the Program is using the interim facility, and the full-scale hatchery is under construction, the Program will seek to collect enough juvenile fish and eggs each year to rear a total of 50 females and 50 males to breeding age, with the 100 relatively unrelated fish coming from up to three source populations, dependent upon availability. The Program should include fish from at least two of the potential broodstock source populations. Once the full-scale facility is in use, the Program will collect more broodstock; up to enough juvenile fish and/or eggs each year to rear up to 300 adult fish from each of the three source populations annually for four years, dependent upon availability. Returning naturalized adults may be incorporated into the broodstock, although returns are not expected until at least 2015.*

Specific take guidelines will be established by federal fisheries agencies via the permitting process and guidance from NMFS. The number of fish available will ultimately be limited by the health and abundance of each potential source population. It is quite likely that take numbers will fluctuate depending on annual adult escapement surveys. A balance will need to occur between the goals of protecting the source stock genetic diversity and building the genetic diversity of the reintroduced population. As noted in Section 6 of the HGMP “any effort to capture the genetic diversity of a source population inherently makes trade-offs between Program capacity (and resilience of the source population to fish collection) and the genetic diversity represented in the broodstock population.” (Bork and Adelizi 2010).

#### 5.1.2 Life stage(s) collected

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The life stage(s) chosen for collection may vary based on several factors, including the population status of each donor system, the potential impact to the donor population, the accessibility of each life stage, stipulations of collection permits, and guidance from the adaptive management process. As detailed in Section 6 of the HGMP, removal of 950 eggs from a Chinook salmon source population is the equivalent of removing a maximum of 1 – 3 adult spawners given the significant mortalities observed across life stages in the wild (Bork and Adelizi 2010), and may be equivalent to the removal of less than one adult (Quinn 2005). Therefore, it is recommended that the majority of individuals be collected at the egg or early juvenile (e.g., fry) stages to reduce negative impacts to source populations, particularly for the Butte and Mill/Deer populations. The life stage(s) chosen for collection, however, needs to be weighed against the potential negative impacts that collection methods may have on the source population (e.g., redd pumping for egg collection may reduce survival of remaining eggs in the wild). Furthermore, egg collection will increase relatedness among broodstock unless only a few eggs are collected per redd to minimize full-siblings and a large number of redds are sampled to obtain adequate broodstock numbers. Consider, for example, 100 eggs collected from a single redd (all individuals would be full- or half-siblings) versus 100 eggs collected from 100 different redds (all individuals would be unrelated or at least considerably less related). Juvenile collection is advantageous since it avoids concerns about redd disturbance and negative impacts to source population adult escapement is still low. Alternatively, adult collection is advantageous for avoiding extensive selection to captivity for hatchery propagation or significant mortality prior to mating in the wild for translocation. While collection of adults from the Butte and Mill/Deer populations may not be a feasible option due to typically low annual adult returns, adults may be collected from Feather River Hatchery spring-run Chinook spawning program.

### 5.1.3 Selection on phenotypes

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Interactions between an individual's phenotype and biotic and abiotic environmental factors can lead to differential fitness and are the central driver of local adaptation. Phenotypic characters that are advantageous in one setting at a particular time might be disadvantageous in another, since phenotypic diversity in a population is caused by a combination of genetic variation, the environment, and their interactions. Age and size at maturity, size at smoltification, and fecundity have all been shown to be partially controlled by genetic factors in salmonids (Carlson and Seamons 2008). As recommended by the TAC, spring-run source stocks should be chosen that have life history, behavioral, and physiological characteristics that maximize the probability for successful reintroduction to the San Joaquin River (Meade 2007). These characteristics are extensively evaluated in the Stock Selection Strategy document (SJRRP 2010c) for each of the primary source populations being considered for reintroduction purposes. However, there is a high likelihood of substantial, unpredictable selective pressures on the introduced fish and, therefore, it is generally recommended that collections do not target particular phenotypes that could skew the current diversity found in the source populations. Ideally, this non-targeted approach will leave the source populations with the same proportion of each phenotypic characteristic as was present prior to collection.

Listed below are several examples of selection on phenotypes and recommendations for avoiding selection. These examples are not exhaustive of the phenotypic characteristics that may be beneficial to evaluate. These examples were chosen based on their potential variation within source populations and their ability to impact fitness.

- **Body size:** The weight and length of an individual should not prohibit collection. Collection methods should not size-select (e.g., collection net mesh size).
- **Smoltification timing -- ocean-type (subyearling outmigrants) versus stream-type (yearling outmigrants):** Collecting a wide range of juvenile sizes will likely capture both juvenile life history types, which are not believed to segregate as spawning adults (P. Moyle, pers. comm.). Additionally, collection from redds or returning adults will capture both juvenile life history types, although ocean-type returning spawners are generally larger than stream-type returnees (Roni and Quinn 1995). Therefore, if performed randomly, with respect to phenotype and temporal/spatial sampling, all life stage collections should capture both types.
- **Spawn timing:** If adults are collected, individuals should be taken throughout the spawning season of this ESU (see Section 2 Table 1). Collection of eggs or juveniles that is sufficiently diverse (e.g., spatial/temporal and unrelated) should not cause a bias in regard to this phenotype.
- **Age of sexual maturation -- varies from two to five years of age:** If length is not selected upon, selection on age at maturity is also unlikely to occur. A notable exception is jacks, which will likely be smaller and demonstrate different mating strategies compared to older males. The precocious male life history strategy of Chinook salmon is believed to be under the influence of both genetic and environmental factors (Heath *et al.* 2002) and should be collected in proportion to the relative number of offspring they contribute to the source populations, if collections occur during adult spawning periods. If performed randomly, egg and juvenile collections should capture suitable proportions of individuals that will mature at different ages.
- **Egg size and fecundity:** Within population egg size and fecundity is partly influenced by fish length (Healey and Heard 1984, Kaufman *et al.* 2009). Collections of juveniles and adults should not skew source population egg size and fecundity proportions when following the above recommendations for fish size and age at sexual maturation. Collection of eggs should occur across individual egg and redd size ranges, again in proportion to the existing egg and redd size distributions found for each source population.



An exception to this recommendation of avoiding selection on phenotypes is that diseased or morphologically/skeletally deformed individuals should not be collected. Additionally, since fall-run Chinook co-occur in all proposed source locations, Genetic Stock Identification (GSI) should be performed prior to hatchery propagation or direct translocation to select for fish that are spring-run Chinook in origin. For juveniles and adults this can be accomplished by taking a fin clip while for eggs a small subset (~3 – 5 eggs) should be collected per redd to conduct GSI prior to translocation into the Restoration Area to ensure that only spring-run are used for reintroduction purposes (see the appendix for additional details regarding methodology).

#### 5.1.4 Spatial and temporal sampling

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It is uncertain if fine-scale genetic structure exists within potential spring-run source populations, since it has never been investigated with a large number of informative markers and spatially and temporally variable sampling within each system. A study of returning spawners of Chinook salmon reported extremely fine-scale homing to specific stream reaches in the Middle Fork Salmon River (Neville *et al.* 2006), which enabled fine-scale genetic structuring. Because of the potential for fine-scale structure, collection of eggs should occur throughout the spawning grounds, regardless of redd density, to increase the probability of collecting genetically distinct sub-populations, if they exist. Similarly, collection of juveniles should occur throughout the spring-run rearing grounds. In addition to spatial structuring, temporal structuring may occur if genetically related individuals are more likely to migrate to and from spawning grounds at particular times during each season. For instance, Atlantic salmon smolts have been shown to outmigrate in kin-structured groups (Olsen *et al.* 2004). It is recommended that individuals be collected throughout the spawning, rearing and outmigration season, depending on life stage(s), to capture any fine-scale temporal spawning diversity. The date, precise location, and number of individuals collected during each sampling event should be recorded. Fin clips should be collected for all older juveniles and adults for genetic analysis.

#### 5.1.5 Collection methods

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The primary impact of collection methods for source populations will be unintended mortality and bias during collection that inadvertently targets particular source individuals. Details of collection methods being considered for each source population can be found in Section 6 of the HGMP (Bork and Adelizi 2010) and the Reintroduction Strategy (SJRRP 2011) documents.

### Summary of recommendations for conserving genetic diversity of the source populations

- *Primarily egg and early juvenile life stages should be collected from the source populations. Collect from a large number of redds to reduce relatedness.*
- *Caveat to above recommendation: employ collection methods that reduce mortality and collection bias (e.g., if redd pumping/excavation is used, choose methods that will have lowest potential impact on uncollected eggs in each redd).*
- *Avoid selection of particular phenotypes during collection, except exclusion of diseased individuals.*
- *Genetic analysis should be conducted for all individuals (fin clips) or a subsample of eggs from each redd intended for reintroduction purposes to ensure samples are spring-run in origin.*
- *Conduct spatially and temporally diverse sampling during collection.*
- *Maintain records detailing the date, precise location, and number of individuals collected during each sampling.*

## 5.2 Conserving genetic diversity of the hatchery population

An Interim Facility and Conservation Facility, described in Section 1.2, are intended to be used in support of SJRRP reintroduction efforts, though final decisions have yet to be made regarding how many fish and from which potential sources these may be drawn. As described in Section 5.1, measures should be taken to maximize genetic diversity in collecting individuals from the source populations to form the foundation of the hatchery population, both for supportive rearing and broodstock applications. The conservation hatchery broodstock approach, in conceptual terms, aims to capture a representative sample of the genetic diversity in a source population; increase the census and effective size of that sample through a generation of hatchery breeding, while not changing the genetic makeup of the sample; and then introduce progeny into the river system where selection can act on phenotypes and their underlying genetic diversity. This approach is complicated by many factors, including but not limited to: domestication selection, difficulties in capturing a representative sample, and loss of diversity in the hatchery due to small effective population size. Any broodstock program is at best a compromise between the ideal approach and the realities of hatchery production.

Scientific studies of salmonid hatchery fish impacts on their wild population and ESU counterparts have mainly focused on fishery enhancement or production hatcheries that use external stocks on rivers with declining wild stocks (Araki and Schmid 2010). The SJRRP is in the position of reintroducing native spring-run Chinook salmon into newly-available (rewatered and restored) historic habitat, geographically removed from extant spring-run Chinook populations. Because the SJRRP must create a new population from very limited source stock options, practical considerations will likely weigh heavily in early reintroduction actions and decision-making. First,

consideration of basic needs associated with San Joaquin River population demography (providing sufficient numbers of founding individuals to avoid founder effects, the extinction vortex, Allee effects, and vulnerability to environmental or catastrophic events) will be necessary to increase likelihood of establishing a population in early phases of reintroduction. Second, the SJRRP is constrained by target dates mandated by the Settlement. Consequently, the Program is unable to await results from experimental studies prior to introductions; the Settlement requires the introduction of spring-run Chinook salmon into the river prior to December 31, 2012. This underscores the need for early restoration actions to be designed in keeping with the goal of maximizing the information gain such that results will inform both scientific understanding and adaptive management. Assuming efforts during the Reintroduction Period and Interim Period are sufficient to establish a VSP, hatchery operations will then shift accordingly, with a reduction of influence on the naturalized population leading to eventual phase-out (Section 7).

Conserving and promoting genetic diversity and integrity of the hatchery broodstock requires a careful balancing of two goals: maximizing survival of hatchery fish while minimizing their negative impacts on wild fish. Achieving these goals can be done through: maintaining genetic diversity and population size, achieving the proper genetic integration with natural populations, minimizing domestication selection, and promoting local adaptation.

#### 5.2.1 Maintaining genetic diversity in the hatchery setting

The importance of starting the initial captive broodstock with as many genetically diverse founders as possible (including spatial and temporal diversity within source populations as well as a representative diversity of all available stocks within the ESU) is discussed above in Section 5.1 and in Section 6 of the HGMP (Bork and Adelizi 2010). This founding genetic diversity acts as the limiting variable for subsequent crosses and an important determinant of program success. It also has the potential to protect against the effects of genetic drift, increase short- and long-term viability, and reduce the risk of extinction (see Fraser 2008). Substantive discussion exists regarding the inevitable loss of rare alleles that will occur, given the limited numbers of source fish available for the SJRRP (HGMP Tables 6.2 and 6.3). Methods are proposed for hatchery rearing and/or propagating the SJRRP spring-run Chinook salmon hatchery population to mitigate these anticipated losses where possible (see HGMP Table 8.1, which gives potential mating strategies depending on the number of source populations used and whether returning adults are included in broodstock).

#### 5.2.2 Achieving and maintaining targeted census and effective population sizes

The importance of maintaining diversity is related to maintaining sufficient census and effective population sizes (discussed in Section 4.1 of this GMP). Hatchery practices play a critical role in capturing genetic diversity and maintaining large effective population size through crossing protocols and release strategies, a role that will be particularly important during the Reintroduction Period. Two distinct periods comprise the early San Joaquin River restoration effort: first, the Reintroduction Period (2012-

2019), wherein the focus is to maximize the genetic contribution of all source stocks and parents to the population (via supportive rearing and/or broodstock propagation), minimize inbreeding depression of broodstock, and maximize  $N_e$  and  $N_e/N_b$  (where  $N_b$  equals number of breeders) ratios in the hatchery. Following the establishment of a population (possibly post-Reintroduction or Interim Period – 2020-2024), the focus on maximizing effective population size of the broodstock ( $N_e$  hatchery or  $N_{eh}$ ) continues, with added emphasis on  $N_e$  of the in-river population ( $N_e$  wild or  $N_{ew}$ ), and the combined population ( $N_{eh}+N_{ew}$ ) per TAC spring-run recommendations (Meade 2007) and in keeping with HSRG recommendations for a new integrated reintroduction program (HSRG 2009b).

All broodstock should be genotyped for the purposes of both PBT and creating breeding matrices (HGMP Section 8). This will enable managers to minimize inbreeding and relatedness among initial hatchery broodstock, using relatedness estimates (e.g.,  $F_{IS}$ ) and subsequently using pedigree analysis for reducing relatedness among hatchery progeny (see appendix for methodologies to estimate relatedness and pedigrees). To maximize the utility of these genetic data, complementary field data will be critical, and PIT tagging should be conducted prior to release of individuals for complementary tracking and identification purposes (though it is possible that PIT tagging may become unnecessary if PBT proves sufficient for individual-level identification). Markings should enable (where possible) the differentiation of direct-release fish, supportively reared fish (reared from source populations and released from the hatchery) and hatchery propagated (single-generation hatchery crosses) fish. Monitoring applications of genetic and field data and their contribution to adaptive management are discussed further in Section 6.2.

Several recommendations share the common goal of reducing variance in certain population parameters in order to maximize effective population size. Such variance can significantly reduce the genetic effective number of breeders relative to the actual number of spawners, with detrimental effects on  $N_e$  (see Section 4.1). Every adult selected for use in the hatchery broodstock should have an equal opportunity to and probability of producing progeny. Equalizing founder representation in the initial captive broodstock affords all donor sources the opportunity to contribute to the population. Equalizing family sizes assures that each individual in the broodstock contributes the same number of offspring to the next generation. This affords all individuals an equal opportunity to contribute to the population and has the effect of halving the rates of inbreeding and genetic drift that might otherwise be realized in an ideal population (see Rodriguez-Ramilo 2006 and references therein). Equalizing captive population sizes *across generations* has the similar effect of reducing variance of inter-annual contributions to the population.

Achieving roughly equal male:female sex ratios is essential to reducing relatedness among hatchery-produced progeny. Equalizing sex ratios (1:1 mating) is the simplest means of countering the erosive effects of drift, though factorial mating strategies (e.g., partial factorial mating or nested designs) may also be suitable and in some instances more efficient (Pollard and Flagg 2004). The multiple paternity of factorial matings may also increase effective population size (Pearse and Anderson

2009, but see additional discussion in Lotterhos 2011). Proposed crossing protocols are discussed in detail in the HGMP (Bork and Adelizi 2010, Section 8) and include alternative scenarios for differing source stock availability. Mixed-sperm fertilizations are strongly discouraged for several reasons. Such matings, by observation, result in unequal sex ratios and increase the relatedness of progeny by creating multiple half-sibling families. Moreover, mass mating/sperm pooling from a number of individuals has been shown to increase the variance of family size and thus reduce the effective number of breeders over what would otherwise be attained in single-pair matings of Chinook salmon (Withler 1988, Withler and Beacham 1994). The sperm-competition that occurs in mass matings will inevitably result in “winners and losers” resulting in unintentional artificial selection, as sperm potency and fertilization rates are correlated with age and size at maturation (Campton 2004).

Additional recommendations for maximizing  $N_e$  address the need to account for characteristics of and impacts to the wild San Joaquin River population when considering hatchery rearing and release practices. While the hope is that the majority of family size variance can be minimized up front by attempting to evenly distribute collection efforts among different family groups within each source and by hatchery rearing processes, some additional equalization may be required prior to fish release, with excess fish culled or diverted for off-site experimental purposes (not released in-river). Equalizing family sizes at release can aid in maintaining high  $N_e$ , and hatchery release practices must avoid the potential negative consequences of releasing “extra” fish if it is not in the best interest of overall effective population size. However, it is also recognized that some inherent trade-off exists between perfect equalization of family size and the pursuit of equalization to the detriment of retaining genetic diversity or even fitness (see Fraser 2008 for discussion); this balance should be evaluated annually by the Hatchery and Monitoring Technical Team to ensure that the appropriate compromise is reached based on the varying annual source or broodstock availability, unpredictable stochastic hatchery survivorship (both supportive rearing and broodstock production) in a given year, and relatedness and diversity of surviving families. Lastly, avoiding (intentionally or unintentionally) shortened generation times in the hatchery setting is also critical to prevent inadvertent selection for a jack phenotype or an increase in the generations in captivity.

While the importance of mitigating for potential risks of inbreeding and depression is well characterized in the hatchery setting, the potential relevance of outbreeding depression remains uncertain, particularly for the level of (intentional) outbreeding being considered in this context (populations within the same ESU). Given the unpredictable and likely system-specific nature of outbreeding effects and the reintroduction timeline constraints of the Settlement, the optimal approach is to conduct scientific studies in parallel with the earliest reintroductions, performing experimental releases of controlled hatchery outcrosses (encompassing at least two generations) of the different source populations and determining success as a proportion of returns (measured as proportion of the escapement gene pool relative to the starting percentage). The success of individual strains in comparison to their respective outcrosses is likely to vary considerably under different environmental conditions and over the course of multiple generations, and so such an experiment requires long-term

data to account for natural environmental conditions in the San Joaquin River system. Some information about short-term viability or even hatchery survival may also emerge shortly after initial crosses. Should early results indicate that outcross progeny have lower fitness (in the wild or in lower hatchery performance/survivorship relative to within-strain crosses), this would be taken as evidence of outbreeding depression and an adaptive management strategy would allow for the elimination of the poorly performing crosses or discontinue outcrossing in the hatchery altogether. Physical and genetic marking techniques will be essential in identifying the different source populations or hatchery crosses, as well as making inferences about the parentage or source of wild spawning fish in the San Joaquin River. Furthermore, power analyses should be performed to evaluate the efficacy of using relative proportions of returns to estimate relative survival by determining the minimum releases and sampling efforts necessary to make inferences (Araki et al. 2007a).

Outbreeding depression as a consequence of hybridization between spring- and fall-run Chinook is far more likely a relevant concern for this system (see Section 4), and genetic methods (GSI, PBT; see appendix) should be used during hatchery propagation to avoid intentional or inadvertent crosses between ESUs.

### 5.2.3 Achieving the proper genetic integration with natural populations

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Following the Reintroduction Period, hatchery supplementation will shift to a less influential role, eventually settling into a four-year mean Proportionate Natural Influence (PNI) above 0.67 in keeping with HSRG recommendations (HSRG 2009a); see also HGMP Objective 4) PNI is approximately estimated by  $PNI = pNOB / (pHOS + pNOB)$ , where pNOB refers to the mean proportion hatchery broodstock composed of natural-origin fish and pHOS refers to the mean proportion of naturally-spawning fish composed of hatchery-origin fish. The timing of employing this HSRG recommendation will depend upon the success of the reintroduction effort, but will almost certainly be useful after the Interim Period and may be applicable at the middle or end of the Reintroduction Period, as a naturalized population is established in the San Joaquin River. During the early Reintroduction Period, the emphasis during this period is on capturing and maintaining genetic diversity of source populations. The Hatchery and Monitoring Technical Team should evaluate and report on the appropriateness of the HSRG recommendations annually. Moreover, the Technical Team should consider any new recommendations from the upcoming California HSRG for implementation, as appropriate. The four-year average pHOS should begin trending down beginning in 2020 (per TAC spring-run recommendations, in 2027 four-year mean pHOS is less than 15%). Focus on achieving eventual hatchery phase-out and reliance on natural in-river spawning is important to the overall conservation and local adaptation of the reintroduced population. Increased abundance dependant on ongoing hatchery supplementation has the potential to obscure underlying causes of population decline. For example, should habitat restoration efforts in the Restoration Area prove insufficient to support a VSP without ongoing supplementation, this fact could be masked by high hatchery output (McClure et al. 2008).

### 5.2.4 Minimizing domestication selection in the hatchery

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Domestication selection - selection for traits that are advantageous or neutral in captivity but disadvantageous in the wild - is a known issue in the conservation of salmonid genetic diversity in the hatchery environment (Araki et al. 2008, Fraser 2008, Bailey et al. 2010). Using quantitative modeling of fitness effects of supplementation on wild population fitness, Ford (2002) showed that selection in captivity may significantly reduce a wild population's fitness during supportive breeding and that even continual introduction of wild individuals into the captive population may not eliminate this effect entirely. Therefore, minimizing both intentional and unintentional selection in the hatchery to the greatest extent possible will aid in reducing the genetic risks associated with a captive breeding program, namely, decreased fitness in the wild. Strategies to reduce domestication selection are already addressed, as recommended by the HSRG (see Mobrand et al. 2005), in extensive detail in the HGMP (sections 3.1 and 9; Bork and Adelizi 2010). Furthermore, according to Ford's (2002) model, conserving or restoring a population's habitat is important for preventing fitness losses that occur during supportive breeding.

Reducing the number of generations in the hatchery is a practice recommended as a means of reducing adaptation to captivity and decreasing the likelihood that hatchery-selected traits will be conferred to subsequent generations (Frankham 2008). Cryopreservation of milt may serve a similar purpose, though is more commonly used in the fisheries setting to compensate for early maturation of males (see below). The Reintroduction Strategy document (SJRRP 2011) currently proposes retention of some proportion of broodstock in the hatchery as a strategy to minimize impacts to source populations (by mining a given source population only once). However, the use of *hatchery progeny in the hatchery* to serve as broodstock for one or more subsequent generations (as occurs with a segregated hatchery program) is discouraged for a conservation hatchery facility, except under extreme circumstances (e.g., no source population is available in a particular year) and is likely to further amplify the effects of domestication selection (e.g., Araki et al. 2007a, Araki et al. 2007b).

A preferred alternative to retaining hatchery progeny in the hatchery for use in subsequent breeding is to incorporate some proportion of returning adult spawners with hatchery ancestry into the broodstock for a single generation. The HGMP currently allows the use of returning adults with hatchery parents as part of the broodstock, but limits the percentage of fish used for ongoing supportive breeding (see HGMP table 8.1 and Section 8.2; Bork and Adelizi 2010). This practice would avoid at least some of the domestication selection that would be incurred by keeping hatchery progeny in the hatchery for multiple generations. It could potentially even confer a selective advantage, with returning fish having already survived any in-river natural selection. Fish with hatchery ancestry have shown decreased fitness relative to their wild counterparts, exhibiting earlier run-timing, spawning in lower (less favorable) areas of the watershed, and producing reduced numbers of progeny (e.g., Williamson et al. 2010). However, the relative fitness of hatchery-origin fish is also dependent on the degree of difference between the hatchery and wild fish, whether broodstock are locally or non-locally derived, and the strength of selection and the number of traits upon which it is acting (reviewed in Araki et al. 2008).



The SJRRP has agreed to adhere to HSRG recommendations for conservation hatchery practices that increase naturalization of the captive environment as described in Flagg and Nash (1999) and as detailed in Section 3 of the HGMP (Broodstock Maturation and Reproduction, Enriched Environments, Growth Rate Modulation, Rearing Density, Anti-Predator Conditioning, Release Size, Release Time and Volitional Release, and Imprinting and Homing; Bork and Adelizi 2010).

Early male maturation (jacking) is a natural salmonid life history trait that has evolved as a successful evolutionary strategy (reviewed in Berejikian et al. 2010). However, it is also significantly influenced by growth rate at specific times of year, with the early growth achieved in the hatchery setting shown to increase early maturation (e.g., Shearer et al. 2006). Increasing the proportion of jacks in a population can have negative consequences (e.g., loss of returning anadromous adults, skewing of female:male sex ratios, and ecological and genetic impacts on wild populations and other native species (see Pearsons et al. 2009 and references therein). In a captive broodstock program it is undesirable to produce mature males when females of the same stock are not mature. However, the exclusion of jacks entirely from mating protocols would exclude an important natural evolutionarily stable alternative life history stage from the population. Furthermore, the inclusion of precocious males has been shown to increase effective population size (Saura et al. 2008).

Natural rates of jacking vary widely, although most Chinook salmon stocks exhibit rates around 5-15% (Heath 1994). Based on scale-aging studies, jacks constituted only 0.03% of total 2010 Feather River Hatchery spring-run estimated escapement in both 2007 and 2010 (Grover and Kormos 2007; Kormos, CDFG unpublished presentation); in contrast, jacks constituted 2.9% of Butte Creek spring-run estimated escapement in 2007. This latter estimate, coming from a spring-run population without hatchery influence, may reflect a more natural jacking rate for Central Valley spring-run fish. Proportion of escapement, it should be noted, does not reflect the realized reproductive success of jacks. The genetic contribution of jacks in Central Valley spring-run populations has not been studied directly. However, genetic analysis of other salmonid species has shown the genetic contribution of jacks constitute 60% of total paternity in Atlantic salmon ((Saura et al. 2008, ) and 35% and 2% of inferred natural and hatchery spawning, respectively, in coho salmon (Van Doornik 2002). Foote et al. (1997) used allozymes to directly quantify the reproductive success of jacks in stream enclosures, and found their reproductive success 42% on average, but varying widely (3-97%) and not significantly different from older males. Lastly, jack spring-run Chinook in Oregon sired 20% of offspring based on genetic analysis of observed spawning events in the laboratory setting (Berejikian et al. 2010).

Jack usage in the SJRRP hatchery should receive ongoing evaluation by the hatchery and monitoring technical teams and should be governed by the attempt to represent contributions of jacks in proportion to the relative number of offspring they contribute to the source populations. Because this value is unknown for some natural spring-run populations (Mill/Deer) and has not been estimated genetically for any Central Valley Chinook population, 3% (the proportion of jacks in a wild Central Valley spring-run population) may be a reasonable starting point. However, if a very large

proportion of jacks or precocious males are present in the San Joaquin River, a higher level of usage may be required during initial reintroduction efforts in order to meet genetic diversity and population targets.

If high incidences of precocial males occur, the hatchery may also consider cryopreservation of jacks' milt to ensure that in the event of early male maturation in the hatchery setting, sufficient genetic material will be available to fertilize eggs of females in the same cohort (Flagg and Nash 1999, O'Reilly and Doyle 2007). However, efforts should focus on creating rearing conditions that reduce early maturation of males in broodstock populations, starting with a low (e.g., 3%) proportion of jack contribution, and monitoring effects on the population. The overall emphasis should be on achieving return *adult* spawners and using the genetic characterization of these fish to guide subsequent crossing strategies.

### Summary of recommendations for conserving genetic diversity of the hatchery population

- *Start the initial captive broodstock with as many genetically-diverse founders as possible.*
- *Incorporate annual input from source populations.*
- *Equalize founder representation in the initial captive broodstock.*
- *Equalize family sizes in captivity and at time of release.*
- *Equalize sex ratios at spawning.*
- *Estimate relatedness among founders; use this information and pedigree analyses, to construct breeding matrices.*
- *Reduce relatedness among progeny (minimize/avoid matings between related individuals; relatedness thresholds will depend upon the starting genetic composition of the broodstock).*
- *Do not conduct mixed-sperm fertilizations.*
- *Equalize captive population sizes across generations. Avoid artificial shortening of the captive generation length.*
- *Minimize duration of hatchery supplementation.*
- *Minimize the number of generations in the hatchery for hatchery broodstock.*
- *Minimize intentional selection in captivity that would decrease fitness in the wild.*
- *Increase naturalization of the captive environment.*
- *Grow captive-reared individuals at 'natural' rates of growth*
  - *Delay maturation of individuals in captivity*
  - *Emphasize return of adult spawners*
- *Utilize outcrossing in an adaptive management framework, using controlled crosses and experimental releases to gain information on the performance and fitness of resultant progeny; be prepared to discontinue outcrossing if outbreeding depression is detected in F1 or subsequent generations.*
- *Use genetic methods (GSI and PBT) to avoid introgression between fall- and spring-run fish in the hatchery.*

### 5.3 Conserving genetic diversity of the in-river population

Environmental conditions of the Restoration Area will undoubtedly be stochastic during the Reintroduction Period and, given climatic fluctuations and unforeseen events, will likely be variable during the Interim, Growth Population, and Long-term periods as well. As stated in the VSP Guidelines, the genetic diversity of a population needs to be as great as possible for it to remain viable when confronted with considerable environmental and demographic uncertainty over both the short- and long-terms (McElhany *et al.* 2000). Additionally, high diversity allows a population to use a greater

environmental range (e.g., divergent spawning times) than it otherwise could with lower diversity (McElhany *et al.* 2000).

### 5.3.1 Genetic diversity of in-river population during restoration

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The following factors need to be considered in order to restore a naturally self-sustaining spring-run Chinook salmon population with sufficient genetic diversity and high reproductive fitness:

- 1) Suitable holding, spawning, and rearing habitat
- 2) Stocking event details

Factors that affect the genetic diversity of the in-stream population include: effective population size, inbreeding and/or outbreeding depression, introgression, life history traits, environmental factors, and straying. Specific effects that these factors have on the source, hatchery, and reintroduced populations are examined in Section 4 of this GMP (*Biotic and Abiotic Factors*).

#### 5.3.1.A Suitable holding, spawning, and rearing habitat

Suitable habitat for all in-river life stages must be maintained across an appropriate spatial range of the Restoration Area to limit mortality due to unforeseen events (e.g., localized heavy predation, isolated disease outbreaks, pollutants) that negatively impact individual fitness in particular locations. The carrying capacity for any limiting life stage must not be exceeded during supplementation to avoid population collapse (Cuenco *et al.* 1993). Several adult carrying capacity estimates are provided in Table 5. Given the approximate nature of such estimates, along with potential unknown factors, it is recommended that upper carrying capacity estimates not be used as a guideline for stocking. As habitat is restored, carrying capacity thresholds should be re-evaluated. Additionally, once data is collected, it will be important to compare carrying capacity and  $N_e$  estimates (see Section 6.3 and the appendix for details regarding  $N_e$  estimation).

#### 5.3.1.B Stocking event details

##### 5.3.1.B.1 Hatchery propagation or rearing versus translocation of source populations

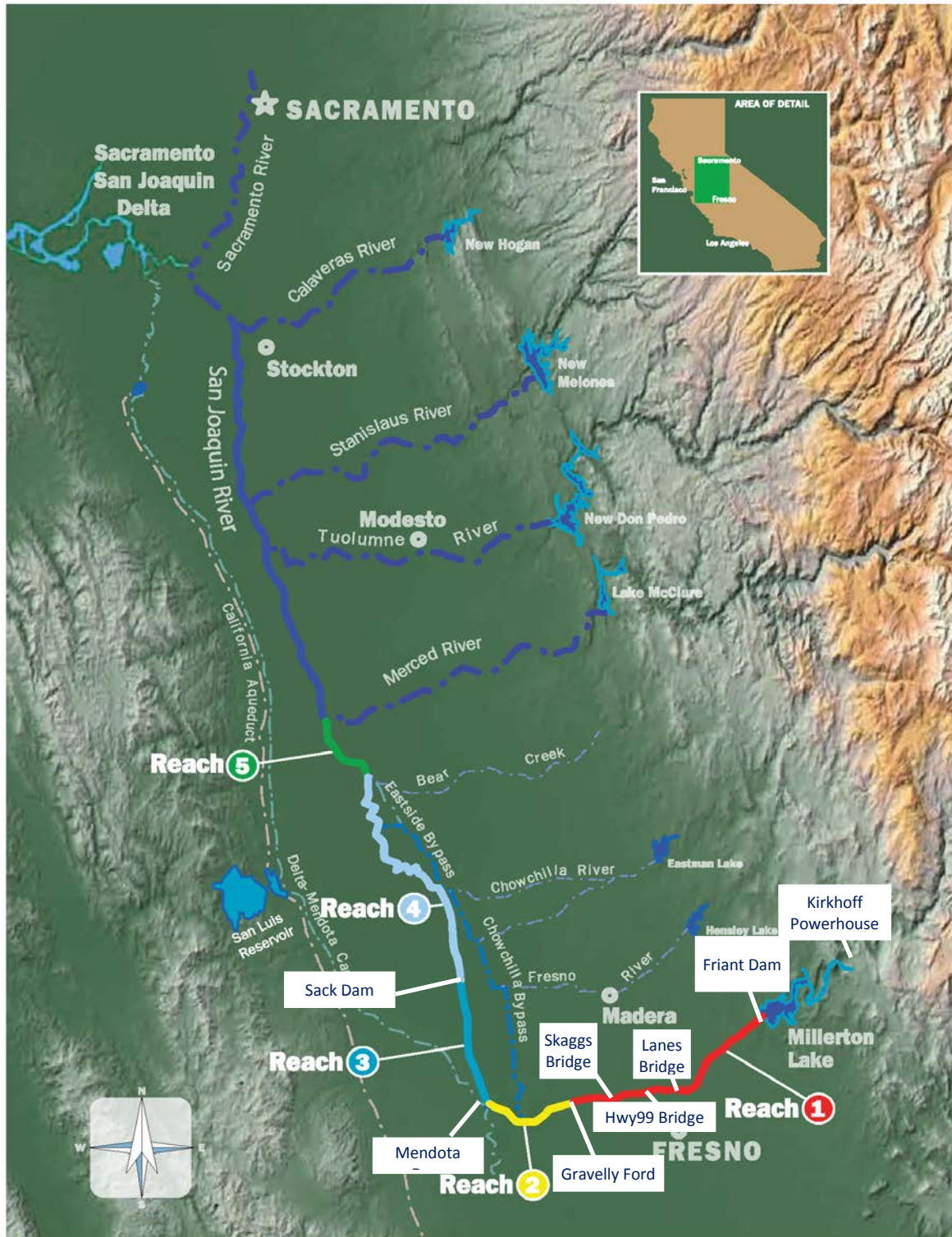
None of the known Central Valley spring-run Chinook populations currently reside in the San Joaquin River basin, so natural population recolonization of the Restoration Area is unlikely to occur and certainly not at a sufficient rate to meet restoration goals outlined in the TAC's spring-run recommendations (Meade 2007). Therefore, previously mentioned source populations will be used to stock spring-run Chinook into the Restoration Area. This stocking will occur by broodstock propagation in a hatchery, hatchery rearing and subsequent release of individuals, or direct in-river restoration stocking of source population individuals directly into the Restoration Area (i.e., translocation).

**Table 5. Potential adult carrying capacity in the Restoration Area**

Summary of potential spawning habitat capacity information that may inform recommended targets of a self-sustaining population of naturally produced spring-run Chinook salmon in the San Joaquin River. Refer to Figure 7 for a map of survey locations. Originally Table 2 in the TAC's Recommendations on Restoring Spring-run Chinook Salmon to the Upper San Joaquin River (Meade 2007)

Source	Extent of Survey	Habitat Area	Potential Adult Population Carrying Capacity		Discussion
			Lower limit based on redd size of 216 ft <sup>2</sup> (Burner 1951)	Upper limit based on redd size of 55 ft <sup>2</sup> (EA Engineering 1992)	
Spawning habitat (Clark 1942)	Lanes Bridge to Kirkhoff Powerhouse RM 255.2 – RM 281.5	266,800 ft <sup>2</sup>	2,470 adults	9,702 adults	Existing habitat is likely less than 1942 habitat, but could create much more spawning habitat for future conditions
Spawning habitat (Fry and Hughes 1958)	Gravelly Ford to Friant Dam RM 229 – RM 267.5	1,000,000 ft <sup>2</sup>	9,259 adults	36,364 adults	Includes estimates downstream to Gravelly Ford, which probably would not be used for spawning by spring-run Chinook salmon
Spawning habitat (Cain 1997)	Gravelly Ford to Friant Dam RM 229 – RM 267.5	303,000 ft <sup>2</sup>	2,806 adults	11,018 adults	Numbers based on existing habitat. Could create much more habitat for future conditions
Spawning habitat (R. Ehlers, pers. comm., in Cain 1997)	Gravelly Ford to Friant Dam RM 229 – RM 267.5	1,820,000 ft <sup>2</sup>	16,852 adults	66,182 adults	Includes estimates downstream to Gravelly Ford, which probably would not be used for spawning by spring-run Chinook salmon
Spawning habitat (Jones and Stokes Assoc 2001)	Friant Dam to Skaggs Bridge RM 267.5 – RM 234.1	408,000 ft <sup>2</sup>	3,785 adults	14,836 adults	Based on existing habitat, similar to Cain and Stillwater Sciences. Could create much more habitat for future conditions
Spawning habitat (Stillwater Sciences 2002)	Friant Dam to Highway 99 Bridge RM 267.5 – RM 243.2	357,000 ft <sup>2</sup>	3,306 adults	12,982 adults	Based on existing habitat, similar to Cain and Jones and Stokes. Could create much more habitat for future conditions





**Figure 7. San Joaquin River Restoration Area**

Colored, numbered reaches upstream from Merced confluence comprise the “Restoration Area,” with callouts identifying localities referenced in text. Adapted from SJRRP map:

[http://restoresjr.net/program\\_library/02-Program\\_Docs/Scoping\\_Materials/STA\\_1\\_Maps\\_SJR.pdf](http://restoresjr.net/program_library/02-Program_Docs/Scoping_Materials/STA_1_Maps_SJR.pdf)

### 5.3.1.B.2 Translocation from source populations into the Restoration Area

When individuals are translocated directly into the Restoration Area, the entire life cycle of the next-generation progeny exists in the Restoration Area's natural environment and individual fitness is linked with local adaptation. According to the Reintroduction Strategy document (SJRRP 2011), meeting the population targets described in the FMP (SJRRP 2010b) through translocation alone is not possible, based on the status of existing stocks. However, transferring enough individuals (i.e., eggs, juveniles, or straying/salvaged adults) to initiate a non-hatchery influenced portion of the population may be a viable option to supplement hatchery operations. With the exception of the FRH, adult collection from the primary source populations is likely not a feasible strategy, except on rare occasions of very high source population adult escapement, since these populations typically cannot afford to lose many, if any, spawning adults to maintain long-term viability. Capture and immediate outplanting of eggs or juveniles should proceed cautiously to protect the genetic integrity of the source populations, since large numbers are needed to obtain detectable rates of adult escapement. If the number of juveniles collected is reduced to avoid a source population genetic bottleneck, then the reintroduced population may suffer from a founder effect, depending on the census size and degree of relatedness between individuals. The primary genetic advantage of translocation over hatchery production is that domestication selection will not occur. If a translocation approach is used for an early life stage, it is imperative that a larger number of individuals be collected from the source populations than is needed for hatchery propagation, since overall mortality rates will be approximately 100-fold higher in the wild versus the hatchery environment (Quinn 2005; HGMP Section 6, Bork and Adelizi 2010).

### 5.3.1.B.3 Hatchery production or rearing prior to reintroduction into the Restoration Area

Eggs propagated in the hatchery that are released into the Restoration Area at the eyed-egg stage will be exposed to natural selective pressures throughout almost all of their life cycle, though artificial selection in the hatchery may have already affected parental contributions. A primary advantage of hatchery propagation and/or rearing is that it typically allows for higher early survival than in-river rearing (Ryman and Utter 1987). Controlled crosses, informed by genetic tagging or pedigree information, can maximize genetic diversity and increase effective population size. It is possible, however, to introduce maladapted traits or lower the overall effective population size (i.e., the Ryman-Laikre effect) if certain precautions (see sections 4.1 and 5.2) are not taken during hatchery rearing or propagation (Ryman and Laikre 1991).

Given uncertainty about the most successful strategy to employ, it is recommended to attempt several alternative approaches under an adaptive management framework. Genetic monitoring via PBT can then be used to gauge the effectiveness of each approach so methods that achieve higher escapement will be favored in subsequent years. It should be noted, however, that collections from source populations are expected to occur for only a seven year period (2012 – 2019) and the Conservation Facility (i.e., SJRRP hatchery) is anticipated to shut down in 2025, as outlined in Appendix 1 of the HGMP (Bork and Adelizi 2010). Altering the fundamental



reintroduction strategy is not recommended until a clear trend is established, which may take a number of years to become evident. It should be noted that one potential gap in the ability of PBT to monitor the successes of alternative reintroduction strategies is the translocation of eggs from source populations into the Restoration Area. This gap is because translocated eggs cannot be genotyped prior to outplanting and their parents are unknown. It is recommended that a small subset of co-occurring eggs within each redd are collected for genotyping so that sibship analysis may later be used for outmigrating smolts and returning adults to assign back to family groups. Accurately determining relatedness in natural populations can be quite difficult (Csillery *et al.* 2006), particularly when attempting to distinguish between closely related individuals, so it is possible that the success of egg translocation will not be reliably monitored. Monitoring of hatchery propagated or reared eggs that are transferred to the Restoration Area, however, should not be problematic given that their parents are known and genotyped so that PBT can occur (see Section 6.3 and the appendix for details regarding methodology).

### 5.3.1.C Genetic factors to consider for different strategies

#### 5.3.1.C.1 Multi-stock versus single stock approaches

The multi-stock approach includes incorporation of all available known Central Valley spring-run Chinook salmon stocks, including the FRH stock, in the reintroduction effort. Additionally, opportunistic collection will occur from other Central Valley tributaries (e.g., spring-run strays and fish salvaged from the Delta pumping facilities). As stated in the Stock Selection Strategy document (SJRRP 2010c), a multi-stock approach has been recommended by the SJRRP Genetics subgroup due to the challenges in collecting sufficient numbers of individuals from any one source population and the uncertainty regarding future success of any one stock in the Restoration Area.

As noted in the Reintroduction Strategy document (SJRRP 2011), the genetic benefits associated with a multi-stock approach versus a single stock approach to reintroduction include an increase in overall genetic diversity and reduction in inbreeding risk, potential for greater initial  $N_c$  and  $N_e$  due to likely higher overall allowable take, Program flexibility with regard to reintroduction strategies, and the availability of diverse reintroduction methods that may lead to higher overall survival and better inform the adaptive management process. The risks include outbreeding depression, hatchery influence and the possibility of a fall-run Chinook salmon phenotype for FRH collections, and challenges in monitoring the independent success of each source population's establishment in the Restoration Area due to the high likelihood of introgression among the spring-run populations. Marks, tags and genetic analyses (e.g., PBT) will be used to monitor the independent success of each source population's establishment in the Restoration Area and appropriate adjustments in supplementation strategies will follow these assessments.

#### 5.3.1.C.2 General release strategies

It is recommended that outplanting occur at appropriate seeding density, body size, dates, time of day, and in habitat conditions that favors survival of each particular released life stage. Specific outplanting details will be determined by the SJRRP Fisheries Management Work Group or one of its subgroups. Additionally, individuals originating from all source populations should be released at every planned release event, in order to facilitate statistical comparison of source population success under similar environmental conditions. Release events should occur multiple times throughout the appropriate season or, in the case of hatchery juveniles or adults, on a volitional basis after proper acclimation to the Restoration Area.

Salmon are capable of returning to specific reaches of a river for spawning (Neville et al. 2006, Quinn et al. 2006) so eggs should be placed in upstream portions of the Restoration Area (i.e., Reach 1) to promote natural spring-run Chinook grounds in the upstream portion of the Restoration Area and reduce the potential of fall-run Chinook introgression. Eggs, either transferred directly from source population habitat or from the hatchery, should be outplanted in several locations throughout Reach 1 (see Figure 7 for a map of the Restoration Area). Multi-location outplanting throughout suitable habitat of the Restoration Area will reduce the risk of large year-class mortality due to a particular unknown environmental variable (e.g., localized predation) and encourage dispersal.

For hatchery propagated or reared fish, volitional release of juveniles or adults is recommended to mimic natural migration timing. Rearing in the same water as is found in the Restoration Area is strongly recommended to increase imprinting (Flagg and Nash 1999). If this is not an option, a period of acclimation prior to release will be needed to reduce stress and increase survival (Flagg and Nash 1999). Translocation of juveniles or adults from source populations should follow a similar release protocol to that of egg translocation, other than using different locations/reaches based on habitat preferences of different life stages. Regardless of life stage released, outplanting should occur at multiple locations within suitable reaches of the Restoration Area.

#### 5.3.1.C.3 Methods of collection

One of the goals while collecting spring-run Chinook for reintroduction purposes is to avoid sampling bias. It is possible for selection to act during collection and transfer since some individuals may be better able to avoid trapping, survive transport, and recover from handling stress. This inadvertent selection, which could lead to differential survival, will negatively affect the  $N_e$  and genetic diversity of the introduced population. As long as collection occurs in multiple locations throughout the suitable geographic area and throughout a given season, there should not be a bias in collecting related individuals during the later juvenile and adult life stages.

Collection of eggs from source population redds is being given serious consideration as a reintroduction strategy by the SJRRP (SJRRP 2011). Redd sampling has been shown to produce a higher  $N_b$  in a hatchery than artificial spawning of adults (Berejikian et al. 2011) and a large portion of egg in a given redd will remain in source population waters. However, it is currently unknown if disturbing a redd increases

mortality of the uncollected eggs (Berejikian *et al.* 2011). If so, this could reduce reproductive success and overall abundance of the source population. The Reintroduction Strategy document provides a detailed assessment of the pros/cons of egg collection (SJRRP 2011) and the other life stages as well. Additionally, the Reintroduction Strategy document provides estimated collection numbers for each life stage, based on alternative survival rates and desired adult escapement and broodstock goals (SJRRP 2011). If source population redd pumping or excavation is initiated, it should occur throughout the spawning area to increase overall genetic diversity of the collected individuals. Additionally, only a small fraction of individuals to be outplanted or used as broodstock should be from the same redd to avoid overrepresentation or excessive use of highly related individuals. Therefore, multiple redds should be pumped or excavated for each source population to decrease overall relatedness of collected individuals. It is recommended that the number of redds to collect from be assessed using simulations that take into account variables such as the genetic diversity of the source population, number and sex ratios of breeders used in hatchery propagation (if applicable), and breeding strategies to estimate different inbreeding levels based on alternative approaches, similar to the approach taken by Fave *et al.* (2008). Collecting from many redds from each source population to decrease average relatedness will need to be weighed against the potential negative impacts of such activities to the source population.

#### 5.3.1.C.4 Life stage released

There are trade-offs when considering which life stage(s) to release into the Restoration Area. Earlier life stages will suffer from greater mortality but may benefit from natural selective pressures that increase long-term fitness and local adaptation. Translocating eggs directly from source population locations into the Restoration Area will maximize the time for natural selection to act and eliminate concerns of artificial selection. Alternatively, juveniles reared in the hatchery (e.g., unfed fry or smolts) will have greater overall survival than eggs upon release but will have increased exposure to artificial selection. A recent study of coho salmon in Oregon found that stocking unfed fry from a hatchery produced fish more similar to wild coho salmon than stocking hatchery smolts, in terms of jacking incidence, body size upon return, and run timing (Theriault *et al.* 2010). Release of older life stages, however, may have different potential benefits (e.g., higher survival to adulthood and thus higher  $N_e$ ). Translocation of adults may occur on a limited basis (SJRRP 2011), though handling stress is a serious concern.

While modeling can be used to make informed predictions, the best life stage and release method cannot be predetermined with certainty. Therefore, it is recommended that several alternative strategies be employed, with monitoring used to gauge successes and failures. Specifically, genetically tracking individuals with PBT should allow for reliable estimates of relative smolt outmigration and adult escapement of various strategies (see Section 6.3 for further details). If certain release strategies are proving ineffectual based on several years of monitoring results (e.g., direct release of eggs or fry have limited survivorship to smolt stage), these strategies will be

discontinued. Permitting results to guide future decision-making is the hallmark of a long-term adaptive management process.

### 5.3.2 Genetic diversity of the post-supplementation in-river population

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Conserving the genetic diversity of the post-supplementation population entails similar efforts to those already described for the in-river population during restoration, with additional considerations involving hatchery phase-out and long-term population viability. The following factors are important to consider when attempting to restore a naturally self-sustaining spring-run Chinook salmon population with sufficient genetic diversity and high reproductive fitness:

- 1) Executing Hatchery phase-out
- 2) Maintaining large effective population size and high genetic diversity
- 3) Retaining (or increasing) suitable holding, spawning, and rearing habitat
- 4) Ensuring long-term population viability

#### 5.3.2.A Hatchery phase-out execution

Conservation hatchery production of spring-run Chinook salmon will decrease starting in 2020 and cease entirely after 2025, pending establishment of self-sustaining spring-run Chinook populations within the target parameters. An overview of the anticipated hatchery phase-out process and target parameters is given in Section 7. Assuming that population targets are met, the genetic diversity of the post-supplementation population will subsequently be maintained only through natural processes (i.e., migration and mutation). Genetic monitoring of the post-supplementation population will allow estimates of inbreeding, detection of lowered effective population size, and inference of maladapted source strains (see sections 6.3 and appendix for methodology recommendations). Adaptive management (including the option of hatchery re-activation, if necessary) provides further insurance against severe population declines.

#### 5.3.2.B Maintaining large effective population size and genetic diversity

Following the successful Reintroduction, and Interim Population Milestone Periods, and moving into the Growth Population period, it is critical to retain not only sufficient numerical and demographic diversity into the future, but also genetic diversity – all of which will enable a self-sustaining natural population and the ultimate cessation of hatchery supplementation of spring-run Chinook salmon in the upper San Joaquin. This post-supplementation natural population is expected to continue to act as an independent VSP and, although it is designated an experimental population, may be included in the 5 year status review of the spring-run ESU. Regardless of its regulatory status, its success will likely improve the overall biological viability of the ESU and in that sense will be considered as a contributing population to the spring-run ESU.

##### 5.3.2.B.1 Effective population size

The importance of effective population size to the longer-term viability of populations has been described elsewhere (Section 4.1). The post-supplementation population should be able to maintain sufficient numbers such that minimum population size does not dip below 500 successfully spawning adults in any given year, as dictated by the preliminary SJRRP population objectives outlined in the FMP (SJRRP 2010b). Effective population size is more sensitive to sustained population declines (Lande and Barrowclough 1987), and semelparous, age-structured salmonid populations show particular vulnerability to such fluctuations (Waples 2002). If population size stays high after supplementation ceases, genetic simulations show that the risk of inbreeding negatively impacting the population is marginal (Waples and Do 1994, Fave et al. 2008). Therefore, the benefits of stopping supplementation after two generations are thought to outweigh the risks to effective size. Monitoring efforts will be critical to determining whether these genetic and demographic goals are met (see Section 6.3).

#### 5.3.2.B.2 Genetic diversity

Effective population size alone cannot measure the genetic health of the post-supplementation population, as losses of heterozygosity and allelic diversity may occur at varying rates (Allendorf and Luikart 2007), making  $N_e$  an insufficient sole indicator of the genetic health of a population. Standard genetic diversity indices (e.g., allelic richness) should continue to be employed to accurately gauge the genetic diversity of the post-supplementation population over time.

Shifts in diversity are anticipated as the post-supplementation population begins to respond to selection of the natural environment. Over time however, the population should increase in census and effective population size and maintain sufficient genetic diversity to locally adapt to the Restoration Area. This may involve shifts in genetic diversity indices (see appendix) over time and relative to the source populations. These shifts should be monitored to ensure that such fluctuations do not represent a threat to population persistence (e.g., evidence of inbreeding, genetic bottlenecks, population crashes). Selection may be detected by comparison of  $F_{ST}$  measures for coding versus non-coding SNPs (e.g., Zayed and Whitfield 2008), detection of outlier loci, comparison of neutral and non-neutral DNA sequence substitutions, and a variety of other methods (see reviews in Vasemagi and Primmer 2005, Helyar et al. 2011). Drift can be assessed through temporal analysis of allelic richness and genetic bottleneck detection. Section 6.3 and the appendix detail the genetic diversity indices to be monitored and provide guidance on methodology. If monitoring detects significant reduction in genetic diversity indices, effort should be made to determine the cause(s) (e.g., environmental factors or reducing census size) and ameliorate, if possible. If genetic diversity continues to decline, the conservation hatchery may need to reinitiate.

#### 5.3.2.C Retaining (or increasing) suitable holding, spawning, and rearing habitat

Habitat availability continues to be a critical variable for the persistence of both the in-river supplemented and post-supplementation populations (see Section 4.3), with a correct balance between population size and habitat availability being an important determinant of long-term population persistence. Sufficient suitable habitat must be



available for all life stages within the Restoration Area to support ongoing holding, spawning, and rearing requirements and to minimize the negative impacts of natural (e.g., predation) and stochastic (e.g., climate, disease) events. However, the population size should be monitored so that it does not exceed the carrying capacity given the available habitat, and adaptive management should allow for increases of habitat availability if required and appropriate. Census size following the cessation of hatchery supplementation is an indicator of how the population will trend, in terms of increasing/decreasing abundance, once established and whether habitat restoration has sufficiently mitigated for the environmental variables causing initial decline and whether available spawning habitat (though mostly downstream of its original locale), is sufficient to support the population.

#### *5.3.2.D Ensuring long-term population viability*

Although it is not technically necessary to evaluate the San Joaquin River by ESU Recovery standards, the NMFS VSP guidelines (McElhany et al. 2000; this document, Section 3) offer helpful guidance in evaluating the San Joaquin population as an important contributing population to the overall health of the Central Valley spring-run Chinook ESU (though the in-river population will likely continue to be considered an experimental population indefinitely within the Restoration Area). Ensuring long-term population viability will rely heavily upon the feedback obtained during and following Reintroduction Period activities (Section 6.3).

Straying (Section 4.2) is an important variable associated with long-term population viability in the sense that the San Joaquin population should exhibit sufficient natal homing to maintain a viable population within the Restoration Area. The post-supplementation straying rates of upper San Joaquin River fish into other San Joaquin or Sacramento River tributaries should be assessed to evaluate what proportion of the population is “lost” on an annual basis to other populations. Straying rates should be evaluated during the course of supplementation to provide a baseline for comparison to post-supplementation rates. Post-supplementation straying should be evaluated to determine whether these rates change over time following cessation of hatchery operations (as the population adapts to local conditions without continued hatchery input). Straying into the system should also be evaluated on an ongoing basis to determine what, if any, gene flow might be accounted for by contributions from any spring- or fall-run fish from other San Joaquin or Sacramento River tributaries.

### Summary of recommendations for conserving genetic diversity of the in-river population

- *Use a multi-stock approach to ensure adequate genetic diversity on which selection can act to enable local adaptation.*
- *Provide suitable and sufficient habitat for all spring-run Chinook salmon life stages and periodically re-evaluate carrying capacity thresholds.*
- *Direct transfer, hatchery propagation, and hatchery rearing strategies should be employed during the Reintroduction Period given uncertainties in most suitable approach.*
- *Releases should occur at appropriate densities, body sizes, times of year and day, habitat conditions, and in multiple events (either planned or volitionally). Family and source population contributions should be distributed across release events.*
- *Multiple collection methods should be employed (e.g., trap and haul, rear and haul, redd pumping or excavation) to capture egg, juvenile, and perhaps adult life stages. Collection should occur throughout habitat range and appropriate season to capture sufficient genetic diversity.*
- *Outplanting by direct transfer from source populations should primarily occur for egg or juvenile life stages.*
- *Outplanting by hatchery propagation or rearing should occur for egg and all juvenile life stages.*
- *Hatchery supplementation should occur for a minimum number of generations (see Section 5.2).*
- *All strategies employed should be monitored to evaluate success with suitable modifications occurring based on consistent trends using an adaptive management framework. Genetic monitoring should continue post-supplementation to identify shifts in genetic diversity that may threaten population persistence and for comparison purposes with the source populations.*
- *Monitoring of eggs directly outplanted from the source populations should be attempted via sibship analysis since PBT is not feasible. If sibship analysis is not successful, this reintroduction strategy may not be properly monitored using genetic analysis.*
- *Assess straying rates into and out of the Restoration Area (during and after supplementation) to ensure adequate homing is occurring.*



## SECTION 6 MONITORING GENETIC DIVERSITY

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As explained in Schwartz et al. (2007), the purpose of a genetic monitoring program is to “[quantify] temporal changes in population genetic metrics or other population data generated using molecular markers”. Additionally, with a large number of genetic markers (typically hundreds to thousands), signatures of natural selection could be detected to gain insight into the genetic basis for adaptation to particular environmental conditions in the upper San Joaquin River. The development of a genetic monitoring program is an often-missed component to comprehensive monitoring of species diversity and is particularly warranted during large scale enhancement (Laikre *et al.* 2008), such as hatchery supplementation. There is an extremely high potential for Program activities to alter the genetic diversity found in the source, hatchery, and/or reintroduced population during the course of the Chinook salmon restoration to the San Joaquin River. Therefore, genetic monitoring of all populations should be performed to routinely assess genetic impacts and inform the adaptive management process and overall restoration goals.

Eilers (2008) provides a partial summary of population monitoring occurring for spring-run Chinook populations and other salmonids in Central Valley river systems. In brief, this includes monitoring spring-run populations using videos, weirs, rotary screw traps, fyke nets, beach seines, snorkel surveys, trawls, tags, and carcass surveys. This variety of monitoring approaches has enabled examination of metrics such as smolt emigration, adult escapement, habitat use, behavior, and diversion effects. Although the genetic diversity of Central Valley spring-run populations has been intermittently assessed (see Section 2), genetic monitoring is not a component of any spring-run Chinook annual monitoring efforts, with the exception of FRH monitoring. Monitoring genetic diversity is dependent to a large degree upon non-genetic monitoring efforts, to the extent that sampling and monitoring observations are intimately linked. To successfully conduct genetic monitoring of all potentially impacted spring-run Chinook populations, it is critically important that there is open communication and collaboration between geneticists and field biologists regarding sample collection procedures.

As stated in the TAC spring-run Recommendation 22 (Meade 2007):

*The information available from monitoring salmonid populations in other Central Valley river systems should be compiled and a critical assessment of monitoring needs and alternative approaches should be conducted prior to reintroduction. The monitoring and evaluation program should be designed to address and evaluate these and other key issues affecting the design, implementation, priorities, and success of the reintroduction program and for informing future decisions regarding refinements or modifications to the reintroduction strategy.*

As advances in the field of population genetics/genomics continue to expand, so too should the methods employed for monitoring populations. Genetic diversity, in a broad sense, is composed of both neutral and non-neutral variation. While measuring neutral variation is currently the most common method to assess overall diversity, newer methodologies and approaches can assist in providing a more comprehensive overview of a population's genetic diversity and integrity. For example, as genotyping and sequencing costs continue to decline, use of considerably more markers or individual sequencing may be feasible. A combination of established and emerging technologies should be considered pre- and post-reintroduction in order to maximize the ability to adaptively manage the restored population and gain valuable scientific insight into the strategies that promote or inhibit long-term reintroduction success and local adaptation.

### 6.1 Monitoring genetic diversity of source populations

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Maintenance, loss, or gain of population genetic diversity can be evaluated only when compared against previous diversity estimates. Given the high risk of reduction in genetic diversity of the source population due to translocation, broodstock collection or hatchery rearing, it is necessary to conduct thorough genetic monitoring of the source populations. This will enable corrective actions to be taken in a timely manner to avoid further depletion of source population genetic variation attributed to upper San Joaquin River restoration efforts.

It is essential that baseline measurements of the genetic diversity indices listed below are conducted for each source population prior to commencement of fish collection. These measurements are required to accurately assess the impacts of collection on the source populations over time.

The following genetic diversity indices should be evaluated during routine monitoring of each source population:

- 1) Effective population size ( $N_e$ )
- 2) Expected heterozygosity ( $H_e$ )
- 3) Allelic richness ( $A_R$ ) for microsatellite markers
- 4) Hardy-Weinberg Equilibrium (HWE)
- 5) Linkage disequilibrium (LD)
- 6) Allele frequency change ( $F_{\text{temporal}}$ ) (microsatellites)
- 7) Genetic differentiation:  $F_{ST}$  (SNPs),  $G''_{ST}$  (microsatellites)
- 8) Inbreeding estimates:  $F_{IS}$
- 9) Correlation between genetic and spatial distance (isolation by distance using a Mantel test)
- 10) Hybridization level between spring- and fall-run for FRH, if feasible
- 11) Genetic stock identification, for individuals collected for reintroduction purposes, to ensure all collected fish are spring-run in origin

The importance of each of these indices is discussed in the appendix of this GMP. In addition to these genetic diversity indices, it is recommended that phenotypic diversity is

examined for life history traits such as those mentioned in Section 5.1.3 (Selection on Phenotypes).

### 6.1.1 Logistics of sampling

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#### 6.1.1.A Frequency

An initial baseline evaluation of each source population should occur prior to any collections for reintroduction purposes. It is recommended that at least three years of recent baseline data (with samples being collected within the last five years) should be obtained. It is recommended that the CDFG Tissue Collection Archive and current sampling be used to obtain these baseline samples. Monitoring of each source population should occur at least once every 3 – 4 years (once per generation), beginning three years after collections commence and ending no earlier than one full generation (four years) after collections cease to detect generational differences in genetic variation. Generational monitoring is a minimum recommendation and ideally source population monitoring will occur annually to quickly detect temporal variation in genetic diversity. Annual monitoring could be conducted with minimal impact to the source populations via carcass surveys. If only generational monitoring occurs and if statistically significant and biologically relevant changes in genetic diversity indices become apparent then annual monitoring should commence and collection strategies should be re-evaluated by the SJRRP Hatchery and Monitoring Technical Team to minimize any effects that may be caused by these collections. Archiving of all samples and curation of a Central Valley Chinook salmon genetic database are strongly recommended (see appendix for further details).

#### 6.1.1.B Sampling sizes and distribution

A power analysis should be conducted to assess the number of individuals needed to detect genetic changes over time for each source population. Individuals should be collected throughout the geographic range of each source population. The sampling effort should occur throughout the appropriate time period for a given life stage (e.g., throughout spawning season for returning adult collections). Spawning adults, sampled live or by carcass surveys, are preferred for collection compared to other life stages since these individuals have a high probability of contributing to the next generation and the sampling is less invasive compared to egg or juvenile collections.

### Summary of recommendations for monitoring genetic diversity of the source populations

- *Conduct a power analysis to determine the minimum number of individuals that should be sampled to detect genetic changes over time.*
- *Measure the following genetic diversity indices:  $N_e$ ,  $H_e$ ,  $A_R$ ,  $HWE$ ,  $LD$ ,  $F_{temporal}$ ,  $F_{ST}$ ,  $G''_{ST}$ ,  $F_{IS}$ , genetic/spatial distance correlation,  $GSI$  (for individuals to be used for reintroduction).*
- *Conduct baseline genetic evaluation of each source population prior to any sample collections.*
- *During sample collections, conduct periodic genetic evaluations of each source population at least once every generation, encompassing spatial and temporal distributed individuals (with at least 3 – 4 sampling sites/population with the exception of FRH, which is a single site).*
- *Deposit all genotypic data into a common database for access by participating salmonid researchers across agencies and academia.*

## 6.2 Monitoring genetic diversity of the hatchery population

### 6.2.1 Background

Monitoring the genetic diversity of the hatchery population has two primary goals:

- 1) Capture and retain a large proportion of the source populations' genetic diversity in the hatchery population.
  - a. In later years of reintroduction, hatchery population composition, matings, and releases may be influenced by observed successes of alternative reintroduction approaches.
- 2) Produce large numbers of individuals with low levels of relatedness for release into the Reintroduction Area.

The Program's adaptive management approach will allow modification of these goals if, for example, one stock thrives to the exclusion of the others or if rapid local adaptation occurs.

The benefits of a genetically diverse salmon population are well documented (Fraser 2008). The Reintroduction Strategy document notes that the hatchery may be used for a broodstock, as currently anticipated, or for supportive rearing, where individuals are reared in the hatchery to reduce mortality and then released in the river as juveniles (SJRRP 2011). As outlined in the FMP (SJRRP 2010b), the hatchery population is likely to be the primary source for the in-river population, so the hatchery population's genetic diversity will be a controlling element in the in-river population's diversity. Therefore, genetic characteristics of the hatchery population in the San Joaquin River should also be evaluated over the life of the hatchery operation to determine:

- Whether genetic diversity of the hatchery population is being retained/promoted through collections from the source populations
- Whether fall-run fish are successfully excluded from hatchery crosses
- Whether crossing protocols (both within and potentially among source population strains) are effectively avoiding the negative consequences of inbreeding depression
- Whether crossing protocols are effectively avoiding the inclusion of offspring from hatchery fish in second-generation production
- Whether domestication selection is impacting hatchery population fitness, if more than one generation of mating is conducted in the hatchery

Capturing and retaining the source populations' diversity will give the in-river population the best chance of success, and is vital to avoiding deleterious founder effects. The methods for measuring whether domestication selection is impacting hatchery population fitness will be developed on an ongoing basis.

#### 6.2.2 Monitoring hatchery population collection

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Given the uncertainties regarding source population availability and hatchery population collection methodologies, hatchery population collection should be carefully monitored to ensure collected fish include the range of life histories and genetic diversity representative of the source populations (see Section 5.1.3 for details). While any single season of collections may not capture the genetic diversity of the source population, collections over time should encapsulate much of the genetic diversity in the source populations. The HGMP provides a detailed discussion of this problem in Section 6.2 (Bork and Adelizi 2010). After fish are collected, fin clips should be taken and genotyped for PBT, and fish should be marked for later identification.

The HGMP (Bork and Adelizi 2010) requires this approach as well:

*All captive reared broodstock will be genotyped for PBT (See HGMP Section 12 for more details) and tagged using an intraperitoneal, passive integrated transponder (PIT) tag after reaching a minimum length of 85 mm. PIT tags will be used for monitoring individual fish throughout captivity.*

For all source populations collected, the following indices should be monitored:

- 1) Location and number of fish/eggs collected for each collection.
- 2) Mortality or observed stress on collected fish/eggs and on fish/eggs sampled but released.
- 3) Effective population size of the collected hatchery population ( $N_e$ )

- 4) Expected heterozygosity of the collected hatchery population ( $H_e$ )
- 5) Allelic richness of the collected hatchery population ( $A_R$ )
- 6) Hardy-Weinberg Equilibrium (HWE)
- 7) Linkage disequilibrium of the collected hatchery population (LD)
- 8)  $F_{IS}$  of the collected hatchery population
- 9) Pedigree analysis
- 10) Pairwise relatedness to conduct mating matrices
- 11) Genetic stock identification to ensure all collected fish are spring-run in origin

Methodology for each of these indices can be found in the appendix. A power analysis is needed prior to monitoring to ensure that the sample sizes and genetic markers are capable of detecting statistically significant differences.

### 6.2.3 Monitoring broodstock growth and reproduction

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After collection, fish comprising the new hatchery population will be reared to sexual maturity and spawned to produce progeny for reintroduction (captive propagation and rearing) or they will be released to the river to allow them to out migrate and return as adults (captive rearing). Fish may also be reared in the hatchery to adulthood and then released to the river to spawn; because direct release of the adults to the river does not require monitoring of broodstock growth and reproduction, it is not addressed in this section.

All hatchery fish, regardless of approach, will experience some mortality during rearing, and the HGMP has set a goal of 85-90% survival from egg to fry stages and 75% or higher survival from egg to smolt stages over the duration of the Program and a 50% or higher survival from smolt to adult (Bork and Adelizi 2010), based on average survival in other programs (e.g., Pollard and Flagg 2004). Hatchery population collection should provide sufficient fish/eggs to achieve Program goals in spite of this mortality if sufficient numbers of individuals are available from source populations. However, if the mortality is not random, but rather concentrated in particular families or populations, this level of mortality could significantly reduce effective population sizes and genetic diversity, resulting in increased inbreeding and a lower chance of successful reintroduction. All captive reared broodstock should be genotyped for PBT and PIT tagged after reaching a minimum length of 85 mm. PIT tags will be used for monitoring individual fish throughout captivity. Using PIT tags, the hatchery population should be monitored during maturation to detect differential mortality.

Fish health policy compliance should be monitored using both visualization and diagnostic assays, and any observed disease outbreaks during inspections should be reported, with dead fish identified by both family and source population.

Rearing practices designed to minimize domestication selection are outlined in HGMP Section 3 (Bork and Adelizi 2010). Adherence of hatchery operations and conditions to recommended natural hatchery rearing practices should be monitored during hatchery population maturation.



If the broodstock approach is used, the development of a mating matrix should allow reproduction that maximizes effective population size while staying random with respect to life history traits, but the actual matings should be monitored to demonstrate compliance with the mating matrix and to document the in-hatchery success of these matings. If adults are released to the river to spawn, sampling of in-river smolt (per Section 6.3, below) should reveal matings and allow for PBT in future generations.

For all hatchery population fish, divided by source population, the following indices should be monitored on an ongoing basis (monthly, for 1-2, and annually for 3-7), in order to detect potential changes in genetic diversity:

- 1) Disease occurrence, following American Fisheries Society professional standards as described in the American Fisheries Society Bluebook (Thoesen 2007), with occurrences reported by family and population.
- 2) Mortality of fish/eggs, reported by family and population.
- 3) Annual effective population size ( $N_e$ )
- 4) Annual expected heterozygosity ( $H_e$ )
- 5) Annual allelic richness ( $A_R$ )
- 6) Hardy-Weinberg Equilibrium (HWE)
- 7) Linkage disequilibrium (LD)

For items 3 through 7, no additional tissue samples need to be taken from the hatchery fish; calculations can be done using the known genotypes, which are associated with the individual PIT tags.

For the hatchery population, divided by source population, the following information should be monitored/collected during reproduction, as applicable:

- 1) Estimated eggs per female.
- 2) Age, size, and origin of each parent involved in each cross, including free-choice matings in-river, as determined by analysis of offspring.
- 3) Survival of each cross to the eyed egg stage.

#### 6.2.4 Monitoring genetic diversity of hatchery population offspring

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Offspring population genetic diversity should be compared for significant differences from previous diversity estimates, and significant divergences should be handled as outlined in the Section 7, Contingency Plans. Power analysis should be conducted to ensure that sample sizes are sufficiently large to detect significant divergence. All captive reared broodstock should be genotyped for PBT and PIT tagged after reaching a minimum length of 85 mm. PIT tags will be used for monitoring individual fish throughout captivity. An evaluation of the concordance between PBT and PIT tags for individual identification is recommended.

The following should be evaluated for the offspring, within each source population and across the entire hatchery population, as applicable:



- 1) Disease occurrence, following American Fisheries Society professional standards as described in the American Fisheries Society Bluebook (Thoesen 2007), with occurrences reported by family and population.
- 2) Mortality of fish/eggs, reported by family and population.
- 3) Effective population size ( $N_e$ )
- 4) Expected heterozygosity ( $H_e$ )
- 5) Allelic richness ( $A_R$ )
- 6) Hardy-Weinberg Equilibrium (HWE)
- 7) Linkage disequilibrium (LD)
- 8) Inbreeding estimates:  $F_{IS}$
- 9) Pedigrees.
- 10) Run timing
- 11) Changes in life history trait distribution

If disease outbreaks begin to occur in the hatchery, routine genetic or biochemical pathogen screens should be considered to control the spread of disease that may threaten the persistence of the entire hatchery population. Section 4.3 lists potential diseases that may impact the hatchery population.

#### Summary of recommendations for monitoring the genetic diversity of the hatchery population

- *Collect fin clips for genetic analysis of fish collected from source populations and from their offspring*
- *Conduct annual genetic monitoring; any given fish should only be genotyped once, and can later be identified by PIT tag.*
- *Report matings conducted and any deviations from the mating matrix, including reason for the deviation.*
- *Measure the following genetic diversity indices annually:  $N_e$ ,  $H_e$ ,  $A_R$ , HWE, LD, and  $F_{IS}$ .*
- *Conduct PBT coupled with PIT tagging to track differential family survival. Evaluate concordance between these two identification approaches.*

### 6.3 Monitoring genetic diversity of the supplemented in-river population

#### 6.3.1 Background

Monitoring the genetic diversity of the restored San Joaquin in-river population has two primary goals:

- 1) Identify success/failure of alternative reintroduction strategies
- 2) Assess overall diversity through time to determine the restored population's genetic integrity

Achieving the first goal will help drive the adaptive management process by identifying and targeting the reintroduction strategies with the highest reproductive success. Additionally, reintroduction strategy monitoring is of substantial scientific value and can help guide future reintroduction efforts. The purpose of the second goal is to identify temporal reduction, maintenance, or gain of genetic diversity in the population as a whole (i.e., comparing diversity within the restored population over time). This population-wide genetic monitoring can identify population trends (e.g., bottlenecks, hybridization, straying, inbreeding) of critical importance to long-term population persistence, for all periods of the restoration.

### 6.3.2 Monitoring alternative reintroduction strategies

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Given the uncertainties regarding habitat conditions, ecosystem dynamics, climatic fluctuations, and other unforeseen factors, the strategy with the best chance for successful spring-run Chinook reintroduction will include several alternative reintroduction strategies. As of this GMP creation, the specific reintroduction strategies to employ are still being debated, with genetic consequences to the source and in-river populations being one of the factors influencing the decision. Potential reintroduction strategies currently being considered include:

- 1) Hatchery propagation, hatchery rearing, translocation, or a combination
- 2) Release of several life stages across adequate spatial and temporal ranges
- 3) Use of source populations from Butte Creek, Mill Creek, Deer Creek, and/or Feather River Hatchery along with potential input from other spring-run Chinook ephemeral populations (e.g., Clear Creek, Battle Creek, Yuba River) and strays from tributaries near the upper San Joaquin (e.g., Stanislaus and Mokelumne rivers)

Though it is unlikely that all the potential reintroduction scenarios listed above will be used in every possible combination, the number of different options under consideration quickly creates a complex combination of scenarios that need to be evaluated independently. With adequate sampling, individual tagging (physical and/or genetic), and the use of statistics, however, it should be possible to detect significant correlations between chosen methods and reproductive success. It is possible that different strategies may have temporally variable success rates depending on changing environmental conditions.

For each reintroduction strategy employed, the following indices should be monitored:

- 1) Outmigrating smolt production
- 2) Adult escapement
- 3) Straying rates

These measurements of reintroduction success for the various strategies should be compared in relative proportion to each other to gauge success. A power analysis is

recommended prior to reintroduction initiation to ensure that adequate sample sizes will be used for each strategy in order to obtain results with statistical support.

The time period between smolt outmigration and adult escapement will occur in the ocean and is outside the monitoring scope of the SJRRP. However, the life stages between egg and smolt development will occur within the Restoration Area and can be monitored to identify if a specific stage is experiencing significantly greater than average mortality (e.g., by comparing with predicted survival rate ranges for different life stages provided in the Reintroduction Strategy document (SJRRP 2011)). This finer-scale monitoring of specific life stages will be of great benefit, particularly during the early stages of introduction, and knowledge gained will be informative for adaptive management. Sampling throughout the reaches of the Restoration Area will be required for non-migrating life stages, which will require more effort than sample collection at a weir. Therefore, it is recommended that sampling for survival of specific life stages occurs once every two years for the first eight years of reintroduction (2012 – 2020). For genetic analysis, fry and parr should be collected from locations throughout the Restoration Area to identify particular reintroduction strategies with differential mortality. This will occur by using PBT to genetically identify each individual and assign it back to the reintroduction strategy used for its release. The only exception to this is the translocation of eggs from the source populations and for this strategy sibship analysis will be attempted (see Section 5.3) but may not be feasible. The specific number of individuals to collect per location should be based on a power analysis to assess sample sizes needed to obtain statistically significant results. After 2020, it is recommended that specific life stage monitoring is conducted if smolt outmigration begins to significantly decline, in order to detect at what stage(s) higher than usual mortality rates are occurring.

Straying into and out of the Restoration Area will need to be monitored in collaboration with monitoring efforts taking place outside the Restoration Area. Other Central Valley locations (i.e., nearby tributaries in the San Joaquin River basin or the Sacramento River basin) will likely receive Restoration Area strays and will also contribute strays into the Restoration Area. If other tributaries aren't sufficiently monitored, the incidence of straying out of the Restoration Area cannot be accurately measured (i.e., straying will be underestimated). Additionally, collaboration between CA salmonid researchers to share genotypic information (i.e., common genetic database) is critical for accurately determining straying rates via PBT among the Central Valley tributaries and hatcheries. The genetic consequences of straying are examined in Section 4.2 of this GMP.

Outmigrating juveniles and returning adults will be genetically identified by PBT, using a minimum panel of 96 SNPs. In the early years of reintroduction it is likely that all migrating juveniles and adults can be genetically monitored. As restoration proceeds and outmigrant and escapement numbers hopefully increase, genetically screening every individually may become logistically or economically infeasible. Annual collections may be capped (e.g., 5,000 smolts and 5,000 adults) if necessary to ensure timely genetic analysis. The exact number of individuals analyzed each year may vary over time depending on evolving genotyping technology and costs but estimated predictions

of outmigration and escapement numbers should be used to ensure that sampling occurs throughout the season to reduce sampling bias. The maximum collection limit may be adjusted based on adaptive restoration monitoring goals and availability of funding. This sampling should occur every year of outplanting and for an additional approximately three generations (i.e., 12 years) after outplantings cease. All collected samples should be archived in the CDFG Tissue Collection Archive.

Depending on reintroduction strategies employed, specific knowledge gained from monitoring the different approaches may include:

- 1) Reproductive success of hatchery propagation strategies
  - a. Broodstock crosses and mating strategies (free-choice mating versus breeding matrices)
  - b. Success of various release strategies (life stage, release location, timing)
- 2) Reproductive success of translocation strategies
  - a. Success of various release strategies (life stage, release location, timing)
- 3) Differential habitat use or migration timing employed by distinct family cohorts
- 4) Straying rate (into and out of system)
  - a. Different rates depending on supplementation method (hatchery propagation, hatchery rearing, translocation)
  - b. Different rates depending on release strategy (life stage, location, timing)
- 5) Gene flow/introgression rate occurring in the Restoration Area
  - a. Between spring-run source populations
  - b. Between spring- and fall-run Chinook ESUs

From an adaptive management perspective, monitoring focused on gauging the success of alternative reintroduction strategies may conclude when spring-run Chinook cease being intentionally reintroduced to the Restoration Area. It is preferable, however, that monitoring continues for several generations after reintroductions cease, in order to inform scientific conclusions regarding long-term success of particular strategies.

### Summary of recommendations for monitoring reintroduction strategies for the in-river population

- *When feasible, physical tags (e.g., PIT tagging) should be used in conjunction with genetic PBT as a back-up/alternative approach to comprehensive monitoring.*
- *Fin clips should be taken from all fish outplanted into the Restoration Area for genetic analysis (PBT and genetic diversity metrics).*
- *For eggs or small fry outplanted into the Restoration Area through direct transfer, a small subset of each family should be collected for sibship analysis.*
- *Annually take fin clips from smolt outmigrants and adult returnees in the Restoration Area and archive all samples in the CDFG Tissue Collection Archive.*
- *Collect at a downstream weir and span the entire migration time range.*
- *Every migrating smolt and adult should be sampled until sample sizes exceed a maximum target for genetic analysis (e.g., ~5,000 smolts and ~5,000 adults).*
- *All fish (or the maximum target) should be sampled at a downstream weir every year of outplanting and for an additional three generations (12 years) after outplantings cease.*
- *Every two years from 2012 - 2020, collect fin clips from fry and parr from locations throughout Restoration Area and genetically assess production of life stage intervals leading up to smoltification.*

#### 6.3.3 Monitoring genetic diversity of restored San Joaquin in-river population

Maintenance, loss, or gain of population genetic diversity can be evaluated only when compared against previous diversity estimates. Frequent genetic monitoring of the in-river population will enable corrective actions to be taken in a timely manner to avoid depletion of genetic variation that could ultimately lead to unsuccessful restoration.

The following genetic diversity indices should be evaluated during routine monitoring of the in-river population:

- 1) Effective population size ( $N_e$ )
- 2) Expected heterozygosity ( $H_e$ )
- 3) Allelic richness ( $A_R$ )
- 4) Hardy Weinberg Equilibrium (HWE)
- 5) Linkage disequilibrium (LD)
- 6) Allele frequency change ( $F_{\text{temporal}}$ ) (microsatellites)
- 7) Genetic differentiation:  $F_{ST}$  (SNPs),  $G''_{ST}$  (microsatellites)
- 8) Inbreeding estimates:  $F_{IS}$  and  $F$  estimates from pedigree reconstruction
- 9) Hybridization level between spring- and fall-run ESUs

The importance of each of these indices is discussed in the appendix of this GMP. These indices will provide an overall assessment of population genetic diversity. PBT will be used to track individual migration and survival. In addition to these genetic diversity indices, it is recommended that phenotypic diversity is examined for life history traits such as those mentioned in Section 5.1.3 (Selection on Phenotypes).

Specific knowledge gained from the in-river population (not specific to reintroduction strategies) may include:

- 1) Detection of population bottlenecks, inbreeding depression, outbreeding depression, and effective population size.
- 2) Differential habitat use or migration timing employed by distinct family cohorts
- 3) Short- and long-term trends in reproductive success
- 4) Straying rate (into and out of system)
- 5) Gene flow/introgression rate occurring in the Restoration Area between:
  - a. fish originating from Restoration Area and strays
  - b. hatchery-origin and wild-origin fish ("wild" fish may derive from hatchery supplementation but have spent no time in a hatchery themselves)
  - c. spring-run source populations
  - d. spring- and fall-run Chinook ESUs
- 6) Comparison of genetic and phenotypic diversity (compared to source populations and temporally within the in-river population) to assess if increased overall genetic diversity is correlating with increased biocomplexity

An additional monitoring option for future consideration, depending on disease emergence in the Restoration Area, is genetic pathogen screens. Genetic pathogen detection methods are being continually developed (e.g., Kelley et al. 2006) and would enable routine monitoring of various reaches to measure isolated or widespread disease prevalence.

Collecting fin clips samples of outmigrating smolts and returning adults at a downstream collection site (e.g., weir) will be the primary method of sample collection. Additionally, carcass surveys can be used to distinguish between strays migrating into the system and spawning versus those that migrate out again prior to spawning. Monitoring should continue annually during all phases of the restoration.

After assessment of reintroduction strategies ceases, genotyping of all migrants into and out of the system is no longer necessary for the purpose of adaptive management. Routine (post-supplementation) monitoring of genetic diversity indices should include ~100 individual samples collected across spatial and temporal distributions and occur every four years. Returning adults or carcass surveys are an appropriate life stage for collection since these individuals have a high probability of contributing to the next generation versus egg or juvenile life stages. Sampling effort should occur throughout the appropriate time period and habitat range for a given life stage (e.g., throughout spawning season for returning adult collections). If possible, genetic studies should be conducted on the same individuals that are used for other



studies (e.g., testing contaminant levels) to increase the potential for synergy across scientific disciplines and reduce handling stress. Increasing monitoring back to Reintroduction Period levels is recommended in at least two scenarios. First, after potential large-scale selective events (e.g., unseasonably warm and/or low-water years, in-river disease outbreaks, environmental contamination events, habitat fragmentation), monitoring should temporarily increase in order to appropriately evaluate patterns of selection (e.g., via  $F_{ST}$  outlier loci) and survivorship and to assess population status. Second, in the event of hatchery reactivation, monitoring intensity should resume at Reintroduction Period frequency levels to allow for proper adaptive management of the population.

As stated in the TAC spring-run recommendations (Meade 2007), with the exception of minimum population targets, Program performance should not be based on results from any one year. Caution should be exercised in modifying the fundamental reintroduction strategy until a clear trend is established, which may take a number of years to become evident. It is recommended that modifications based on genetic monitoring do not occur until a trend is established over at least a 2 – 3 year period, keeping in mind that some trends may take even longer to observe.

#### Summary of recommendations for monitoring the genetic diversity of the in-river population

- *Collect fin clips for genetic analysis*
- *Genetic and phenotypic monitoring should be conducted annually throughout all restoration phases.*
- *Collect at a downstream weir and span the entire migration time range (i.e., outmigrating smolts or returning adults).*
- *During the reintroduction phase, every migrating smolt and adult should be sampled until sample sizes exceed a maximum target for genetic analysis (e.g., ~5,000 smolts and ~5,000 adults). Conduct PBT to track straying and differential survival.*
- *Post-reintroduction, collect 100 returning adults (at weir or by carcass survey) every four years. If a large-scale in-river selective event or hatchery reactivation occurs, resume reintroduction phase monitoring.*
- *Measure the following genetic diversity indices:  $N_e$ ,  $H_e$ ,  $A_R$ ,  $HWE$ ,  $LD$ ,  $F_{temporal}$ ,  $F_{ST}$ ,  $G''_{ST}$ ,  $F_{IS}$ ,  $F$ , hybridization levels.*

## SECTION 7 HATCHERY PHASE-OUT

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The TAC spring-run recommendations (Meade 2007), the FMP (SJRRP 2010b), and the HGMP (Bork and Adelizi 2010) all provide perspectives on the hatchery phase-out. Those are reviewed here to facilitate examination of the genetic considerations around the phase-out.

### 7.1.1 TAC Spring-run Recommendations

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The TAC spring-run recommendations noted that a self-sustaining population should be re-established by December 31, 2024, the end of the Interim Period, to be followed by a Growth Population Period from January 1, 2025, to December 31, 2040. The TAC provides recommendations for naturally produced spring-run Chinook salmon population targets for those periods.

During the Interim Period, which begins January 1, 2020, the TAC recommends a five-year running average target for total river escapement of at least 2,500 fish, with allowable population fluctuation between 500 and 5,000 spawners (Meade 2007). The TAC further recommends that 500 spawners should be the minimum target for fish returning to spawning areas for any given year after 2019. The TAC also recommended that if these targets are not met, “monitoring data should be reviewed, and restoration strategies and efforts should be assessed by the TAC in consultation with implementing agencies to recommend refinements in management actions to improve returns.”

During the Growth Population Period, the TAC recommends a target for the 5-year running average of spawners should increase from 2,500 to 30,000 spawners, and the rate of increase of the number of spawners (cohort replacement rate) should be greater than 1.00.

The TAC developed these targets based in part on genetic models, using the figure of 500 spawners from Allendorf et al. (1997) and Lindley et al. (2007). The TAC also considered other sources that gave similar figures: Cass and Riddell (1999) recommend 100 female spawners, roughly 300-500 fish when males and unsuccessful spawners are taken into account, and Hedrick et al. (1995), focusing on Sacramento winter-run Chinook salmon, and counting fish both spawned in the wild and in restoration hatcheries, recommend 500+ annual spawners.

Finally, the TAC cites Allendorf et al. (1997) for the proposition that long-term maintenance of genetic diversity within a population requires an effective population size of 500 and then follows Lindley et al. (2007) to suggest that for Central Valley Chinook salmon, the proportion of fish that make up the effective population is 20%, so the actual minimum escapement needed is estimated to be approximately 2,500 fish.

### 7.1.2 FMP Recommendations

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The FMP (SJRRP 2010b) reiterates the goals set by the TAC spring-run recommendations, but provides some additional targets and observations. “After the initial 10-year Reintroduction Period, the target for the proportion of hatchery and other artificially produced fish will be less than 15 percent of the population, except potentially during periods of prolonged drought.” The 15 percent figure includes strays from out-of-basin hatcheries.

The FMP recognizes three uses for the hatchery during the reintroduction:

*First, spring-run Chinook salmon may be stocked with fish that are incubated and/or raised in a hatchery prior to release in the Restoration Area. Second, it is likely that large numbers of study fish may be needed for juvenile Chinook salmon survival studies and for calibrating rotary screw traps. Third, if monitoring determines that the natural production of juvenile Chinook salmon is too low during the relatively dry water year types (e.g., Critical-Low and Critical-High year types)[...] hatchery fish may be used to supplement the population in those years. A long-term source of eggs for the hatchery will have to be identified to avoid sacrificing naturally produced San Joaquin River adult Chinook salmon.*

### 7.1.3 HGMP Recommendations

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The HGMP notes that, “Under the Settlement Agreement, the hatchery should be phased out by 2025, unless required for years with abnormally low flows insufficient to support the salmon population. Hatchery use in the post 2025 period will be assessed annually by the Hatchery and Monitoring Technical Team.” (Bork and Adelizi 2010).

HGMP Objective 7 calls for the phase-out of “Conservation Facility operation based on an adaptive management approach and achievement of restoration objectives.” In support of that objective, the HGMP established two standards. First, Standard 7.A. establishes that, beginning in 2020, the four-year moving average hatchery proportion of the total natural spawning population (pHOS) should be declining to reach 15% or less in 2027. Second, Standard 7.B. requires that “quantitative natural population targets (e.g.,  $N_e$ , census size, genetic diversity) and other community and ecosystem indicators of reintroduction success are derived and periodically evaluated to determine the schedule for phase-out of Conservation Facility production.” Finally, Indicator 7.B.ii. requires that “Reductions in hatchery production are made based on achievement of goals in HGMP Table 1.1, allowing for annual variation of up to 50% from the goals to accommodate natural fluctuation, per the FMP.” (Bork and Adelizi 2010).

### 7.1.4 Genetics Considerations in Hatchery Phase-out

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Under the existing HGMP, hatchery operations will begin to ramp down in 2020, the last year of broodstock collection from source populations (Bork and Adelizi 2010), if escapement meets the Program targets discussed above. Egg production is forecast to decrease by more than 50% in 2024, and by 2025 only age-5 females from 2020 will be spawned and there are unlikely to be a large number of those fish. By the planned phase-out date, the hatchery should have had the opportunity to spawn two generations of broodstock from the source populations, and the Program should have data on which fish from which populations are returning. The dates established in the HGMP are subject to revision if scientific evidence suggests it is necessary to achieve overall reintroduction goals, and indeed the TAC spring-run recommendations (Meade 2007), the FMP (SJRRP 2010b), and the HGMP (Bork and Adelizi 2010) all call for adaptive management of the phase-out.

While no data are available on effective population size for the source populations, phase-out based on the targets discussed above has a sound genetic basis and draws heavily from the VSP guidelines established in Allendorf et al. (1997) and Lindley et al. (2007). An effective population size of 50 individuals is accepted as large enough to minimize random small-population genetic effects (Frankel and Soule 1981; Nelson and Soule 1987; Allendorf et al. 1997), and an effective population size of 500 is accepted as large enough to maintain long term adaptive potential (Franklin 1980; Allendorf et al. 1997). Allendorf et al. (1997) and Lindley et al. (2007) take these figures and then assume that, for Chinook salmon populations, the effective population size is 20% of the census population size. Using these figures, Allendorf et al. (1997) and Lindley et al. (2007) arrive at a census size of 250 fish to minimize random small-population genetic effects and 2500 as large enough to maintain long term adaptive potential, spread over a 4 year generation time, as follows. The census size requirements are calculated as the average annual escapement ( $\hat{S}_t$ ) multiplied by the average age at reproduction (generally set at three years, but four years for these populations, based on the HGMP), to account for multiple generations. Applying this factor, the escapements correlating to these guidelines are average escapements of 63 and 625 spawners, respectively, well below the minimum numbers established by the TAC spring-run recommendations (Meade 2007) and echoed in the FMP (SJRRP 2010b). It is unclear from the TAC spring-run recommendations and FMP discussions whether these estimates accounted for the generation multiplier in determining the necessary census size; that is, it is unclear whether their numbers account for the relationship between  $N_b$  and  $N_e$ . As noted above, Cass and Riddell (1999) and Hedrick et al. (1995) provide ample support for the TAC and FMP targets in either case. Importantly, these targets will have to be adjusted to account for the actual generation times found in the reintroduced population. A failure to account for the actual generation time would likely result in a misapplication of the phase-out criteria.

The targets established by the TAC spring-run recommendations and FMP allow an ample cushion for the protection of genetic diversity. This cushion also accounts for variation in the actual  $N_b/N_e$  ratios, away from the 20% assumption. For example, Waples et al. (2010) estimated Chinook salmon  $N_b/N_e$  at  $\sim 0.04 - 0.32$  for the stream-type life history and  $\sim 0.05 - 0.36$  for the ocean-type life history. Using the TAC target of 500 fish, this would give an actual minimum escapement between approximately 1388

and 12500, based on the most extreme numbers. Ongoing monitoring of the reintroduced population will allow estimation of  $N_e$  for the reintroduced population, which should change the escapement targets. Further, the average generation time in the reintroduced population may vary significantly from the four year expectation, which would also require adjustment of the genetic guidelines.

Even if the reintroduced population is meeting the targets discussed above, Hatchery phase-out will have implications for the source populations, the hatchery population, and the in-river population, discussed below. If the reintroduced population does not meet those targets, supplementation should continue until the targets are met and the genetic considerations outlined throughout this GMP will continue to be relevant. Finally, if the targets are met and the hatchery is phased out, but the average annual escapement subsequently drops below the target levels and additional supplementation is required, reactivation of the hatchery would require several genetic considerations, discussed below.

#### *7.1.4.A Source Populations*

For the source populations, the end of broodstock collection in 2020 will end any ongoing impacts to those populations from this collection. Monitoring of those populations should continue for another four years, in order to detect any impact on adult escapement from broodstock collection, but the termination of broodstock collection is unlikely to have a significant population impact on the source populations. If the collection of broodstock from the source populations continues or is reinitiated after 2020, due to low returns in the San Joaquin River or for other reasons, impacts to the source populations are likely to remain low, given the numbers of fish anticipated to be removed from those populations. Use of collection methods or collection at levels not anticipated in the HGMP might have a more serious effect.

#### *7.1.4.B Hatchery Population*

If the population targets are met and the phase-out goes ahead as scheduled, the hatchery population will begin to shrink in 2021, after collection of new broodstock from the source populations ends, and will continue to shrink as the remaining broodstock fish mature, depending on survivorship to maturity and age at maturity in the hatchery. The shrinking population may pose some problems for management of genetic diversity in the hatchery population: smaller numbers of fish available for crossing may result in unequal sex ratios or too few fish for factorial mating. The HGMP provides a detailed breakdown of fish estimated to be available for crossing during the waning years of hatchery production. See HGMP Appendix 3 (Bork and Adelizi 2010). Either survivorship to maturity and age at maturity in the hatchery would reduce the hatchery effective population size and could accelerate loss of genetic diversity. To counter these concerns, the Conservation Facility should cryopreserve enough milt to achieve equal sex ratios and provide for a factorial mating, as required by the HGMP, based on the number of females estimated to be available in the last several years of hatchery operations. See HGMP Appendix 3 (Bork and Adelizi 2010) for a breakdown of hatchery fish availability for broodstock mating. While low numbers of fish available for

mating in the last two years of hatchery operations give the impression of low effective population size, the effective population size is calculated based on the generation time, which includes the previous, larger generations, so the small numbers should not be cause for concern.

If the average adult escapement falls below target levels, the hatchery population may need to be re-established to prevent genetic bottlenecks or to avoid extinction, as contemplated in the FMP (SJRRP 2010b) and the HGMP (Bork and Adelizi 2010). Any reactivation of the hatchery will have significant genetic impacts on the reintroduced population. Low adult escapement may have a variety of causes, from systemic issues like disease or inbreeding to stochastic causes like low water years or poor ocean conditions. When determining the appropriate response to low escapement, the underlying cause must be determined in order to formulate an appropriate response. If the underlying cause is a systemic issue that indicates genetic problems for the population as a whole, such as poor disease resistance (based on fish health monitoring in the hatchery and instream monitoring), that leads to a very different hatchery response than stochastic events unlikely to be related to the population's genetic diversity.

If the underlying cause of low escapement indicates genetic problems (e.g., inbreeding depression; poor fitness linked to genetic issues), the hatchery reactivation should draw fish from outside of the reintroduced population. Initial supplementation to avoid total loss of the reintroduced population may need to use returning adults from the reintroduced population to allow time for the development of a new broodstock, but for the long term, additional diversity should be introduced from the source populations, provided that either the source populations are the only viable source for additional fish, or that the Hatchery and Monitoring Technical Team still believes that the previously used sources are the best sources for additional introductions. The best source for the additional fish can be determined based on the relative success of the source populations in the Restoration Area, judged by genetic monitoring of the reintroduced population. If offspring of crosses between populations are most successful after the  $F_2$  generation, additional outcrossing should be undertaken. Supplementation using additional fish from the source populations would likely require a broodstock approach, to minimize impacts to those populations, and thus would require a lead time of 2-3 years, depending on the life stage collected to start the broodstock. Finally, because supplementation with source stocks would be attempting to change the genetic identity of the reintroduced population, the supplementation should not be limited by the HSRG recommendations outlined in HGMP Section 1.9 (Bork and Adelizi 2010) and should instead be determined based on the underlying genetic problem.

If the underlying cause of low escapement does not appear to be a genetic problem, the hatchery should use fish from the reintroduced population, while seeking to maintain an  $N_e$  that is as high as possible. Using these fish has two advantages. First, from a permitting standpoint, the fish are more likely than the source population fish to be available in sufficient numbers for the creation of a second broodstock using adult fish, given their experimental status. Use of adult fish means that the fish will spend less time in the hatchery prior to crossing, easing domestication concerns and allowing for



an immediate population impact. Second, the fish in the reintroduced population will likely have been exposed to one or more generations of selection in the Restoration Area, and using these fish is more likely to allow supplementation without swamping out any local adaptation. Any supplementation that is not attempting to correct a genetic problem should abide by the HSRG recommendations outlined in HGMP Section 1.9 (Bork and Adelizi 2010).

#### 7.1.4.C In-River Population

Finally, the hatchery phase-out will impact the genetic characteristics of the in-river population. Among other goals outlined above, the establishment of the reintroduced population with broodstock from the source populations should attempt to include as much genetic diversity from the source populations as possible, although this goal has to be balanced with ongoing natural selection in the new environment. The end of Conservation Facility operations also marks the end of major infusions of genetic diversity from other populations (assuming that the hatchery is not reactivated). Thus the genetic diversity of the in-river population after phase-out will be maintained only through migration and mutation, and lost due to selection or random genetic drift. Selection strength and effective population size will affect the rate of loss of genetic variation, and close monitoring of the in-river population will be necessary to ensure that inbreeding does not impact the population, as outlined in the monitoring section. While the targets discussed above should provide a sufficient effective population size to avoid inbreeding, if the assumptions described above do not hold (generation time,  $N_e/N_c$  ratio, unexpectedly low genetic diversity or high founder effects), additional supplementation may be necessary.

#### Summary of recommendations for Hatchery Phase-out

- *The HGMP, FMP, and TAC census targets for phase-out have a sound genetic basis, provide an ample safety net, and should be followed.*
- *The conservation facility should cryopreserve enough milt from excess males throughout the reintroduction period to achieve equal sex ratios and provide for a factorial mating in the final years of hatchery operation, when females are expected to significantly outnumber males due to the early return of males as precocious smolt or jacks*
- *If average adult escapement indicates a need for the hatchery population to be re-established to prevent genetic bottlenecks or to avoid extinction, fish may be drawn from either the source populations or the experimental population, depending on the underlying cause of the low escapement. Stochastic or environmental causes would allow for use of the experimental population, while causes with an underlying genetic basis may require additional fish from the source populations.*
- *If the monitoring of the experimental population shows unexpectedly low genetic diversity or high founder effects, additional supplementation with source-stock fish should be considered.*

## SECTION 8 CONTINGENCY PLANS

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There is a high probability, perhaps almost certainty, that not all aspects of spring-run Chinook salmon restoration to the upper San Joaquin River will go exactly as planned. Presented below are triggers (i.e., scenarios) for foreseeable issues that may occur during the course of restoration along with particular re-evaluation and monitoring strategies aimed at ameliorating these issues. The purpose of a trigger is not to cease all standard practices. Instead, the trigger should be taken as an opportunity to re-evaluate practices and/or environmental conditions so that managers better understand the causes behind the trigger event in the hopes of reducing negative consequences to the source, hatchery, or in-river population.

### 8.1 Contingency plans: source populations

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The source populations should be minimally impacted by the levels of take identified in the HGMP (Bork and Adelizi 2010), and allowable take from these populations will be determined by the NMFS permitting process and ongoing evaluations of the source population status, not by this GMP. Further, any management actions directly affecting source populations beyond limiting broodstock collection is beyond the scope of the Program. For these reasons, the source populations are not generally addressed in the contingency plans. However, the source stock availability may be limited by low escapement in those populations, so that possibility is addressed.

#### 8.1.1 Contingency plan for limited broodstock availability

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##### 8.1.1.A *One or more source populations are unavailable.*

###### Trigger

Low adult escapement results in a decision by NMFS to forbid collection from one of more of the source populations. Note that low adult escapement is already impacting these populations and population trends suggest the problem may worsen in the near future (GrandTab 2010).

###### Re-evaluation

If at all possible, individuals should be mined from all three primary source stock populations, even if the mining is at a low level and several years are needed to develop a full scale broodstock. Collection from additional populations should resume as soon as source population levels recover sufficiently. If the FRH is used as a sole source, active management to prevent continuing introgression with the fall-run Chinook will be even more vital to maintenance of the spring-run phenotype. Undertaking the entire reintroduction with a single source is a worst case scenario because the source populations exhibit low to moderate diversity levels, and the inbreeding resulting from a relatively small sample from one population presents opportunity for problems related to low genetic diversity (founder effects) and lack of adaptive capacity.

### Monitoring to inform re-evaluation

Ongoing monitoring of source population  $N_c$  provides sufficient data to assess the viability of those populations and their availability as broodstock.

## 8.2 Contingency plans: hatchery population

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Section 6.2 details monitoring the genetic diversity of the hatchery population. One of the primary goals of hatchery population genetic monitoring is to capture and retain a large proportion of the source populations' genetic diversity in the hatchery broodstock.

Reactivation of the hatchery would require additional contingency planning; see Section 7 for additional discussion of hatchery reactivation.

### 8.2.1 Contingency plan for hatchery genetic diversity

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#### *8.2.1.A Failure to capture source population diversity.*

##### Trigger

After four years of broodstock collection, much of the genetic diversity detected in the source populations is not found in the hatchery broodstock. This could result from poor or differential survival of collected fish, from failure to collect a representative sample of the source population, from maladaptation of source fish or hybrids to the new environment, and from random sampling error. While noting that the goal of a captive breeding program should be to maintain as much genetic diversity as possible, Fraser (2008) endorsed maintenance of 90% of the genetic diversity in a population, based on a variety of measures. Because the in-river population is likely to be small for the first several generations, the broodstock should capture an even higher percentage of the diversity to allow the naturalized population to reflect 90% of the diversity in the source populations. If less than 95% of the known diversity of any one source population is present in the hatchery after 4 years of collections, this contingency plan should be triggered. While some of that diversity may be maladaptive to the new environment, the relatively high bar of 95% seeks to preserve as much of the source population diversity as possible. This trigger may need to be adjusted in the future.

##### Re-evaluation

If the broodstock collection does not produce a population with 95% of the diversity from each of the source stocks after 4 years of collection, collection methods should be reevaluated to determine a collection method that will capture a more diverse broodstock. The source populations may need to be reevaluated to determine if they have lost diversity during the collection period. If additional collection methods cannot be developed, additional generations of source population fish may be collected to better represent the source population diversity, within the constraints of the NMFS permits..

### Monitoring to inform re-evaluation

The hatchery population will be genotyped for PBT, and this genotyping will allow continuous monitoring of the population's diversity through standard genetic diversity indices detailed in the hatchery monitoring section, Section 6.2.

#### *8.2.1.B Unequal sex ratios, high relatedness among broodstock, or unequal family sizes.*

##### Trigger

Extremely unequal sex ratios that prevent execution of the planned breeding matrices, high relatedness among broodstock, or unequal family size significantly reduce the effective population size in the hatchery, leading to more rapid loss of genetic diversity in the broodstock. If the broodstock's effective population size, after four years of collections, leads to an expected or actual > 5% reduction in the  $N_e$  of any one source population present in the hatchery, this contingency plan should be triggered.

##### Re-evaluation

If the broodstock exhibits unacceptably unequal sex ratios, high relatedness among broodstock, or unequal family size, such that the above trigger is reached, broodstock collection numbers may need to be increased in order to increase the effective population size, or the duration of the supplementation extended, to ensure representation of the source population genetic diversity in the reintroduced population. Additional collections will depend on permitting decisions by NMFS but should be undertaken to protect the genetic integrity of the reintroduced population. If additional source population fish are unavailable, outcrossing between source population fish should be considered to reduce relatedness in the broodstock, which will require a pedigree for all hatchery fish. If research suggests that outcrossing is maladaptive, intentional outcrossing should be avoided.

##### Monitoring to inform re-evaluation

The hatchery population will be genotyped for PBT, and this genotyping will allow continuous monitoring of the population's sex ratios, relatedness, and family size, both for outmigrating smolts and returning adults.

#### *8.2.1.C High mortality in hatchery population.*

##### Trigger

An environmental factor in the hatchery leads to high mortality or differential survival of fish from one family or source population, or low survival of progeny at any life stage while still in hatchery. Low survival of adults released for in-river mating drastically reduces the effective population size. If the observed change in census size indicates that the broodstock's effective population size, after four years of collections, leads to an expected loss of more than 5% of the known diversity of any one source population present in the hatchery, this contingency plan should be triggered. If the

observed diversity of offspring in the hatchery or in the river indicates a loss of more than 5% of the known diversity of any one source population present in the hatchery, this contingency plan should be triggered.

### Re-evaluation

In all cases, the environmental factor leading to reduced survival should be ameliorated, if possible. Regardless of the underlying cause, additional broodstock collection should be undertaken to increase population size and reduce loss of genetic diversity. Strong selection in the hatchery will reduce the diversity of the in-river population, reducing the likelihood that it will have sufficient diversity to adapt to river conditions. Moreover, the selective pressures in the hatchery will not be identical to those in the river, so selection in the hatchery should be avoided as much as possible. If hatchery conditions cannot be ameliorated, and high levels of mortality on the broodstock continue in spite of additional collection, the strong selection combined with a lack of favorable genotypes in the population may eventually lead to extirpation, and supplementation using new source populations with novel genetic diversity should be considered.

### Monitoring to inform re-evaluation

Hatchery mortality should be monitored closely to determine if particular families or source populations are more susceptible. Frequent testing for diseases and contaminants in the hatchery is also strongly recommended. PBT may be used to evaluate the parentage of diseased fish.

## 8.3 Contingency plans: in-river population

Given the stochastic environmental conditions likely to be experienced by the in-river population, there is a high likelihood that reintroduction will not be proceed exactly according to plan. The following contingency plans are provided as a genetic guide to identify foreseeable triggers, monitoring needs, and re-evaluation of strategies.

As previously detailed in Section 6.3, monitoring the genetic diversity of the in-river population has two primary goals:

- 1) Identify success/failure of alternative reintroduction strategies
- 2) Assess overall diversity through time to determine the restored population's genetic integrity

### 8.3.1 Contingency plans for alternative reintroduction strategies

*8.3.1.A Relative performance of a particular reintroduction method: source population, hatchery propagation, translocation, life stage, release location or time*

### Trigger

One of the potential reintroduction methods listed above is performing poorly (i.e., statistically significant lower proportional smolt outmigration and/or adult returns) relative to other comparable methods over at least a three year period.

#### Re-evaluation

A decision will need to be made regarding if the benefits of the particular method (e.g., increased genetic diversity, less risk to source population, ease/cost of method, etc.) outweighs the poorer reproductive fitness. If benefits do not clearly outweigh the lower performance then the method either needs to be adjusted based on scientific expertise or discontinued to re-allocate resources to the most efficient strategies. If the method is adjusted (e.g., releasing a poorly performing life stage at a new location) then this new adjustment will also be subject to the trigger and discontinued if it is performing poorly. Alternatively, if the entire in-river population is performing poorly (regardless of reintroduction method) the entire reintroduction strategy will need to be re-evaluated, along with examination of biotic and abiotic factors that may be involved in poor overall performance.

#### Monitoring to inform re-evaluation

Because multiple methods will be combined during each supplementation event (e.g., hatchery propagated fish released at fry life stage in a particular section of the river at a particular time) meticulous records will need to be collected and evaluated to identify the specific factor(s) leading to reduction in fitness. Monitoring success of alternative reintroduction methods will occur through both physical and genetic tags, as described in Section 6.3.

*8.3.1.B      Straying rate of each particular reintroduction method: hatchery propagation, translocation, life stage, release location or time*

#### Trigger

One of the potential reintroduction methods listed above is producing fish with a high straying frequency over at least a three year period. For example, any method that is producing strays at a frequency greater than five percent (see Section 4.2), particularly without any attributed environmental driver (e.g., low flow rates).

#### Re-evaluation

If many unrelated methods are producing high rates of straying, the cause may be an unknown environmental factor. Any environmental conditions favoring straying need to be adjusted accordingly. If only particular methods result in straying, these methods will need to be adjusted based on scientific expertise or discontinued.

#### Monitoring to inform re-evaluation

Rates of straying into the Restoration Area will be actively monitored by physical and genetic tagging during every year of supplementation. However, it will not be



possible using San Joaquin River monitoring alone to determine the rate of straying into other river systems. Monitoring of all nearby river systems, which will be the most probable destination for strays, is likely outside the scope of the SJRRP monitoring program. Therefore, the SJRRP will need to actively coordinate with monitoring that is occurring in these systems. A common genetic database, as discussed in the appendix, will be of great assistance in monitoring strays for all Central Valley spring-run Chinook salmon populations.

### 8.3.2 Contingency plan for continued genetic integrity of in-river population

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#### 8.3.2.A *Disease, contaminants, habitat, and other environmental factors*

##### Trigger

An environmental factor leads to high mortality or differential survival of the in-river population. For example, a 15% reduction in any life stage attributed to a particular environmental factor. Some factors may be readily identified (e.g., physiological signs of a particular disease) while others may be less obvious (e.g., contaminants in a particular river section).

##### Re-evaluation

In all cases, the environmental factor leading to reduced survival should be ameliorated, if possible. Additional action taken will, in part, depend on the particular environmental cause of poor survival. If only a particular section of the Restoration Area is affected, individuals should be re-distributed to other sections, if feasible, until conditions improve. If the problem is systemic (e.g., disease) and the strength of selection is strong enough, then the population should be given the opportunity to select for favorable genotypes in the wild. Strong selection combined with a lack of favorable genotypes in the population may eventually lead to extirpation. If this occurs, supplementation of new source populations with novel genetic diversity, preferably from the CA Central Valley, should be considered.

##### Monitoring to inform re-evaluation

Environmental conditions in all reaches of the Restoration Area should be routinely monitored, minimally on an annual basis, so that unfavorable conditions don't become firmly established. Mortality of fish will be informed by monitoring of smolt outmigration and adult returns along with different life stages throughout the Restoration Area. Frequent testing for diseases and contaminants throughout the Restoration Area is also strongly recommended to potentially promote environmental conditions unfavorable to a particular disease or reduce contaminant levels.

#### 8.3.2.B *Introgression*

##### Trigger

Introgression between fall-run and spring-run Chinook salmon is occurring at a >5% level within the Restoration Area (see Section 4.2).

#### Re-evaluation

Identify potential environmental factors leading to introgression (e.g., physical barrier separating spring- and fall-run returnees isn't completely effective). A physical barrier, perhaps more than one, need to be completely operational. Identify potential genetic factors leading to introgression (e.g., previous introgression between run types in the FRH is leading to unpredictable or greatly overlapping return times of spring- and fall-run ESUs). Consider removing individuals that return during this overlapping time from the Restoration Area prior to spawning (i.e., select for divergent run timings). This will likely encourage clear segregation between spring-run and late fall-run ESUs.

#### Monitoring to inform re-evaluation

The extent of introgression will be monitored annually through genetic analysis of outmigrating smolts and adult returns described in Section 6.3.

#### *8.3.2.C Reduction in standard genetic diversity measurements ( $N_e$ , $A_R$ , etc.)*

#### Trigger

Genetic diversity indices decline significantly from the weighted average of the founding source populations over a three year period.

#### Re-evaluation

Identify if supplementation strategies are causing differential survival or reduced reproductive fitness and adjust strategies, if necessary. If reduced genetic diversity is occurring after supplementation, determine if environmental factors can be attributed to reduced diversity and remove or adjust, if feasible. If inbreeding levels or reduction in effective population size become serious concerns, consider increasing or reinitiating supplementation from source populations through translocation or hatchery activities. If an anthropogenic cause cannot be determined, the reduction in genetic diversity may be due to natural processes (e.g., local adaptation), which should proceed without intervention.

#### Monitoring to inform re-evaluation

These indices will be routinely monitored as detailed in Section 6.3.

#### *8.3.2.D Increased proportion of hatchery fish*

#### Trigger

The hatchery proportion of the in-river population is greater than 15% of the in-river population after the initial ten year Reintroduction Period (i.e., starting in 2027)

over any four year period. This trigger directly indicates that one of the FMP Population Objectives is not being met (SJRRP 2010b).

#### Re-evaluation

These hatchery origin fish may be from intentional Restoration Area supplementation or strays from other systems. If the supplementation is intentional then it can be reduced in an attempt to stay below the 15% range, though it may be difficult to make accurate adjustments. However, reinitiation of the hatchery will almost certainly be due to extenuating circumstances (e.g., substantial reduction in census or effective population sizes, inability of in-river population to sustain itself) and this <15% hatchery origin recommendation may need to be temporarily suspended. If straying is the cause of the increased proportion of hatchery fish then environmental conditions and/or the practices of other nearby hatcheries that promote straying should be evaluated and, if possible, ameliorated to favor homing. If high rates of hatchery straying, outside the range of typical straying in wild populations (see Section 4.2), are continually problematic after intentional supplementation ceases then all hatchery fish may need to be removed from the Restoration Area prior to spawning.

#### Monitoring to inform re-evaluation

Proportion of hatchery fish in the in-river population will be assessed each year during monitoring detailed in Section 6.3.

#### *8.3.2.E Alteration in rate of straying*

##### Trigger

Straying rates, into or out of the Restoration Area, exceed 5% during a three year period.

##### Re-evaluation

If many unrelated methods are producing high rates of straying, the cause may be an unknown environmental factor. Any anthropogenically influenced environmental conditions favoring straying need to be adjusted accordingly. If using a weir for collection, consider removing marked hatchery strays from the system prior to spawning.

##### Monitoring to inform re-evaluation

Rates of straying into the Restoration Area should be monitored by physical and genetic tagging during every year of supplementation. However, it will not be possible using San Joaquin River monitoring alone to determine the rate of straying into other river systems. Active coordination with monitoring programs that are occurring in other CV river systems is recommended and a common genetic database, as discussed in the appendix, will be of great assistance in monitoring strays for all CV spring-run Chinook salmon populations.

## SECTION 9      CONCLUSIONS

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Throughout the course of restoration, it is impossible to foresee and plan for all potential genetic risks given the ecosystem complexities and interactions. The primary goals of this GMP were to acknowledge known risks that may compromise genetic diversity and recommend actions for avoiding or ameliorating these risks. Genetic factors relating to fitness do not work in isolation and it is important that the overall health of the restored population is assessed in an integrative fashion across biological sub-disciplines.

The monitoring actions recommended are considered essential for determining the relative successes of alternative reintroduction strategies in the short-term and fluctuations in genetic diversity that can impact fitness over the long-term. In addition to these routine monitoring actions, we strongly recommend that less routine studies are initiated to aid in both long-term restoration success and be of general benefit to the scientific community. A few of the research topics that could be addressed include: fitness outcomes of outbreeding and mechanisms of outbreeding depression; identification of loci under selection that are associated with fitness; response to climate change (e.g., temperature tolerance); and epigenetic modifications implicated in rapid phenotypic response to new environments. Newly emerging conservation genomic technologies have made these types of studies feasible (Allendorf et al. 2010, Frankham 2010). It will be unfortunate if the restoration of spring-run Chinook salmon to the upper San Joaquin River is not treated as an opportunity to make scientific breakthroughs that will lead to continued improvements in future restorations.

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## SECTION 11 APPENDIX

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### 11.1 Overview of recommended genetic parameters

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#### 11.1.1 Calculation of $N_e$

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While it is possible to estimate  $N_e$  using demographic methods (Caballero 1994), these methods often overestimate  $N_e$  since it is difficult to include certain factors, such as variance in reproductive success. There are several genetic estimates of  $N_e$  that can be measured, with the variance  $N_e$  ( $N_{eV}$ ), inbreeding  $N_e$  ( $N_{eI}$ ), and coalescent  $N_e$  ( $N_{eC}$ ) being among the most common. Both  $N_{eV}$  and  $N_{eI}$  estimate contemporary  $N_e$ , which can help gauge restoration success, evaluate existing adaptive potential, and guide management actions.  $N_{eV}$  is based on changes in allele frequencies over time, which can change rapidly during population bottlenecks. It is measured using a temporal approach (e.g., Jorde and Ryman 1995) and the precision and accuracy of the measurement increases as the number of generations between sampling events increases (Waples and Yokota 2007). This temporal method measures the harmonic mean  $N_e$  in the interval between sampling periods, though it does not provide any information for the current sampled generation (Waples 2005). In contrast,  $N_{eI}$  is often based on patterns of heterozygosity (Pudovkin *et al.* 1996), linkage disequilibrium (Hill 1981), coancestry (Nomura 2008), or sibship (Wang 2009) and, in general, measures the  $N_e$  of the previous generation. It is measured using a single-sample approach, thereby enabling  $N_e$  estimation without using historic samples. A third genetic  $N_e$  estimate method is the coalescent  $N_e$  ( $N_{eC}$ ) method, which estimates long-term  $N_e$  over many generations since the most recent common ancestor (Kuhner 2006). Since  $N_{eC}$  can be strongly influenced by past demographic events (e.g., a historic bottleneck), it should not be used to assess current population trends but can serve as a historic baseline for comparison with contemporary  $N_e$  estimates and to ensure that population targets set by managers are realistic (reviewed by Hare *et al.* 2011).

It is recommended that both microsatellite and SNP markers are used and compared for  $N_e$  estimation to evaluate precision. Additionally, it is recommended that multiple  $N_e$  estimates (e.g.,  $N_{eV}$ ,  $N_{eI}$ ,  $N_{eC}$ ) using multiple sampling (e.g., single-sample or temporal) and computational (e.g., Approximate Bayesian Computation (Tallmon *et al.* 2008)) approaches, are conducted in order to evaluate precision and hopefully achieve the most accurate consensus with the smallest confidence intervals. This is particularly important given the often large and variable  $N_e$  ranges observed with different methods. It is difficult to recommend particular approaches for future estimation since methods of  $N_e$  estimation are rapidly progressing. As effective population size estimation methodology continues to improve, advances should be incorporated into  $N_e$  estimation for populations affected by Program activities. As an initial strategy, using the single-sample linkage disequilibrium method in conjunction with the temporal method is recommended to obtain independent estimates of  $N_e$ . A list of contemporary  $N_e$  estimators, along with strengths and assumptions of each, can be found in Luikart *et al.* (2010). Factors that can lead to inaccurate estimates of  $N_e$  (e.g., overlapping generations) need to be recognized and taken into account (see Luikart *et al.* 2010 for

review and recommendations). To avoid bias from overlapping generations, age structure information should be collected and cohorts reconstructed, if possible, to obtain  $N_b$  estimates that can be used for  $N_e$  estimation (Waples 2005). This could be accomplished by collecting outmigrating juveniles of the same age for  $N_e$  estimation. It is recommended that at least 10% of each population's predicted effective population size is sampled to obtain precise and accurate results (Palstra and Ruzzante 2008). Census population sizes ( $N_c$ ) can also be estimated using the same molecular genetic data (e.g., genetic mark-recapture) or by using traditional demographic approaches (reviewed by Luikart *et al.* 2010). Evaluation of  $N_e/N_c$  over time can be used to assess whether specific ecological factors (e.g., mating system or survivorship) are leading to a predictable reduction in the ratio and how ecosystem management might maintain or increase this ratio (Luikart *et al.* 2010). Finally, it is important to note that each  $N_e$  estimate must be properly matched to the time period that it is applicable to and that this may vary depending on the method used for estimation and the sampling process (Waples 2005).

### 11.1.2 Importance of additional genetic diversity indices

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To accurately assess the genetic diversity of populations impacted by restoration efforts, other standard genetic diversity indices should be calculated in addition to  $N_e$ .

- 1) Estimates of unbiased heterozygosity ( $H_e$ ) (for SNPs and microsatellites) and sample-size corrected number of alleles per locus ( $A_R$ ) (for microsatellites) are recommended to assess genetic diversity for each locus.
- 2) Hardy-Weinberg Equilibrium (HWE) can be disrupted by evolutionary forces such as mutation, migration, selection, drift, or non-random mating. Individual loci that are not in HWE may be an indication of genotyping errors.
- 3) Linkage disequilibrium (LD), the non-random association of alleles among loci, can be caused by bottlenecks, recent introgression, cryptic population substructure, small population size, or selection. Additional tests mentioned in this section are needed to determine which evolutionary force(s) may be responsible for observed LD. Particular locus pairs that are in LD may be an indication of physical linkage. It is recommended that LD is examined for both locus-pairs and at the population level.
- 4) Changes in allele frequencies ( $F_{\text{temporal}}$ ) can be caused by bottlenecks, introgression, substructure, and selection (Schwartz *et al.* 2007).  $F_{\text{temporal}}$  is very sensitive to population declines since frequencies can change without the loss of alleles (Schwartz *et al.* 2007). If  $F_{\text{temporal}}$  is significant, additional tests for genetic bottlenecks should be conducted to evaluate the rate of allelic loss relative to heterozygosity loss. Detection of recent genetic bottlenecks may enable corrective actions to be undertaken to minimize the fitness declines and the fixation of deleterious alleles (e.g., Hedrick 1995). There is not currently an existing program to calculate  $F_{\text{temporal}}$  but statistically

- significant changes in allele frequencies, along with 95% confidence intervals, can be calculated based on equations in Waples (1989).
- 5)  $F_{ST}$  (SNPs) or  $G'_{ST}$  (microsatellites) can be used to detect differentiation among sampled populations. See Meirmans and Hedrick (2011) for a recent review of the advantages and drawbacks of different statistics for quantifying population structure. Marker neutrality should be tested prior to population structure analyses since loci influenced by selection may potentially confound results (reviewed by Helyar *et al.* 2011)
  - 6) Testing for a correlation between genetic and spatial distance can identify if related individuals are aggregating spatially (i.e., isolation by distance). Samples (eggs, juveniles, adults) will need to be collected throughout the spawning or rearing range. This could have important implications for future sampling (e.g., source population mining), allowing for collection strategies that better minimize the likelihood of obtaining highly related individuals.
  - 7)  $F_{IS}$  provides a genotypic estimation of inbreeding to assess if a source population should be mined and the impact that mining may be having on each population's genetic health. It should be noted, however, that other factors (e.g., reduced population size from poor ocean conditions) may also be responsible for increased inbreeding levels so causation of increased inbreeding will require additional research. For the in-river population, multiple generations of PBT will enable pedigree reconstruction for direct estimates of inbreeding ( $F$ ). Calculation of  $F$  using 3 – 5 generations of pedigree information has been reported to provide reliable estimates (Balloux *et al.* 2004).
  - 8) Levels of hybridization between spring- and fall-run ESUs should be monitored at both the FRH and in the upper San Joaquin River. Ideally, PBT will be used to assess hybridization and may be used in conjunction with physical tagging.
  - 9) Genetic stock identification (GSI) should be conducted for any individuals collected for reintroduction purposes to ensure that only Chinook salmon that are spring-run in origin are brought into the hatchery for propagation and/or rearing or directly translocated into the Restoration Area. Additionally, if individuals straying into the Restoration Area cannot be identified via PBT (i.e., not present in the common database recommended in Section 11.3), GSI may be used to assign them back to their respective populations.
  - 10) Pairwise relatedness estimates should be used to minimize inbreeding and the loss of genetic diversity in the hatchery through the use of mating matrices. A study evaluating the type I and type II error rates of four relatedness estimators recommended that  $M_{xy}$  and  $R_{QG}$  be used to determine which Snake River sockeye salmon pairs to spawn in a hatchery and reported that this approach should be generally applicable for other stocks without



pedigree information (Kozfkay *et al.* 2008). There are, however, many other relatedness estimators and comparison of them is recommended for the SJRRP Conservation, using known pedigrees from potential source populations (e.g., FRH), if available.

All of these genetic diversity indices can be evaluated using the same genetic data set for each population and collectively enable evaluation of the genetic integrity and diversity within and among monitored populations through time. In Section 11.4 there is an overview of recommendations for genetic monitoring of each population (Appendix Table 1) and software programs that may be used for each recommended genetic metric (Appendix Table 2).

## 11.2 Overview of parentage based tagging (PBT)

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PBT involves the genotyping of individuals for use as intergenerational tags (Anderson and Garza 2006). PBT relies on the use of a reference database, which contains potential parents, so that progeny can be genetically assigned back to their parents. If adequate records are maintained and compiled into a database, PBT may provide information such as: individual identification, pedigree reconstruction, inbreeding estimates, straying rate estimates, age, survival rates (of families, reintroduction methods, particular life stages, etc.), reproductive success, and heritability estimates for particular phenotypic traits. Using simulations, Anderson (2010) found that the panel of 96 SNPs recommended for SJRRP genetic analyses, which is the same panel used by the Southwest Fisheries Science Center (SWFSC) for PBT and GSI, assigned progeny to incorrect parents <0.5% of the time in populations studied. Typically, progeny were not assigned to parents, though these parents were in the database, at a rate of <0.1. While the FRH spring-run population was included in these simulations, it should be noted that assignment accuracy in the Mill, Deer, and Butte Creek populations was not assessed. These 96 SNPs, which were chosen for their ability to conduct PBT and GSI for California Chinook salmon, were described in a recent study by the SWFSC (Clemento *et al.* 2011). The use of these markers by multiple laboratories should facilitate sharing of genotypic information for Chinook salmon throughout the Central Valley if a common database is implemented, as recommended (see Section 11.3 below).

Some of the benefits of PBT, in comparison to physical tags, include: no issues with tag recovery, ability to conduct non-lethal sampling, opportunity for additional information (e.g., examining loci under selection), and reduced concerns regarding differential mortality. Some concerns regarding PBT include the need for additional empirical studies for evaluation purposes and the need for further comparisons of the time/cost of genetic tagging versus physical tagging.

## 11.3 Retaining/sharing collections and genotypic information

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### 11.3.1 Archiving samples

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All tissue samples collected in support of Program restoration efforts and monitoring should be deposited in the CDFG Tissue Collection Archive for long-term storage and potential future use to assess changes in genetic diversity over time or for use by researchers not affiliated with the SJRRP. For long-term storage it is recommended that samples are stored in 95% ethanol at -80° Celsius. Alternatively, tissue/DNA desiccation or liquid nitrogen storage may be investigated.

#### 11.3.2 Database

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All genotypic data should be deposited into a common database so researchers with a vested interest in accessing genetic information for Chinook salmon throughout the Central Valley may access it. The curator of the database, along with other details (e.g., information it contains, standardization of genotyping across laboratories, dissemination of findings resulting from database query), will need to be collectively decided upon by participating researchers. An example of such a database already in existence is the one maintained by the Genetic Analysis of Pacific Salmonids (GAPS) consortium to facilitate genetic data standardization and sharing for Chinook salmon throughout the Pacific Coast (Seeb *et al.* 2007). The initiation of such a database for CA Central Valley Chinook salmon is strongly recommended to facilitate a better understanding of connectivity and diversity across the region. This database will need to be sufficiently funded to ensure continuous maintenance and upgrades.

#### 11.4 Recommended genetic analyses and software programs

**Appendix Table 1. General overview of recommended genetic analyses for each population impacted by Program activities**

Population	Time Period	Frequency	Life Stage(s)	Recommended Genetic Analyses
Source <sup>a</sup>	Initial baseline: prior to 2012; Monitoring: Reintroduction Period	After initial baseline obtained, once per generation (i.e., once every 3-4 years)	Adults (alive or carcass)	$N_e$ , $H_e$ , $A_R$ , HWE, LD, $F_{temporal}$ , $F_{ST}$ , $G'_{ST}$ , $F_{IS}$ , hybridization <sup>b</sup> , Mantel test, GSI
Hatchery <sup>a</sup>	Reintroduction Period	Annually	Initially juvenile; PIT tagging will allow subsequent estimation of genetic parameters during broodstock rearing	PBT, $N_e$ , $H_e$ , $A_R$ , HWE, LD, $F_{temporal}$ , $F_{IS}$ , pairwise relatedness, GSI
In-river	Reintroduction Period	Annually or Biennially	Outmigrating smolts & returning adults (annually); fry & parr (biennially)	PBT <sup>c</sup>
In-river	Reintroduction Period	Annually	Eggs (directly translocated)	Sibship analysis
In-river <sup>a</sup>	Reintroduction Period and beyond	Annually during Reintroduction Period then once every four years	Adults (alive or carcass); $N_e$ estimates may benefit from juvenile collections if adults aren't aged	$N_e$ , $H_e$ , $A_R$ , HWE, LD, $F_{temporal}$ , $F_{ST}$ , $G'_{ST}$ , $F_{IS}$ , Mantel test, GSI

<sup>a</sup> Prior to commencing analyses, power analyses are recommended to ensure that adequate sample & loci numbers are used to detect statistical significance

<sup>b</sup> Hybridization levels between spring- and fall-run at the FRH should be measured via PBT (see Section 6.1)

<sup>c</sup> Used to monitor survivorship and reproductive fitness of different reintroduction strategies and directly estimate inbreeding ( $F$ ) via pedigrees (see Section 6.3)

**Appendix Table 2. Suggested software programs for the recommended genetic analyses**

<b>Genetic Analysis</b>	<b>Suggested Software Programs<sup>a</sup></b>	<b>References</b>
Parentage Based Tagging (PBT)	<i>SNPPIT</i>	Anderson (2010)
Contemporary $N_e$ (single sample LD method)	<i>LD-Ne</i>	Waples & Do (2008)
Contemporary $N_e$ (temporal method)	<i>TempoFs</i>	Jorde & Ryman (2007)
$H_E$ , $F_{IS}$ , $F_{ST}$ , Mantel test	<i>Arlequin</i>	Excoffier et al. (2005)
$G''_{ST}$	<i>GenoDive</i>	Meirmans & Van Tienderen (2004)
$A_R$	<i>FSTAT</i>	Goudet (1995)
HWE, LD	<i>GDA</i>	Lewis & Zaykin (2001)
Genetic bottleneck	<i>Bottleneck and M_P_VAL</i>	Piry et al. (1999); Garza & Williamson (2001)
Sibship analysis	<i>Colony2</i>	Wang (2009)
Pairwise relatedness	<i>Coancestry</i>	Wang (2011)
Genetic stock identification (GSI)	<i>ONCOR</i>	Kalinowski et al. (2007); Anderson et al. (2008)
Power analysis	<i>POWSIM</i>	Ryman & Palm (2006)

<sup>a</sup> Many other programs are currently capable of conducting these genetic computations (some with alternative assumptions) and new programs are constantly being created. It is recommended that a review of available programs is undertaken prior to commencing any analysis.