Mission statement and five-year plan Coho salmon captive broodstock program

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### Summary

The objective of this mission statement is to clarify the role of the Fisheries Ecology Division (FED) and the ongoing captive broodstock program (CBP) in supporting recovery of coho salmon in the Central California Coast Evolutionarily Significant Unit (CCC ESU). The statement represents a consensus view among the research staff of FED regarding the overarching goals of the program and more specific research areas to be pursued in the next five years. FED is committed to continuation of the CBP and to collaboration with the Monterey Bay Salmon and Trout Project (MBSTP) to ensure the successful rearing of coho salmon for supplementation activities within the CCC ESU, with a specific emphasis on the focal and supplemental streams identified in the Final CCC Coho Salmon Recovery Plan (NMFS 2012) for the Santa Cruz Mountains Diversity Stratum. We envision two primary goals for the program in the near future:

- 1. Reduce the extinction risk of the southernmost populations of coho salmon by continuing production of adult broodstock in our laboratory facilities.
- 2. Expand ongoing research efforts to provide information necessary to support, improve and adapt recovery plans, with the objective of restoring viable coho salmon populations within the Santa Cruz Mountains Diversity Stratum.

# Introduction

Coho salmon (*Oncorhynchus kisutch*) have been in decline in California, and the Santa Cruz Mountains in particular, for decades. These declines triggered a federal Endangered Species Act (ESA) listing for the CCC ESU as threatened in 1996, followed by an upgrading to endangered status in 2005. The ESU is also listed as endangered under the California Endangered Species Act (CESA). Extensive details on causes and potential options for reversing these trends are provided in both the state (CDFG 2004) and federal (NMFS 2012) recovery plans. Although there are many contributing factors, coho salmon are basically suffering a decline from three main challenges, which interact with negative feedback loops to limit spawning and rearing success:

- 1. The capacity and productivity of freshwater habitat have been diminished as a consequence of anthropogenic land management and water resource practices.
- 2. Variability in ocean conditions has resulted in periods of poor marine survival for coho salmon in the California Current system, and the frequency of poor ocean years may be increasing in response to climate change. Reductions in freshwater

habitat capacity increase the vulnerability of populations to variable marine conditions.

 Declining population sizes have resulted in a loss of genetic diversity and associated life history pathways that took advantage of previously available habitats and environmental conditions.

Declining abundance, diminished capacity of habitats, and loss of diversity have collectively rendered coho salmon populations less resistant to natural and humancaused environmental perturbations and less resilient when those disturbances occur. Recognizing the potential extinction of the CCC ESU, the SWFSC FED, in collaboration with MBSTP, California Department of Fish and Wildlife (CDFW), and the Southwest Regional Office of NOAA Fisheries (SWR), began in 2001 to collect individuals from Scott Creek, in Santa Cruz County, the southernmost extant population of coho salmon in the CCC, to form a captive broodstock program. This effort was initiated in recognition that recovery planning, habitat restoration, and reversal of potential climate change conditions would take much longer than the species could persist at the southernmost edge of their range.

In this statement we clarify the role of FED within the framework of multiple agencies working together to recover coho salmon within the CCC ESU. Our specific goals will differ in some respects from those of other agencies, despite a common objective of reducing extinction risk and supporting recovery. As a division of the Southwest Fisheries Science Center, our responsibility is to provide relevant data and analyses to inform management decisions. To that end, our focus is on evaluating multiple methods and approaches to coho salmon recovery, with the CBP providing one mechanism with which to conduct such evaluations.

### Background

The CBP has been operating since the FED moved to Santa Cruz in December 2000. The mission and overall goals of the program have been implicit but not formally stated for collaborators such as the MBSTP, CDFW, and SWR. At the initiation of the CBP, there was an intent to continue operations for 10 years, then evaluate the program's success and decide if it should continue and, if so, whether it should be modified. We believe it is appropriate at this time to provide a brief review of the program, establish the rationale for continuing its operation, and develop clear goals for the next five years. We also believe it is appropriate to clarify the role of FED, as a research facility, in assisting recovery of coho salmon through production of a captive broodstock.

Scientific staff of FED met in spring 2012 to conduct the 10-year review and evaluate the program's successes and challenges to date (summarized in the next section). We reached a consensus that the program should be continued for the foreseeable future. In the past 10 years, wild populations of CCC coho salmon have continued to decline and have been functionally extirpated from most watersheds in the Santa Cruz Mountains Diversity Stratum. We believe that FED's CBP plays a critical role in preventing the complete extirpation of CCC coho salmon south of the Golden

Gate. Without the supplementation provided collaboratively by the adults produced in our laboratory and MBSTP's Kingfisher Flat Hatchery, coho salmon in Scott Creek, the last extant population in the Santa Cruz Mountains Diversity Stratum, may have gone extinct. However, we also believe that the CBP should not serve solely for production of adult coho salmon. In keeping with the general mission of a NOAA Fisheries Science Center, the CBP should also serve as a research resource, supporting properly designed scientific investigations that address factors related to coho salmon conservation and recovery. In particular, NOAA research should inform and support adaptive management of the MBSTP hatchery. A general outline of anticipated research projects is presented in subsequent sections of this statement. Research objectives for the next five years will be broadly defined and flexible to allow for directional shifts based on new project results. We anticipate that results derived from future research will be directly applicable to recovery efforts promulgated by CDFW and the SWR.

# Current status of the CBP

### Basic description of operations:

The CBP is a joint venture between the FED and MBSTP. The FED supplies rearing tanks, all food, and other disposables, and MBSTP provides facilities for spawning adults and rearing fry to smolt stage as well as expertise in artificial spawning methods for coho salmon. Adults are spawned at the MBSTP, with eggs placed in incubating systems until hatching. Alevins are reared in standard hatchery stacked tray systems through yolk absorption, whereupon fry are transferred to shallow troughs. When juveniles attain a size of approximately 65 mm they are transferred to deeper raceways or large holding tanks and held until maturity. For a more complete overview of the CBP and its operations see Sturm *et al.* (2009).

Coho salmon are currently reared from smolt to spawning adult stage at three facilities: FED, MBSTP (Scott Creek, Figure 1), and Don Clausen Hatchery (Warm Springs Hatchery, WSH, Russian River). FED currently has an agreement with the Army Corps of Engineers to annually rear two separate brood years of Scott Creek coho salmon at WSH. Holding tanks vary in size and volume depending on location: The four tanks at FED are 3.7 m diameter, 1.8 m deep, and hold approximately 22,700 liters of water. At both MBSTP and WSH, there are two tanks that are 6.1 m diameter, 1.5 m deep and hold approximately 45,400 liters of water. Another important difference between the rearing tanks is that FED tanks contain seawater drawn from Monterey Bay, whereas the MBSTP and WSH tanks contain freshwater from Scott Creek and the Russian River, respectively. At all three facilities tanks are flow-through, single pass, and the water turnover rate is at least six times per day. Water velocity within each tank varies with the incoming flow rate, with WSH having the highest velocity (over 1 m/s) and the FED tanks having the lowest (less than 0.5 m/s).

Originally, broodstock fish were natural-origin (wild) coho salmon collected annually from Scott Creek and spawned at the MBSTP hatchery, where the offspring were reared to smolt stage and released as part of a reintroduction and stock



Figure 1. Map of Scott Creek watershed showing location of MBSTP hatchery and the FED weir.

supplementation program. Beginning with brood year 2001, a small number (150) of the offspring were kept for the CBP and raised to maturity. In 2004, mature adults of this first CBP cohort were mated using a spawning matrix, which identified genetic relatedness to prevent inbreeding between broodstock individuals and minimize the chance of inbreeding depression in the resulting offspring. However, as the program continued, returns of wild coho salmon to Scott Creek declined severely and wild adults were no longer available for spawning. Consequently, offspring of CBP fish are now routinely kept as the next generation of captive broodstock fish. In 2011, concerns over the low genetic diversity of the CBP fish prompted crossing CBP coho salmon with out-of-basin (but within-ESU) coho salmon, as noted below.

#### Challenges and Successes

The CBP has successfully perpetuated a population of coho salmon in the Santa Cruz Mountains Diversity stratum during a period when wild populations throughout the CCC ESU have experienced precipitous declines. Fish have been reared to maturity in both seawater and freshwater, and lines of all three brood years in the Scott Creek population have been maintained. Use of the spawning matrix has ensured that the genetic diversity of each annual cohort is as high as possible. Hatchery adults, smolts, and fry have been released into local creeks and rivers that historically contained coho salmon but currently do not.

The CBP has faced numerous challenges during its 10-year operation, including sub-optimal growth rates resulting in adults that are smaller than typical wild fish, poor egg fertilization rates, high egg mortality rates (due to frequent fungal infections, sedimentation, and mechanical abrasions in rearing jars), and occasional operational failures in the hatchery that resulted in poor water quality. Many of these challenges have been successfully addressed through continually evolving changes in operations.

For adults held at the FED rearing facility, modifications from the program outlined in Sturm et al. (2009) include addition of air lifts in the tanks to increase both dissolved oxygen concentration and water velocity inside the tanks, modifying the water input manifolds into all tanks to increase the water velocity, and replacing some of the gel food in the diet with krill top coated in vitamin powder and cod liver oil. Adult rearing capacity was increased in 2010 by the addition of two tanks at the MBSTP hatchery and two tanks at WSH (as described above). Adding these tanks increased the potential number of adult coho salmon produced annually by the CBP from 150 in 2002-2009 to 320 beginning in 2010. Adults at all three facilities now receive the same improved diet. At MBSTP, use of automated (belt or demand) feeding devices has ceased and all feeds are distributed by hand in an effort to reduce adverse social interactions and feeding dominance and size variation.

Modifications have also been made to the spawning and rearing protocols at the MBSTP hatchery since Sturm *et al.* (2009). Adult females are now injected with Ovaplant to synchronize maturation, and ultrasound is used to assess egg ripeness. The upwelling jars previously used for egg incubation have been replaced with a moistair incubator and a temperature controlled water supply. Collectively, these changes have greatly improved egg fertilization rates and survival. Water quality for all stages reared at the MBSTP hatchery has been improved to reduce fluctuations in temperature, avoid excess sediment delivery, and ensure nominal flow rates. In addition to these operational improvements, the genetic diversity of broodstock offspring has been increased through crosses of Scott Creek males and females with fish that originated in other diversity strata within the CCC ESU. However, despite these improvements in rearing strategies, smolts released into local streams continue to result in low numbers of adult returns, reflecting either poor smolt-to-adult survival rates or poor homing fidelity (Table 1).

	Smolts released		Returns to Scott			
	(Scott Creek only)		Creek		<b>T</b>	Smolt to
Brood Year (BY)	Release Year	Ν	at age 2 (jack) <sup>†</sup>	at age 3 (adult)	Total return for BY	adult survival (%)
2005	2006	729	1	2	3	0.41
2006	2007	2,279	7	6	13	0.57
2007	2008	3,141	8	1	9	0.29
2008	2009	1,874	0	0	0	0.00
2009	2010	600	2	0	2	0.33
2010	2011	0	1 <sup>‡</sup>	TBD	TBD	
2011	2012	2,000	TBD	TBD	TBD	
2012	2013	32,000 <sup>§</sup>	TBD	TBD	TBD	

Table 1. Coho salmon smolt releases and adult returns to Scott Creek by brood year. Smolts released into creeks other than Scott are not included.

†Jacks are defined as sexually precocious 2-year old males

<sup>‡</sup> Presumably a stray from adjacent San Vicente Creek where 300 smolts were released in May 2011

§ Preliminary data

## Goal 1 – maintenance of spawning adults

### Justification for continued production: extinction prevention

CCC coho salmon are currently listed as endangered under both the federal ESA and the state CESA. Populations are especially depressed at the southern end of the CCC ESU (i.e., Santa Cruz Mountains Diversity Stratum) where coho salmon have been extirpated from most watersheds and all brood-year lineages have too few individuals to be self-sustaining (CDFG 2004).

The Scott Creek watershed is one of only a handful of watersheds south of the Golden Gate where coho salmon have been observed within the last decade and the only population that, until recently, supported all three brood lineages. However, in the last seven years, the natural population has experienced substantial declines, with three or fewer returning adults being captured at the Scott Creek weir<sup>1</sup> in each of the last three years. Consequently, the population is at high risk of extirpation through both demographic and genetic processes. The small effective population size (number of breeders) combined with low encounter rates between potential mates in the natural environment have resulted in a substantial loss of genetic diversity from the population. The near elimination of brood lineages, coupled with the relatively fixed three-year life history of coho salmon in California, increases the likelihood of extirpation since there is minimal gene flow among brood lineages, and the lack of demographic exchange across lineages diminishes any potential rescue effects. The reduction of the native population to an unsustainably small number of family groups necessitates the continued production of coho salmon through captive breeding as a means of preserving the remaining genetic lineage and reducing the likelihood of extinction through stochastic environmental processes.

### Genetic composition, inbreeding and outbreeding

The current genetic composition of the Scott Creek broodstock is characterized by low heterozygosity, Hardy-Weinberg disequilibrium, and over-representation of a few family groups (inadvertent hatchery-mediated variance in reproductive success). Numerous family group lines already have been extirpated, and most remaining individuals are related to each other at some level, making inbreeding difficult or even impossible to avoid. This is of great concern for several reasons. First, mating between fish related even at low levels is known to result in widespread, lethal deformities in their offspring (Conrad *et al.* 2013). Such external deformities were observed in recent generations of Scott Creek coho salmon offspring, suggesting that inbreeding and inbreeding depression (the deleterious effects of inbreeding) are present in this population. Second, inbred lineages, with their largely monomorphic genetic and phenotypic makeup, are highly susceptible to natural environmental variability, such as disease outbreak or temperature fluctuation (e.g., Arkush *et al.* 2002). Third, small populations are prone to demographic volatility. Low effective population size coupled

<sup>&</sup>lt;sup>1</sup> The weir is located near the mouth of Scott Creek (Fig. 1) and operated annually by FED from December to May to estimate the number of returning adult coho salmon.

with low fitness indicates that even in recovery or expansion, coho salmon populations will continue to be vulnerable.

A stringent method of inbreeding avoidance is essential to maintain overall fitness and persistence of this population. However, even with such a protocol in place, average relatedness among the Scott Creek broodstock has increased steadily since the inception of the program due to the lack of unrelated mates (Fig. 2). To counteract this trend, an experimental outbreeding component was initiated in winter 2010-2011, using Olema Creek broodstock (Coastal Diversity Stratum) obtained from WSH in the Russian River watershed. Olema Creek is a tributary of Lagunitas Creek, which supports the largest coho salmon population south of Mendocino County and is the most phylogenetically similar extant population within the CCC ESU to Scott Creek, making this population the most suitable choice for outcrossing with local fish. Outbreeding experiments continued in winter 2011-2012; however, in this brood year no Olema Creek fish were available, so captive broodstock from the Russian River coho salmon population (Coastal Diversity Stratum) were used instead.

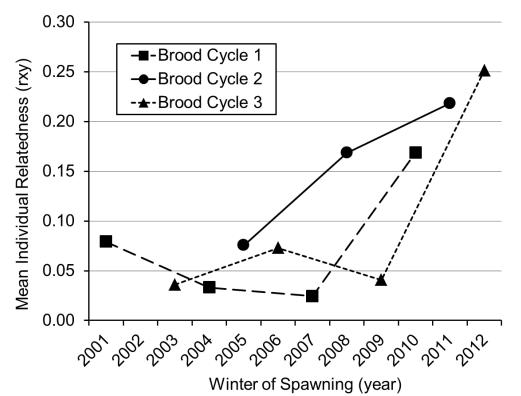


Figure 2. Temporal trend of increasing mean relatedness of coho adults from Scott Creek, for each of the three brood cycles (broodlines), where generation time is three years. Each data point denotes the average genetic relatedness of broodstock individuals at the time of spawning. The Captive Broodstock Program began with the offspring of adults spawning in winter 2001/2002 (Brood Cycle 1 on this plot).

### Spawning matrix past, present and future

The spawning matrix is a genetics-based tool for inbreeding avoidance, or the selection of unrelated mates within a broodstock. In a captive broodstock composed largely of related individuals, the spawning matrix is used to minimize inbreeding

through the spawning of least-related pairs. For genetic analysis, a tissue sample along with a unique identifier is collected from every broodstock individual. Polymerase chain reaction (PCR) is used to amplify 18 neutral microsatellite DNA markers that are variable in California coho salmon populations. Multi-locus genotype data is then analyzed to determine the relatedness of each pair of broodstock fish and, from a matrix of relatedness estimates ( $r_{XY}$ , Queller and Goodnight 1989), a spawning matrix is constructed. The spawning matrix is female-focal, with males ranked by their relatedness to each female such that the most desirable and least related mates appear at the top of each female's list of potential mates. Males related to the focal female above a critical  $r_{XY}$  value are flagged as crosses to avoid since they would constitute a high level of inbreeding

Since inception of the CBP, technological additions to the genetics laboratory infrastructure (e.g., capillary sequencer), along with optimization of molecular genetic protocols, have decreased the turnaround time from receipt of sample to inclusion in the spawning matrix from five workdays to about three. Future improvements to the spawning matrix may include an in-season, dynamically updated matrix that would reprioritize males based on the number of times spawned.

A major research area for the future (see below) involves experiments raising and releasing fish at multiple life-stages instead of releasing smolts exclusively, a standard practice in the past. For example, pre-spawning adult fish are now being released into appropriate streams when there are excess CBP adults, exceeding the potential rearing capacity of the hatchery. Directed experiments would compare the success of fish released as fry, smolts, or adults. These comparisons would require an additional component to the spawning matrix to select unrelated maturing adults for release into the watershed.

# Goal 2 – expansion of research efforts

The primary goal of research associated with the CBP is to maximize the effectiveness with which hatchery supplementation methods can be used to prevent the extirpation of coho salmon in the Santa Cruz Mountains diversity stratum and assist with recovery of wild populations. The federal coho salmon recovery strategy (NMFS 2012) requires increased productivity in four of the five diversity strata. Current habitat conditions generally worsen and threats increase from north to south in the ESU (NMFS 2012). Many of our research results will be relevant in the context of recovery elsewhere, but we will focus on the specific needs of this diversity stratum. According to NMFS' recovery strategy, ESU-wide recovery criteria cannot be reached until all four of the targeted diversity strata are considered viable (including the Santa Cruz Mountains diversity stratum). The distinct genetics of these populations and the current condition of their rearing habitats require an understanding of local biological and ecological factors. For example, as the southernmost populations of coho salmon, they may face more extreme impacts of impending climate change compared to more northerly populations.

General research areas to be addressed by FED include the following:

# 1. Coho salmon husbandry

### Factors affecting fertilization rates

The CBP has historically had a problem obtaining fertilized eggs from some females. In some cases, fertilization rate for individual females is less than 20% and complete failure (0%) has been observed. This problem exists for females reared at all three facilities (MBSTP, FED, and WSH). Previous to the 2012 spawning season, the average fertilization rate was around 25% with a hatch-out success around 10%; however, in 2012 the fertilization rate averaged 60% with a hatch-out rate of 50%. Although improved, these rates are still lower than those achieved for coho salmon at Iron Gate Hatchery on the Klamath River (Siskiyou County), where both fertilization and hatch-out success typically exceed 80%. The increases in fertilization and hatch-out success in 2012 were attributed to the changes in food composition and resulting increased size of the females. To determine why some females produce viable eggs while others do not, tissue samples (egg, muscle, and liver) were collected from 14 females that had good spawning success (over 80% hatch out) and 14 females with poor spawning success (under 30% hatch out) in 2012 for lipid profile determination. Although results are still pending, this is a first step into investigating the cause of the historically poor fertilization rate. Any differences in the condition and health of females with high vs. low hatch-out success will provide insight into the rearing requirements needed for successful adult development.

## Factors affecting adult survival and growth

Currently the CBP starts each brood year with approximately 320 age 1+ fish; however, mortality rates can be as high as 35% before maturation at age 3+. Two main causes of this mortality were identified: disease and adverse social interactions. The CBP continues to develop means of rapid detection and treatment of diseases that are routinely contracted by CBP fish. Adverse social interactions may arise from crowding and low flow velocity. Larger tanks (6.1 m diameter) with higher current velocities (like those at WSH and the MBSTP hatchery) may reduce the dominance behaviors expressed by larger fish toward smaller fish, allowing the smaller fish to grow, survive, and reach maturity. With the current setup of replicate tanks at three locations, this hypothesis can be tested. Reducing these interactions may increase the numbers of adult spawners available for the CBP and for release into local watersheds.

# 2. Genetic and molecular investigations

### Pedigree reconstruction to estimate fitness effects

The Scott Creek coho salmon broodstock show evidence of inbreeding, brought about over the past decade by limited mate choices within a very small population. From this state of restricted genetic variation, we can learn about the effect of inbreeding on survival and fitness by reconstructing pedigrees spanning several generations in each of the three brood cycles. Ideally, pedigree reconstruction would involve collection of single nucleotide polymorphism (SNP) data over multiple generations within each of the three brood cycles. Pedigree reconstruction methodology using SNP data has been implemented and demonstrated to be successful in Klamath River coho salmon. Use of the Fluidigm SNP genotyping platform for application of this powerful method in Scott Creek coho salmon requires additional funding, above the cost of genotyping microsatellite DNA markers for the construction of a baseline SNP dataset.

#### Survival of offspring as a function of parental relatedness

Crosses performed in the hatchery between fish of known kinship provide genetic 'tags' that can be referenced for later assignment of offspring to parents (Anderson and Garza 2005). In the absence of knowledge of parentage, internal heterozygosity can be used as a proxy for the inbreeding history of individuals. Internal heterozygosity is an individual metric ranging from 0 (homozygous at all markers) to 1 (heterozygous at all markers). Research on Russian River coho salmon has shown that crosses between even low-level relatives can result in lethal deformities of offspring (Conrad *et al.* 2013). Continued analysis of the relationship between relatedness of parents and fitness of offspring, using existing and future data, will allow us to determine an acceptable level of relatedness between prospective mates in the spawning matrix.

#### Genetic monitoring of adult releases

The combined rearing capacity of the three facilities (FED, MBSTP, WSH) results in adult abundances that exceed the egg and juvenile rearing capacity of MBSTP, providing the opportunity to release ripe adults directly into local streams. Monitoring should be a dedicated component of all adult release events to encourage regular assessment of their success and to facilitate improvement of future release strategies. Ideally, monitoring will include not only the observation of redds and young-of-year in stream following the release of adults, but also a genetic sample and other biological data from non-lethally sampled juveniles. Given genetic data from offspring and released adults of known genotype, analyses of parentage, family groups, and relatedness can provide information about reproductive success and mate choice in the wild. Documentation resulting from monitoring efforts could include an annual or broodcycle progress report on the genetic health of coho salmon in Scott Creek and nearby basins.

#### Outbreeding experimentation to aid recovery in a critically endangered population

An outbreeding strategy may be implemented when a population fails to respond to first-pass captive breeding. Outbreeding of Scott Creek fish began in 2011 to expand the gene pool of this remnant population. The success of outbreeding depends on numerous factors, including genetic compatibility and timing of maturation. For this reason, outbreeding donor populations ideally should be geographically proximal and phylogenetically similar to the recipient populations (Olema Creek and Scott Creek, respectively, in this case). The recent implementation of outbreeding provides an opportunity to compare the fitness of surviving offspring from both captive crosses and outbred crosses, in the event that they return as adults to spawn beginning in 2014. Juveniles sampled in the Scott Creek basin in 2012 and 2013 will be analyzed to determine parentage and hybrid status. With stream surveys ongoing, the FED plans to compare survival of offspring from different cross types in the future.

### 3. Coho salmon life history, behavior and ecology

### Timing of release for coho salmon smolts

The transition from freshwater to marine environments marks a critical phase in the life history of coho salmon. Mortality rates during the early period of marine life are typically high, and it is likely that the timing of smolt outmigration is locally adapted to coincide with the most favorable period for growth and survival at sea. A recent study has shown strong regional patterns in migration characteristics, with populations in Oregon and California migrating over extended periods lasting 2-3 months during the spring (Spence and Hall 2010). Protracted migrations may serve as a hedge against the unpredictable conditions that occur in the Coastal Upwelling Domain of the North Pacific Ocean.

Since the inception of the collaborative MBSTP/NOAA CPB, coho salmon juveniles have been released into several streams in the Santa Cruz Mountain region both to supplement extant populations and in an attempt to re-establish extirpated populations. Traditionally, these coho salmon have been released as smolts during the spring, primarily in mid-to-late March and usually over a short period of time (Mark Galloway, MBSTP, pers. comm.). Sampling conducted in 2003 indicates that the majority of fish released in the upper Scott Creek watershed migrated to sea within 2–3 weeks of release; hatchery-reared fish emigrated earlier than their naturally produced counterparts (Hayes et al. 2004). While such differences were initially viewed as desirable from the standpoint of minimizing interactions between wild and hatchery fish, the subsequent collapse of wild populations requires a reassessment of this strategy. Namely, given that the role of the hatchery has shifted from supplementation to extinction prevention, the practice of releasing all individuals over a relatively narrow window may not match natural and presumably adaptive patterns of migration observed by wild fish in California and Oregon streams.

Improvements in smolt-to-adult survival rates of hatchery-origin fish may be possible with increased understanding of the relationship between timing of release and subsequent survival. We propose initiating experiments addressing this question by examining behavior and estimating marine survival of smolts released during different periods in the spring. The existing monitoring infrastructure in Scott Creek (i.e., weir and instream PIT tag antenna systems) offers opportunities to track downstream movement of smolts following release and to enumerate both outmigrating smolts and returning adults, which would allow estimation of marine survival for different release groups. Over multiple years, these data will allow us to assess interannual variation in timing of highest survival, as well as to explore relationships between migration timing and environmental variables that trigger downstream movement (e.g., temperature, flow, lunar phase, etc.), thereby guiding future release strategies. Additional information on relationships between size at release and its relationship to both survival and frequency of jacking in the population could also be explored (see below).

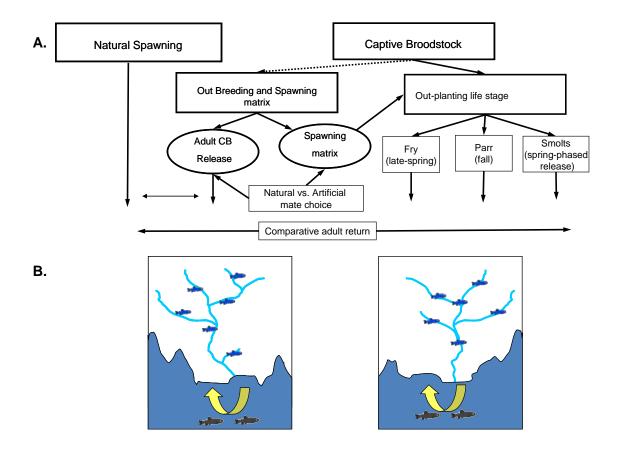
### Stage/age at release

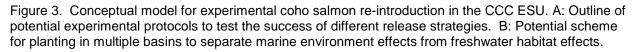
Another important question relating to optimizing returns from hatchery releases is "at what life stage should fish be released"? As noted above, coho salmon from the MBSTP/CBP have been released primarily at the smolt stage (i.e., age 1+) during the spring. Such a strategy may be appropriate if conditions in available rearing habitats are generally poor; however, there are potential disadvantages of this approach. First, juveniles retained in the hatchery for a full year are subject to domestication selection processes associated with artificial rearing environments. Second, fish reared in the MBSTP hatchery but released as smolts outside of the Scott Creek watershed may not have adequate time to imprint on chemical odors in receiving streams and thus may exhibit higher straying rates. Releasing fish at a younger age during the preceding summer or fall may improve imprinting, in addition to reducing the period of time during which they are subject to domestication selection in the hatchery. These potential advantages of earlier releases, however, must be evaluated against potential costs if oversummer and overwinter mortality rates are high.

Experimental spring and fall releases of juvenile salmon in multiple streams in the Russian River system have provided useful data on oversummer and overwinter survival of the different release groups, survival and growth rates in different tributaries, and relationships between survival and environmental variables (e.g., flow, temperature). This information has helped refine release strategies and locations for the Russian River captive broodstock program (Obedzinski et al. 2009; Obedzinski and Nossaman 2012). Comparable experiments are needed in the Santa Cruz Mountains stratum to help determine appropriate release strategies and locations in this region. Such work has recently been initiated by FED and will expand over the next five years.

In addition to juvenile releases, the Russian River program has also experimented with release of maturing captive brood adults. Releasing adults offers two potential advantages over juvenile release. First, it allows spawners to choose their own mates, rather than having mate selection determined artificially through analysis of parental relatedness. Second, it decreases potential domestication effects during the period of juvenile rearing, such as acclimation to pelleted food and the absence of exposure to potential predators. Preliminary results from the Russian River program indicate that adult fish released into the wild have spawned successfully and produced offspring in two coastal watersheds. Adult releases from the MBSTP hatchery have also been made on several occasions in recent years. From adult plantings in 2012, we have confirmed the presence of juveniles in both Scott Creek and San Vicente Creek. Further research is needed to evaluate whether adult releases ultimately provide a net demographic and genetic benefit over more traditional juvenile release strategies.

A conceptual model of how these experimental reintroduction efforts will be conducted and compared for effectiveness is provided in Figure 3.





#### Habitat quality and fish performance

As mentioned above, the relative success of different release strategies may be strongly influenced by physical habitat conditions within a given watershed. Unfortunately, relationships between freshwater habitat quality and coho salmon production are poorly understood, especially within the CCC ESU. To address this knowledge gap, FED is actively engaged in research to identify important habitat elements that support coho salmon production at each life-stage in key streams in the Santa Cruz Mountains Diversity stratum.

In August 2011, FED initiated research to evaluate the effectiveness of large wood additions to San Vicente Creek, which supports one of 16 dependent coho salmon populations identified for recovery in the Federal Recovery Plan for the CCC ESU (NMFS 2012). As part of this effort, 4000 unfed fry-stage coho salmon from the CBP (MBSTP) were elastomer tagged and introduced to 4 sites (*n*=1000 each) in spring 2012 to assess differences in coho salmon density, site fidelity, and growth between wood-augmented and control stream reaches over time. Analogous fry releases were carried out in San Vicente Creek in 2013 and will be repeated at least one additional year (i.e., 2014) to determine the response of all three recognized brood-year lineages

to habitat restoration efforts. We also plan to initiate fry releases in other streams where coho salmon have been extirpated. Introduction of fry to such streams is a vital component of the recovery plan and not possible without the successful operation of the CBP. Re-establishment of coho salmon in multiple watersheds will benefit recovery of the Santa Cruz Mountains Diversity Stratum as a whole. We will work with collaborators in the future to determine specific watersheds most likely to support coho salmon and the life stages most appropriate for introduction.

#### The ecology of coho salmon reintroductions

In order for CBP reintroductions to be maximally effective, it is important to identify and quantify significant causes of mortality at each life stage. For coho fry and juveniles, substantial post-introduction mortality may occur due to density-dependent interactions such as increased competition for resources (e.g., space or trophic resources), and/or increased predation from resident species. Moreover, the strength of these interactions may vary due to the physical, chemical, and biological characteristics of the receiving watershed. We propose to use CBP fish in controlled field studies and laboratory investigations (using artificial stream channels) to assess how community interactions affect the survival and performance of coho salmon. Results of these efforts will advance our understanding of the ecology of CCC coho salmon and assist in the development of appropriate planting strategies for streams within the Santa Cruz Mountains Diversity Stratum.

#### Early maturation

Although the majority of coho salmon mature at age 3, some fraction of individuals (predominantly males) matures at age 2 after spending a single summer at sea. Previous studies indicate some heritability of the jacking phenotype (Heath et al. 2002); however, rearing environment and particularly factors affecting growth also play a role in determining the fraction of individuals that mature early (Gross 1991; Heath et al. 1994). Consequently, conditions within a hatchery likely influence the frequency of jacking within the hatchery population.

Depending on the specific situation, a higher jacking fraction could provide positive benefits by helping rebuild weak brood lineages through both demographic processes and exchange of genes among lineages. However, in extremely depressed populations, increasing jacking rates could have negative effects if there are no females available to spawn with returning jacks.

In the past six years (winters 2006–2007 to 2011–2012), the numbers of adult coho salmon returning to Scott Creek have been extremely low, with only 35 fish recorded during this period and 29 of these being of hatchery origin. Of the returning hatchery adults, approximately 66% have been jacks. Although the sample size is small and there are other plausible explanations, these observations suggest the possibility that hatchery rearing practices are contributing to a high jacking rate. In part, this may reflect the fact that size at release is typically larger for hatchery fish than observed for wild fish at the same time. We propose a more formal examination of the relationships

between size at release, subsequent marine survival, and jacking frequency. Such work could be conducted in concert with studies on effects of release timing.

# Logistical operations of the CBP

At the 20 April 2012 meeting at SWFSC Fisheries Ecology Division, it was decided a steering committee would form to facilitate communication among staff involved with the coho salmon captive broodstock program and to formalize the goals of the program in a written document. This committee will be responsible for ensuring that operational milestones, outlined below, are completed on time. In addition, the committee will meet prior to all Technical Oversight Committee (TOC) meetings of the MBSTP to discuss and come to a consensus regarding recommended planting strategies of all life stages produced by the CBP each year. Members of the committee will then participate in the TOC meetings and facilitate coordination among MBSTP, CDFW, SWR, and FED staff conducting CBP operations at FED. We expect that all final decisions regarding releases will be determined during the TOC meetings.

The following is an annual timeline for the operational, sampling and analytical milestones of the captive broodstock program:

# **CBP Monthly Schedule**

March-April

Team: Salmon Ecology

- Fin clip genetic tissue samples and PIT tag juvenile broodstock (yearlings)
- Desiccate tissue samples
- Data entry: PIT tag number, tissue sample collection date, brood year
- Deliver complete set of samples and electronic metadata to Molecular Ecology team member

# April

Team: Molecular Ecology

- Receive and batch broodstock samples
- Confirm that sample information is complete
- Team: Early Life History
  - Transport age 1+ broodstock from MBSTP hatchery to FED and WSH

# May

Team: Molecular Ecology

- Extract DNA from tissue samples
- Assign DNA repository numbers and enter metadata into repository database

June-August

Team: Molecular Ecology

- Genotyping and sex determination of broodstock samples
- Error-check and finalize genotype data

# September

Team: Early Life History, Salmon Ecology, Molecular Ecology

• Meet to discuss upcoming spawn season logistics

# October

Team: Early Life History and/or Salmon Ecology

- Deliver complete electronic broodstock inventory to Molecular Ecology team
- Accounting of *all* individuals: mature, immature, mortalities, re-tags, etc.
- Deliver re-tag samples and electronic data to Molecular Ecology team

# October-November

Team: Molecular Ecology

- Receive and batch any additional samples (e.g., re-tags)
- Extract DNA and genotype these samples during QT IGH

# December

Team: Molecular Ecology

- Deliver spawning matrix version 1 to Early Life History and Salmon Ecology Teams
- Select adults for release

Team: Early Life History

• Transport adult broodstock from FED and WSH to MBSTP hatchery

# December-February

Team: Salmon Ecology

- Deliver QT samples and electronic data to Molecular Ecology team
- Team: Molecular Ecology
  - Genotype returning adults in QT manner
  - Deliver revised spawning matrix versions to Early Life History and Salmon Ecology Teams

March

Team: Early Life History, Salmon Ecology, Molecular Ecology

Spawn season wrap-up meeting

Team: Early Life History, Salmon Ecology

• Deliver list of actual spawn crosses performed the previous winter

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