Hatchery and Genetic Management Plan for Merced River Rainbow Trout

Merced River Conservation Committee
PO Box 2216
Mariposa, CA 95338

November, 2022

Michael Martin, Ph.D.
Director

TABLE OF CONTENTS

DRAFT

MERCED RIVER RAINBOW TROUT: HATCHERY GENETIC MANAGEMENT PLAN

1. EXECUTIVE SUMMARY

This document details recommendations for the development and management of a hatchery broodstock of an indigenous ancestry Merced River rainbow trout ("MRRT") or Yosemite Steelhead Trout.[1] We give a brief description of the relevant background of MRRT, including its long history of introgression with introduced rainbow trout strains. We then discuss the process of choosing both the location and number of fish to be collected from wild populations and incorporated into a broodstock population. This process includes considerations of maximizing the sampling of wild genetic diversity while not subjecting natural populations to undue harm. Additional considerations include whether or not to use fish that show signs of introgression, which we recommend against. We suggest collection of wild MRRT from any populations found in upper tributaries of the Merced River or neighboring watersheds that show little or no introgression in forthcoming genetic surveys and analyses. Collection will occur over the course of at least four years, and longer if wild population sizes are small. We then describe in detail the breeding procedures that are recommended for retaining genetic diversity, and the suggested steps for minimizing adaptation to captivity among hatchery fish. We recommend that planting locations of hatchery-reared MRRT should include only those areas that are currently planted with non-native rainbow trout, to minimize effects on other wild MRRT populations. We finish by providing recommendations for monitoring of both wild populations and hatchery fish. As we describe, monitoring is essential for ensuring the preservation of wild, native stocks, as well as for active management of the broodstock.

2. GOAL OF THE PROJECT

The goal of this project is to establish protocols and guidelines for the initiation and maintenance of a Merced River rainbow trout or steelhead (*Oncorhynchus mykiss iridius*, "MRRT") broodstock at the Merced River Hatchery, near Snelling, California,[2] or other suitable hatchery location with reliable water supply. Such a broodstock would be designed to

[1] *See*: https://fishbio.com/field-notes/the-fish-report/ocean-away-genetics-yosemites-trout .

[2] https://www.wildlife.ca.gov/Conservation/Inland-Fisheries/Projects/Merced-Rescue-Summary

incorporate as much of the native genetic diversity as possible from those MRRT populations that have been least introgressed with non-native trout (other *O. mykiss* populations), and to maintain this diversity in the hatchery setting. The resulting trout can be used to plant in those areas that are currently planted with nonnative hatchery rainbow trout, with the eventual goal of restoring MRRT to many regions of its original distribution in the upper Merced River watershed, while sustaining angling opportunities within the basin.

This document aims to provide context for the recommendations and guidelines for how this program can use adaptive management to optimize results. We based our recommendations on research that has examined captive breeding and the maintenance of genetic diversity. We also incorporated the guidelines presented by Flagg and Nash (1999) on best practices for operating conservation hatcheries of salmonids. The protocol presented will provide insight that can be used in other, similar situations.

Although there are many hatchery operations with similar aims, rarely has thorough genetic information been available from the beginning of such as project, as it will be for the creation of the MRRT broodstock. The opportunity to track MRRT from wild, isolated populations, through a hatchery, and ultimately back into its former range is unique. This process will provide information about the dynamics of reintroduction, as hatchery-reared MRRT are placed in contact with the non-native rainbow trout that currently inhabit much of MRRT's former range. The monitoring and genetic data resulting from this project could have important implications for the management of other native fish that are faced with the threat of introduced species.

3. BACKGROUND

MRRT is part of the Central Valley steelhead trout (anadromous rainbow) Distinct Population Segment (DPS)-an endemic population of rainbow trout of the San Joaquin River watershed of the southern Sierra Nevada range of California (NMFS 2014). The MRRT historically inhabited the mainstem Merced River and its tributaries, though was likely excluded from the headwater region and other areas of high elevation due to natural geologic passage barriers (e.g., waterfalls in Yosemite Valley or steep gradients in the South Fork, Merced River above Wawona). There has been some disagreement in the literature on whether any rainbow trout (steelhead) reached Yosemite Valley. When reminiscing about trout culture

DRAFT

and planting, Shebley (1927) stated that "*in 1892, steelhead and salmon ascended the Merced River rapids below the Vernal-Nevada Falls*", taking advantage of the high spring floods to surmount the low dams that were present in the river at that time. However, Shebley provided no evidence to support his statement, which was later questioned (Snyder 1993, unpublished Yosemite National Park memorandum). The paucity of suitable spawning gravels in Yosemite Valley (E.R. Gerstung, personal observation) also would indicate that few, if any, salmon ascended that far, although the presence of "speckled trout" (rainbow trout, *Oncorhynchus mykiss iridius*) in Yosemite Valley was noted in some early accounts (Caton 1869; Lawrence 1884; Hutchings 1886; Evans and Wallis 1949; Hubbs and Wallis 1948; Yoshiyama et al. 2001). Yet, California Fish Commissioner B.B. Redding (USFC 1876) had noted even earlier, in 1875:

> "A few years since, they [salmon] spawned near the Yosemite Valley. A dam built for mining purposes, some four or five years since, prevented them from reaching this spawning ground. Last year the dam was removed and the fish have again free access to the headwaters of the Merced, but whether they have returned to their former spawning grounds on this river …I have not learned"
>
> *(USFC 1876b, p 481).*

It appears that salmon, at one time and in unknown numbers, may have approached the vicinity of Yosemite Valley, even if they did not enter the valley proper. However, for the present, the area around El Portal is the best estimate of the historical upstream limit of salmon (spring-run Chinook salmon) in the mainstem Merced River, unless supporting evidence for Shebley's (1927) statement that they ascended farther upstream can be found. Even the vicinity of El Portal may be higher than where most of the salmon historically ascended, considering the lack of archaeological evidence of salmon fishing technology or salmon remains in excavations near El Portal (Snyder 1993). Spring-run salmon were thought to be limited to the lower South Fork, from Savage's Trading Post to Peach Tree Bar (Yoshiyama et al. 2001). Early Californian Gold Rush histories of salmon and steelhead fishing state that:

On the other hand, steelhead reached both Yosemite Valley (Moyle et al., 1996) and up the South Fork to a mile or two east of Wawona (= Yo Semite Crossing) (Clark 1857). Other South Fork observations on trout distributions were by Colonel H.C. Benson, one of the early Park Superintendents who stated in a letter written to Mr. Chester Verteeg on July 12, 1924:

"when we went into Yosemite Park there were no fish in the Park *except those which were enabled to come directly up the Merced River to Yosemite Valley and up the south fork of the Merced to within a mile or two east of Wawona. . .*" (emphasis added; In Hubbs and Wallis 1948). These distributions of steelhead trout in the Merced River to Yosemite Valley and South Fork to east of Wawona have subsequently been confirmed utilizing two independent alternative techniques (habitat and simple rules or environmental envelope models) by Lindley et al. (2006; see Figure 1).

A. Early Rainbow Trout Introductions

Since the middle of the nineteenth century, there was widespread introduction of non-native fish, primarily trout, throughout the upper Merced River (Evans and Wallis 1949). Evans and Wallis (1949) summarize the history of rainbow trout plantings in Yosemite National Park as follows:

> "In 1877; 14 years before the area, now within the park boundaries, had been set aside as a national reservation, the first recorded plants of rainbow trout were placed in the barren waters of Kibbie, Eleanor, Vernon and Laurel Lakes, which lie in the northwestern section of the park. Messrs. Kibbie, Parsons, and Smith, early day settlers, are credited with these first introductions.[3] However, it is believed that some of the early day sheepherders may have removed trout from some streams and carried them to other waters which were accessible to their summer campgrounds even before 1877.
>
> Mr. John L. Murphy planted trout in Tenaya Lake in 1878. In the same year plants of eastern brook trout were made in the Lyell Fork of the Tuolumne River. The oldest record of trout planting in Yosemite Valley dates back to April 1, 1879, when Mr. M. A. Blade of Union City, Pennsylvania, wrote in the Grand Register of the Cosmopolitan House: 'Came in with the Yosemite Fish Commissioners with 20 thousand young trout for the different streams of the valley.
>
> More rainbows were planted in Lake Eleanor in 1880; then for several years no further plantings were recorded.

[3] Colonel Benson, one of the Parks earliest superintendents, reported (1924) that Mr. Kibbie placed rainbow trout in Kibbie, Laurel, Vernon, and Eleanor Lakes in 1877 (Evans and Wallis 1949). The exact origin of those fish was not reported, but the most obvious source was from the Tuolumne River, below Preston Fall (presumably, native Tuolumne River steelhead rainbow trout). After that date, it appears that several non-Yosemite rainbow stocks were introduced to the Park, including Sisson (Mt. Shasta) or Mount Shasta Hatchery strain in 1892, Mt. Whitney Hatchery strain (1945 or 1946 until 1948), and possible not-reported sources at various times throughout pre- and hatchery stocking. Even the presumed-native Eleanor Lake rainbow stock may have been compromised by 1880's introductions of non-native rainbow fish. Beck (1995) reported that Kibbie Lake was planted with presumed non-native rainbow trout in the 1960's until 1972. However, he did not mention any trout plantings after the original plantings of rainbow trout in Laurel and Vernon Lakes.

In the fall of 1892, Mr. W. H. Shebley of the California Fish Commission started from the old Sisson hatchery in Siskiyou County (now the Mt. Shasta hatchery) with a shipment of black spotted, eastern brook, and rainbow trout to plant in Yosemite. Earlier attempts to plant hatchery trout had failed because of the long trip and the time required. On this first successful stocking, the shipment arrived at Raymond by train, from whence it was sent in stages furnished by the Washburn brothers to Wawona. The fish were held overnight in a stream and the next morning transported via an Army ambulance wagon to Mono Meadows, where they were transferred to pack trains for the final stretch of the journey. They were planted in Ostrander and Merced Lakes and in Bridalveil Creek.

After the entire region had been set aside as a national park, a general systematic stocking of the streams and lakes was undertaken. During the early days while the Army had charge of the administration of the park, Col. H. C. Benson took a great interest in fish planting and covered miles of trail stocking lakes and streams which previously had been devoid of fish life. Some of these streams have not been planted since and yet they are still abundantly stocked with reproducing trout.

Although the rainbow was native below the falls, it has been planted into barren back country lakes and streams as well as in the heavily fished Merced River in Yosemite Valley. The rainbow has been planted under the names of Shasta trout, McCloud River rainbow and steelhead."

B. Merced River Rainbow Trout Hatcheries

Two rainbow trout hatcheries were operated in Yosemite National Park (Evans and Wallis 1949): Wawona and Happy Isles hatcheries. Prior to 1927, trout for distribution and introduction into the streams and the lakes of the park were shipped in from other areas or were reared in the Wawona hatchery on Big Creek, which was built in 1895 by the Wawona Hotel Company at Wawona and was operated by the California Fish Commission. The Wawona hatchery closed in 1928 (Leitritz 1970). At the time of the Wawona hatchery closure, Yosemite National Park had another fish hatchery within its boundaries at Happy Isles, located in the floor of Yosemite Valley. Established in 1927, this hatchery was operated and maintained by the California Division (Department) of Fish and Game until 1956 (Leitritz 1970). Leitritz (1970) described these hatcheries and their histories:

"Wawona Hatchery—1895–1928

In 1895, a small hatchery was erected at Wawona, to provide fish for the lakes and streams in the Yosemite Park area. The hatchery was installed by the Yosemite-Raymond Stage Line and turned over to the California Fish Commission, to be operated on condition that 500,000 trout eggs would be hatched and distributed in the vicinity annually. It was managed for a number of years by M. L. Cross. Eggs were shipped to this station from outside sources.

Throughout its existence, Wawona Hatchery usually had difficulty keeping fish in good condition after the first of July. The water warmed rapidly, and although the fish grew well, they generally had to be planted before the end of July. Algal growths in the warm water also caused difficulties. The hatchery was finally closed because it was believed campers had contaminated Big Creek, which was the only source of water. Big Creek was also affected by a prolonged drought, which began about 1914, and caused water supply problems at many other hatcheries."

"Yosemite Experimental Hatchery—1918–1920"

"Yosemite Hatchery—1927–1956

"In an attempt to stock the streams of Yosemite Park with trout fry, the Fish and Game Commission during the fall of 1917 made a survey to locate a suitable hatchery site. A site was located near Happy Isles in Yosemite Valley and application was made to the United States Department of the Interior to lease the property required for operations. Every assurance was given by Yosemite Park officials that the Commission would be granted a suitable lease.

In order to determine the suitability of the location for trout propagation, an experimental hatchery was established at Happy Isles during the fall of 1918 and operations were commenced in the spring of 1919. In all, 400,000 rainbow, Lahontan cutthroat, and steelhead trout eggs were shipped to the station, and the resulting fry planted in the streams and lakes of Yosemite Valley with the cooperation of park officials.[4] The site appeared satisfactory for rearing trout fry, but approval to erect permanent buildings on leased land could not be obtained. The Board of Fish and Game Commissioners, therefore, decided to abandon the project. All equipment was transferred to Wawona Hatchery.

Negotiations were resumed a few years later and arrangements for installation of a permanent hatchery were finally concluded in 1926. The building was constructed and finished in time for operation during 1927. There were 52 troughs, cottages, and an aquarium for display purposes. All species of trout, as well as grayling eggs from Montana, were successfully hatched and reared. The site was one of the most popular for visitors to the park."

C. Merced River Rainbow Trout Egg Sources

Evans and Wallis (1949) generally describe the sources of rainbow trout eggs (and broodstock) for the Merced River and tributaries in Yosemite National Park as follows:

"Trout eggs for artificial propagation must be obtained from one or two sources. They must either be obtained by trapping wild trout of the desired species when the fish are ready to spawn or must be collected from a domesticated brood stock developed for that purpose.

Generally speaking, it is easier to use the latter method as the egg supply obtained from wild trout is often rather difficult to obtain, and the quantity may vary considerably from

[4] The sources of these eggs were not identified in Leitritz (1970).

> year to year. However, in keeping with the National Park Service aim to preserve all fauna in as nearly natural condition as possible, it is generally considered that, when possible, the eggs from wild stock are more desirable for stocking park waters.
>
> Two species, the rainbow and the eastern brook trout, are being raised in the Happy Isles hatchery at present. The eastern brook trout eggs are spawned from hatchery-raised adult stocks at Creede, Colorado.
>
> Formerly, the rainbow trout eggs were collected from an egg taking station, established in 1933, at Lake Eleanor in the northern section of the park. Here the wild rainbow stock was captured for artificial spawning. At present, Lake Eleanor is the only body of water within the park boundaries closed to fishing. This action, of course, preserves the breeding stock. It is planned that within the next few years the Lake Eleanor station will be put back into operation again.
>
> Although during the past few seasons, the rainbow trout egg supply has been secured from the Mt. Whitney Hatchery, the methods used in the taking of the spawn at Lake Eleanor will be described as they are characteristic of methods used elsewhere. During the spring of the year large number of native rainbows run up stream out of Lake Eleanor into the tributaries to spawn. At the mouth of a large tributary, Frog Creek, the National Park Service has constructed a dam with fishways and traps in order to capture the migrating trout for egg collecting purposes."

These introductions of other species have largely occurred to further recreational angling, and have included brook trout (*Salvelinus fontanalis*), brown trout (*Salmo trutta*), coastal rainbow trout of hatchery and wild origin (*O. mykiss irideus*), and California golden trout (*O. m. aguabonita,* "CAGT") The scale of introductions has ranged from the planting of a relatively small number of individuals, to the operation of a full-scale hatchery planting hundreds of thousands of rainbow trout into the Merced River annually. Along with the effects of competition with, and possible predation from, these introduced fish, MRRT was presumed to easily hybridize with coastal rainbow trout and hatchery trout (Hansen et al. 2000; McCracken et al. 1993; Williams et al. 1997), producing fertile offspring that may then introgress their genes back into the native population (Bagley and Gall 1998; Moyle et al. 1995). Contrary evidence of the maintenance of wild populations of rainbow trout with hatchery introductions is presented by Garza and Pearse (2008)[5], Pearse et al. (2011), and Pearse and Campbell (2018)[6]. The patterns of introgression generally reflect the different stocking practices for the different strains, but have possibly affected MRRT throughout most of its historic range. Introgression of MRRT with CAGT appears to be limited or non-existent, possibly occurring in specific locations, such

[5] *See:* Table 1.
[6] *See:* Figure 2.

as Adair Lake, at the headwaters of the Merced River basin in the Merced Range. Finally, hatchery rainbow trout have been planted throughout the upper watershed and in the lower reaches, resulting in potentially compromised fish in the mainstem and the South Fork of the Merced River, as well as their tributaries.

As the general perspective on management has shifted over the past several decades from primarily utilitarian to one including more concern for biodiversity, protection of native fish has become a management priority (Hubbs and Wallis, 1948; Mobrand et al. 2005; Pister 2001). In the Merced River, one fisheries management plan has been written for the South Fork, Merced River (California DFG, 1979). This plan focused on general management objectives which provide these benefits: a) maintain wild trout populations at levels necessary to provide satisfactory recreational opportunities; b) maintain and enhance where possible the habitat required for optimum wild trout production; and c) preserve the natural character of the streamside environment. The specific goals of wild trout management for the South Fork Merced River and its tributaries are: a) protect the aquatic environment of the South Fork Merced River and its tributaries; b) perpetuate a naturally sustained, balanced[7] population of rainbow trout; and c) provide a quality backcountry angling experience characterized by a naturally scenic streamside environment. Hatchery production of MRRT would serve the dual goals of maintaining fish planting for continued angling in the Merced River while using native broodstock and helping restore the extremely diminished range of MRRT.

4. WHAT IS "MRRT"?

The goal of preserving or restoring native MRRT is not a straightforward one. In addressing this goal, one might consider both the phenotype as well as the genotype as the target upon which management should focus. Historically, MRRT was known for its relatively large size, except in tributary populations, where fish were considerably smaller (Clark, 1857; California DFG, 1979). Research on trout has shown size to be at least partially determined by phenotypic plasticity or environmental factors (Keeley et al. 2006; Seiler and Keeley 2009). In tributary populations, MRRT individuals may therefore have the capacity to grow larger if placed in a different habitat, such as migrating from natal waters to the ocean.

[7] The plan defines balanced as "including optimum numbers of adult trout (7 inches and greater) which would maintain an adequate spawning stock and provide sufficient numbers of larger fish or maintenance of quality angling. (Specific numbers to be identified with the implementation of this plan.)"

In this case, broodstock from tributary populations could still produce the larger-size fish typical of the mainstem Merced River.[8] Genetic components, on the other hand, have also been shown to have a large effect on body size (Keeley et al. 2006; Seiler and Keeley 2009). If genetic components are more determinate of body size, using tributary populations as broodstock may not produce the larger phenotype that may be better suited for surviving in the mainstem.

There are also other phenotypic characteristics typically attributed to MRRT that were reported by Hubbs and Wallis (1948). They reported that MRRT (i.e., Yosemite trout that they named *Salmo gairdnerii irrideus*) was the wide-spread, generally nonmigratory, moderately coarse-scaled, relatively deep-bodied, big-headed and big-finned types. They describe the Yosemite form and their analysis of that form as follows, including their conclusions regarding its present status:

> "The Yosemite form has been referred to "*Salmo shasta*, the Shasta rainbow trout of fish-culturists, but a recent examination at Stanford University of the type specimens of *Salmo gairdneri shasta* Jordan, by Hubbs and Follett, failed to disclose any valid reason for even the subspecific recognition of *shasta*. It is possible that the Yosemite waters were originally populated by a slightly differentiated race of rainbow trout. . . .it will probably never be possible to determine what were the characteristics of the indigenous stock or stocks."
>
> "The native stock of rainbow trout has no doubt been modified by hybridization with introduced stocks, representing different races of the same species, or has been more or less completely replaced by the exotic races; the original characteristics of the local form of the Merced River can probably never be learned."

Although conservation standards should ideally include considerations of morphology, identifying the "ideal" phenotype to conserve can be confounded by subjective interpretation, including determination of which phenotypic characteristics are most important, and the cutoff for those characteristics that are present as a gradient between MRRT and its sister taxa. In addition, it can be difficult or impossible to detect introgression using morphology. Particularly in cases of lower levels of introgression, morphological characteristics may be relatively similar to what may be considered a "typical" MRRT. Using morphology to

[8] This phenotypic plasticity has been shown in the Pyramid Lake with Lahontan Cutthroat Trout (LCT), where re-introduction of a non-native LCT was discovered, and subsequent introduction of the original LCT stock produced larger individuals. See: http://www.fws.gov/lahontannfhc/truckee_basin.html

characterize MRRT, therefore, may undermine the goal of avoiding introgression, considered to be the primary threat to MRRT.

Characterizing a MRRT genotype may be difficult due to the extent of introgression throughout the Upper Merced River watershed. The degree or percent introgression of Mount Shasta Hatchery (MSH) or Mount Whitney Hatchery (MWH) strains on the putative MRRT population is not known at this time. Using diagnostic genetic markers or markers with high allele frequency differentials, such as single-nucleotide polymorphisms (SNPs), Simmons et al. (2009) found a mosaic pattern of introgression through non-introgression in McCloud redband trout of the upper McCloud River watershed, California. These upper mainstem and South Fork, Merced River populations are self-sustaining, and presumably filling the ecological role that historic, non-introgressed MRRT population filled. As such, it is difficult to discount these populations as not having some value for conservation. Indeed, determining the level at which introgression should be allowed has been a notoriously difficult task in conservation management, and no single standard has been adopted (Allendorf et al. 2001; USFWS 1996).

Pearse and Campbell (2018) have provided the seminal research on the genetic status of the Merced River steelhead trout. They surveyed genetic variation in Rainbow Trout *Oncorhynchus mykiss* within the upper Tuolumne and Merced rivers, in and around Yosemite National Park, to evaluate both population origins (ancestry) and the evolutionary response to natural and artificial barriers to migration (adaptation). Despite extensive stocking with hatchery Rainbow Trout strains throughout the study area, this analysis revealed that several populations retained largely indigenous ancestry. Adaptive genomic variation associated with anadromy was distributed throughout the study area, with higher frequencies observed in populations connected to reservoirs that are known to support adfluvial life history variants. Fish in southern Central Valley rivers experience temperatures near the upper thermal limit for salmonids and represent an important reservoir of genomic diversity for adaptation to climate change. These results highlight the importance of local adaptation as well as the potential for resident Rainbow Trout populations above barrier dams to contribute to the recovery of steelhead (anadromous Rainbow Trout) once migratory connectivity is restored between upstream spawning and rearing habitats and the ocean.

It seems reasonable that any **MRRT populations that retain largely indigenous ancestry**, should be protected, and care should be taken to avoid further introgression (with

non-native species) in these locations. Those populations of largely indigenous ancestry MRRT that have little or no introgression should be fully protected (isolated from planting) to prevent any further introgression of non-indigenous hatchery Rainbow Trout strains. As such, the standard for MRRT should be based on those populations that contain only MRRT, and not non-native or introgressed individuals. This decision should be revisited pursuant to monitoring and evaluation of both the broodstock and the fish that are planted. As a starting point, however, the conservative approach, and therefore the one recommended, is to consider "MRRT" to be only those fish that do not show introgression genetically. These are the fish that will be the focus of, and source for, the management plan presented here.

5. BROODSTOCK SELECTION

A. Source locations

The selection of source populations to use for broodstock will based upon the findings of genetic analysis of introgression between MRRT and non-native trout. As stated above, our first priority is in using broodstock that shows no introgression, since the aim of this project is to create a broodstock representative only of MRRT. We acknowledge that detecting backcrosses that are many generations old between closely related taxa can be difficult, even using many molecular markers (Boecklen and Howard 1997). Introgression with CAGT and other non-native *O. mykiss* populations may be particularly difficult because of their genetic similarity to MRRT, although there appear to be very few locations in the Upper Merced River watershed with potential populations of CAGT. Because of this similarity, however, low levels of introgression with CAGT are more acceptable than introgression with coastal rainbow trout, which are more distantly related and also not native to the Upper Merced Basin. Nonetheless, CAGT and MRRT are distinct taxa, so introgressed individuals, by definition, do not represent the target species for this project. We, therefore, recommend that care be taken to avoid all CAGT introgression whenever possible, as well as to avoid introgression with hatchery trout under all circumstances (e.g. Araguas et al. 2009).

Meek et al. (2014) reviewed genetic stocking considerations for reintroduction(s) of steelhead rainbow trout into the San Joaquin River watershed, including the Merced River. They consider the many important genetic aspects to consider when determining the source for steelhead reintroduction, and outline the genetic data needs when determining sources for

DRAFT

reintroduction. They discuss the lessons learned from previous reintroductions in relation to a reintroduction scenario in the San Joaquin River, and recommend potential source populations:

> "In summary, steelhead sources used in **reintroductions should, where available, originate from within the target basin,** provided that these actually represent a genetically and ecologically similar stock relative to the historical population or nearby extant populations. Where within-basin stocks are not available or suitable, from extirpation, low abundance and/or diversity levels, or genetic contamination, the use of out-of-basin stocks, or a mix of within-basin and out-of-basin stocks, may be considered with the caveats that the out-of-basin stocks or hybrid offspring from mixed stocks may not be best adapted to the current or future reintroduction area (e.g. Huff et al. 2011) and their use should not endanger any nearby extant populations. Additionally, if mixed stocks are used, it will be very important to monitor the success of the different stocks and their crosses to detect any outbreeding depression (see "Single Versus Multiple Sources" section and the discussion in Baerwald et al. 2011, Sec. 4.2.3). The body of evidence from the available studies suggests that there may be potential for using residualized populations that possess the targeted genetic history in reintroduction efforts, though there is great uncertainty about whether these populations, once given the chance, will regain anadromy. The conditions found in the reintroduction area will also likely play a role in determining rates of anadromy (see Satterthwaite et al. 2009, 2010). In the status review of west coast steelhead DPS's, the NMFS Biological Review Team (BRT) (Good et al. 2005) concluded that the likelihood a resident fish will give rise to anadromous fish with enough frequency to produce a self-sustaining anadromous population is low enough to warrant just focusing on the anadromous form in the risk assessment for the status review of each DPS; however, the presence of numerous, native resident *O. mykiss* is considered a mitigating risk factor for some populations. The studies reviewed herein also suggest that populations that were once anadromous but have been residualized because of man-made barriers might be good candidates for reintroduction downstream in the same watershed. Great caution should be taken, however, and the necessary genetic data collected in advance, to evaluate the relationships of these residualized populations to surrounding populations to ensure they are not predominantly influenced by hatchery out-planting and to ensure they are not inbred. Given the extensive history of *O. mykiss* propagation and introduction, it may be difficult to determine which stocks are 'wild' and which are influenced by past hatchery stocking (Good et al. 2005; Deiner et al. 2006)."

Although the Merced River watershed was not included in the analyses of native *O. mykiss* lineages by Garza and Pearse (2008), results from adjoining tributary watersheds (Tuolumne and San Joaquin Rivers) is interesting, and may be considered in the genetic assessment of the Merced River (Table 1). Based on their analyses, which included the use of both SNP (single nucleotide polymorphism) and microsatellite markers, Garza and Pearse (2008) suggest hatchery rainbow trout stocking (e.g., upper Tuolumne and San Joaquin River watershed) has not eliminated the residualized native steelhead in the above-barrier populations studied. Rather, these selected above-barrier populations may represent the relatively non-introgressed ancestral genetics of steelhead in the Central Valley. Prior to 2015, all Merced River populations were thought to be of the similar origins or genetic lineages.

Pearse and Garza[9] found almost all identified California Fish and Wildlife (CaFW) hatchery trout from an Upper Merced River sample (Yosemite Valley), and a Lower River sample was associated with the Eagle Lake Hatchery trout strain. In the 2018 Pearse and Campbell[10] study, the signal of hatchery ancestry previously observed in a sample of 59 lower Merced River fish (from 2 sites - Merced River Ranch and Merced River Hatchery) confirmed the initial result. They found in the entire Merced River watershed sampled**,** all but one small population in the South Fork, above Wawona has retained its indigenous ancestry population genome (MRRT).[11]

With this study,[12] the trend and pattern of the lack of non-hatchery introgression is confirmed in the upper Merced River watershed (above Lake McClure), and this selected above-barrier populations represents the relatively non-introgressed ancestral genetics of steelhead in the Merced River and the Central Valley.

Recently, molecular tools have given new insights into the genetics of existing populations. DNA provides a metric into the relative contributions of different ancestral populations (such as hatchery strains and fish native to the area), as well as information on steelhead life histories (i.e., whether fish retain anadromous characteristics). This migratory ability persists even in long-isolated above-dam populations, expressed as an adfluvial life history. Recent study in the Upper American River watershed found an isolated *O. mykiss* population from above-dam barriers, represent genetically appropriate sources to develop broodstock for reestablishing a native anadromous population in a river below-dam barriers, which have blocked migration for extended periods.[13]

The development of a suitable MRRT might be confounded by several practical constraints:

1) Broodstock collection from more desirable locations is possible.[14] For example, this could arise if high priority populations (South Fork, Merced River) have too few fish to support collection without imposing harm on the extant population, or if severe logistical

[9] Pearse and Garza, 2015.
[10] Pearse and Campbell, 2018.
[11] Ibid.
[12] Ibid.
[13] Albadía-Cardoso et al., 2019.
[14] Such as Lake Eleanor, Yosemite National Park or upper Cherry Creek, Stanislaus National Forest.

issues prevent collection from those locations.

2) Future genetic evidence or further sampling indicates that introgression with CAGT or non-MRRT hatchery populations is higher than 5% or greater than the amount measured in high priority populations, and that introgression with hatchery trout continues to be significant.

3) Insufficient genetic diversity exists in the primary source populations to avoid inbreeding. Inbreeding concerns could come from genetic estimates of the broodstock, or if monitoring indicates that survival either in the hatchery or the planted populations is very low and that inbreeding depression is a likely cause.

Gow et al. (2011) evaluated the genetic effects of hatchery supplementation on five steelhead rivers in British Columbia, Canada. They examined samples collected over 58 years, a time period that spanned the initiation of native steelhead trout broodstock hatchery supplementation in these rivers. They detected no changes in estimates of effective population size, genetic variation or temporal genetic structure within any population, nor of altered genetic structure among them. They suggested that in order to better manage natural resources undergoing hatchery supplementation, it is important to obtain an understanding of the biological effects of management decisions that use native broodstock (Brannon et al. 2004; Reisenbichler 2004). Genetic monitoring of archived samples collected over time may provide a tool for quantifying the potential for hatchery programs to change the diversity and structure of indigenous gene pools and hence, guide subsequent management plans (Schwartz et al. 2007; Allendorf et al. 2008; Van Doornik et al. 2011).

Although future recommendations for population supplementation await genetic evaluations, continuing monitoring will enable evaluation of potential source locations for MRRT stocks. Ideally, the MRRT that are planted back into the Merced River will form self-sustaining populations. This process may likely involve some degree of interbreeding with the non-native rainbow trout of hatchery-origin stock that may currently exist in parts of the river. Such interbreeding may be difficult to avoid, but we expect that, just as the non-native fish initially replaced MRRT through hatchery stocking, the MRRT may eventually replace the non-native rainbow trout. Should monitoring show low levels of introgression in planted areas, or any other reaches of the Merced River, these locations should be considered for

DRAFT

incorporation into future hatchery operations.[15] Pure, genetically isolated, non-introgressed stock populations of MRRT (in the Upper Merced River watershed) should be excluded from any future hatchery plantings in all Merced River watershed streams and tributaries.

After planting of MRRT stock begins, planted fish that can be recaptured upon maturing become potentially available as future broodstock. The primary considerations in using these fish are that they should not be used during the period in which wild-origin broodstock are still being collected, since incorporation of wild diversity should be the priority. In addition, accurate identification of fish as having derived from MRRT broodstock is vital. If fish released prior to the development of the MRRT broodstock are marked in a similar manner to fish released after, care must be taken to ensure that only MRRT-derived fish are collected. Although physically marking fish will be important for field verification that a fish is of MRRT-origin stock, genotyping such individuals will also allow for identification of individuals, since their parents will be part of the hatchery pedigree. This verification should be performed before any fish collected from areas that have been planted are used for broodstock.

B. Quantity

Sampling statistics dictate that the more thoroughly a source population is sampled for broodstock, the more accurately its genetic diversity will be represented (Lacy 1994). While Lacy's genetic recommendations (1994) focused principally on zoo-managed mammal populations, its findings have obvious relevancy to fish broodstock management. The development of an ideal broodstock would remove all fish from non-introgressed populations, thereby maximizing or capturing the extant genetic diversity presently available to create the broodstock. As a practical matter in the case of the MRRT, there exists the "competing" goal of preserving extant and native MRRT populations that remain in the wild. This goal requires that broodstock collections must be only samplings of wild fish. We therefore must balance these competing requirements, to capture as much wild genetic diversity as possible, without negatively affecting the viability of the extant, wild populations.

The literature includes many recommendations about the number of founders suitable for capturing wild genetic diversity (Ralls and Ballou 1986; Frankham et al. 2002). While it is understood that more is better, many studies also show that the majority of the genetic

[15] This includes below dam-barrier reaches (i.e., Crocker-Huffman, Merced Falls, McSwain, and New Exchequer Dams).

diversity of a population can be captured by relatively small samples. Frankel and Soule (1981) and Miller and Kapuscinski (2003) each suggest that 50 fish is an appropriate minimum number for maintaining genetic diversity, while Allendorf and Ryman (1987) recommend 100 breeding pairs. By compromising only slightly on genetic diversity, several authors have shown that taking only 20-25 unrelated individuals could capture approximately 97 percent of wild genetic diversity (Lacy 1994; Frankham et al. 2002; Ralls and Ballou 1986), assuming an equal sex ratio in the collected fish and that all fish reproduce.

Because there may be few native MRRT populations available to be used as source populations, and given the considerations and recommendations above, a goal of 75 -100 fish from each source population is recommended. The source populations are expected to be in two regional areas: 1) mainstem Merced and a few isolated tributary streams (in the vicinity of Yosemite Valley to El Portal), and 2) the South Fork, Merced River and its tributaries, in the vicinity of Wawona, approximately 4 km upstream to Bishop Creek downstream. The best way to obtain this number of broodstock will be to collect approximately 25 fish from each source, annually for four years. We expect some individuals will not survive the transfer to the hatchery, or will not successfully spawn, producing the range in the ultimate size of the broodstock.

As mentioned earlier, broodstock collection should only be performed at a level that the wild population can support without compromising its persistence. Since tools such as a Population Viability Analysis (PVA) are not currently available for these wild populations, and census estimates have not been performed, it is difficult to make precise estimates of the percentage of these populations that can be harvested without harm. As such, it is important that a rough census be taken of each of the broodstock source populations, prior to pursuing this alternative. Following this, we suggest that the number of fish collected be no more than 10% of the estimated census size of the population, a figure that has been used for collecting ESA-listed salmonids (e.g. Bork and Adelizi 2010). This figure, it should be noted, applies to the collection of adult fish. Given that survival of younger life stages, particularly eggs, can be much higher in a hatchery than in the wild, collection of these life stages can decrease the per capita effect on the wild population while allowing the same or higher number of fish to be collected (Waples 1999; Bork and Adelizi 2010). The collection of eggs would require that care be taken to avoid over-sampling individual family groups, plus the practical difficulties obtaining egg collections *in situ*.

DRAFT

In the case that population census estimates indicate that 25 fish would constitute more than 10% of the adult population, a smaller number is recommended for broodstock collections. Because of the highly variable water year types in the Merced River watershed, collections in dry and critically dry water years should be avoided. The number of years scheduled for collection would then increase accordingly such that the total broodstock population is still at least 75-100 fish from each source. If subsequent genetic monitoring of wild populations indicates that significant diversity exists in a given wild population that has not been captured in the broodstock collection, collection from that population should continue for another 3 years and be reevaluated, after collections.

6. BREEDING CONSIDERATIONS

A. Effective population size

In attempting to transfer the genetic diversity present in wild MRRT to a hatchery broodstock and eventually to fish planted back into the Merced River, the first key is to maximize as much diversity as possible when collecting fish from the wild, as recommended above. The second key component is ensuring that the diversity present in the broodstock is maintained through optimal breeding schemes/protocols (Montgomery et al. 1997; Ralls and Ballou 1986; Sonesso and Meuwissen 2000; Sriphairoj et al. 2007; Yokota et al. 2003). The two primary aims here are reducing the chance of inbreeding with the captive population, and, subsequently, decreasing adaptation to the captive environment. Both of these aims are accomplished by maximizing the effective population size (Ne) (Allendorf 1993; Caballero and Toro 2002; Frankham et al. 2002). Effective population size refers to the size of an *ideal* population that experiences genetic drift at the same rate as the wild population (Lacy 1994; Frankham et al. 2002). Effective population size is estimated for retention of genetic diversity by the following equation:

$$Ht / H0 = [1 - (1/2Ne)]^{t}$$

where genetic diversity is measured by heterozygosity H, and *t* is the number of generations (Falconer and Mackay 1996). The loss of diversity detailed in this equation has been verified empirically by Frankham (1996). Two cited recommendations for how much genetic diversity should be preserved in captive programs include 90% for 100 years (Frankham et al. 2002) and 90% for 200 years (Ralls and Ballou 1986). If we use this Ht/H0

= 0.9 over 100 years, assuming a generation time of 3 years, then a minimum effective population size (Ne) is approximately 156. These numbers of individuals (one half males/one half females) are necessary to maintain 90% of the genetic diversity of the founders for 100 years. If a generation time of 2 years instead of 3 is assumed, the Ne required increases to 237 individuals.

Along with being related to loss of genetic diversity, Ne is a function of the variance in family size following the equation:

$$Ne = (4N - 2) / (2 + Vk)$$

where Ne is effective population, N is the census size of the population, and Vk is the variance in the number of progeny between families (Crow and Denniston 1988). By equalizing each breeding pair's contribution to the next generation, therefore, breeding programs can maximize Ne, and in turn maximize the retention of genetic diversity (Allendorf 1993; Fraser 2008). Along with allowing for the highest Ne, equalizing family sizes has also been shown to reduce the rate of adaptation to captivity, regardless of population size (Allendorf 1993, but see Frankham 2008). We recommend this method be applied to MRRT breeding by equalizing the number of eggs that reach the hatching stage among each pair of breeding adults. While some subsequent mortality is certain, so that family sizes will not be exactly equal by the time these individuals breed, equalizing at this stage should greatly decrease the variance in family size, and be an effective way of increasing Ne. See section 7 below for a more detailed discussion.

Another strategy that has been shown to be effective in increasing Ne is factorial mating (Fiumera et al. 2004; Wang et al. 2002; Waples and Do 1994). Factorial mating describes a system in which individual fish are mated with more than one other fish, in contrast to a monogamous system in which each individual has a single mate. Complete factorial mating dictates that every male is mated with every female, whereas partial factorial mating prescribes multiple matings per individual, but allows some pairs to remain unmated. Fiumera et al. (2004) compared monogamous designs with both completely and partially factorial designs, and found that complete factorial designs had the best capacity to maintain genetic diversity and high Ne, but that partial factorial designs also increased Ne when compared to monogamous systems. The use of only five mates per individual, in fact, produced a Ne that was almost no different than a

DRAFT

complete factorial system, and even three mates per individuals retained close to 95% of the Ne as a fully factorial system (Fiumera et al. 2004).

We recommend that as wild fish are brought into the hatchery, they are mated factorially, with three mates per individual. This design will not only maximize Ne, but will create more opportunities for each fish to contribute to the subsequent generation. If, for example, one fish is sterile, in a monogamous system that fish as well as its mate would have zero offspring. In a factorial system, its mate would have other opportunities to create offspring. This factor has obvious benefits for a system in which we expect wild populations to be relatively small, with correspondingly small numbers of original founders. Our priority is to ensure that every fish collected from the wild has a chance to contribute to subsequent generations. The details of the factorial system are laid out below in Section 7.

B. Inbreeding versus outbreeding depression

The management of small populations often involves balancing the threats of inbreeding depression and outbreeding depression (Flagg and Nash 1999; Edmands 2007). The former has been well documented in both wild and captive populations and arises from the mating of close relatives, leading to a loss of fitness and increased risk of extinction (Frankham et al. 2002; Frankham 2005; Reed et al. 2003; Thrower and Hard 2009; Wang et al. 2002 and references therein). Fitness declines attributed to inbreeding depression are often the result of the expression of deleterious mutations that would otherwise remain unexpressed in larger, more genetically diverse populations (Edmands 2007; Reed et al. 2003; Wang et al. 2002).

Outbreeding depression, on the other hand, has been given less attention, and there is far less empirical evidence of its effect on either wild or captive populations. Outbreeding depression is attributed either to the loss of local adaptation, or to the disruption of co-adapted gene complexes, but researchers have found it difficult to elucidate predictable patterns (Frankham et al. 2002; Tallmon et al. 2004). Emlen (1991) developed a model to assess the fitness effects of outbreeding, which was based on functional genetic distance between the parental populations. Edmands (2007) reviewed the risks from both inbreeding and outbreeding and found that outbreeding depression has led to fitness declines similar in severity to those documented for inbreeding, though acknowledged that the evidence was scarcer. There were indications, however, that effects could be particularly strong after the first generation, leading the author to advise testing the effects of outcrossing for at least two generations. McClelland and Naish (2007) performed a meta-analysis of outbreeding among fish populations, and found a range of

both positive and negative effects, with no strong conclusions and no reliable predictors of outbreeding depression. In particular, salmonids showed no relationship between the genetic distance between parental populations and the size of the outbreeding effect. Lastly, Frankham et al. (2011) developed a decision tree that was based on the four variables from the breeders' equation, taxonomic status, and gene flow within the last 500 years. The predicted probability of OD in crosses between two populations is elevated when the populations have at least one of the following characteristics: are distinct species, have fixed chromosomal differences, exchanged no genes in the last 500 years, or inhabit different environments. Conversely, the predicted probability of OD in crosses between two populations of the same species is low for populations with the same karyotype, isolated for <500 years, and that occupy similar environments. In the former case, we recommend crossing be avoided or tried on a limited, experimental basis. In the latter case, crossing can be carried out with low probability of OD. Their model would find it to be unlikely in the case of Merced River trout populations that do not have relatively high differentiation between parental populations.

Flagg and Nash (1999) emphasized the need to minimize both inbreeding and outbreeding depression in managing captive populations of salmonids. To gauge the relative risks of each, we calculated relatedness among individuals within potential source populations and FST between source populations. Relatedness refers to the probability that two individuals share an allele sampled at random due to descent from a common ancestor, and was calculated in the program SPAGeDiv1.3 (Hardy and Vekemans 2002). We used four different relatedness estimators, which tend to vary in their accuracy based on characteristics of both the population and the markers being used. The four estimators were Moran's I (Hardy and Vekemans 1999; *r*xyQG (Queller and Goodnight 1989); *r*xyLR (Lynch and Ritland 1999), and *r*xy (Wang 2002).

We cannot calculate relatedness within each of the recommended source populations, until completion of the on-going genetic analyses. Kozfkay et al. (2008) point out that molecular estimates of relatedness are often a better relative measure than true evidence of identity by descent, so the values do not necessarily correspond to true relationship status.

In creating broodstock from wild sources, founders are generally assumed to be unrelated (Fisch 2011; Hedrick et al. 2000; Sekino et al. 2004). Rudnick and Lacy (2008) tested this assumption, and found that violating it does not have a large effect on the retention of genetic diversity when appropriate mating pair selection was used. This study assumed that pairs would be available within the sample that were unrelated. The high relatedness values

for the MRRT broodstock source populations, however, indicate that the availability of such pairs may be low, which could lead to an increased likelihood of inbreeding if these populations are kept separate upon being brought into the hatchery.

Another contributing factor is that the fish raised in the hatchery will not be returned to any of the source locations. Because we are not recommending transfer between any of these wild populations, calculating adaptive genetic distance as in Emlen (1991) will not be possible. In the case of MRRT, all fish will be planted in locations where they do not have access to extant pure stock source populations, such that there may be different environmental conditions than any of the source populations have experienced, and specific local adaptations that arose in the source populations may not be as beneficial (Fraser et al. 2011; McClelland and Naish 2007). The loss of these adaptations to due outbreeding, therefore, may not be as large a concern as the simple fact that these fish will be facing different selection regimes both in the hatchery and when planted into the Merced River.

In contrast to outbreeding depression, heterosis is often exhibited when small, inbred strains are hybridized (Coulson et al. 1998; Edmands 2007; Ingvarsson 2001; Nakadate et al. 2003). Heterosis refers to increased fitness as a result of outbreeding, also known as hybrid vigor. Heterosis is the basis for the "genetic rescue" of inbred populations that has been noted in conservation literature, in which the influx of novel genetic diversity leads to recovery (reviewed in Tallmon et al. 2004). Tallmon et al. (2004) point out that, similar to outbreeding depression, predicting heterosis is very difficult, and in many cases the benefit is short-lived.

Given the amount of uncertainty involved with predicting the probability, severity and duration of inbreeding depression, outbreeding depression, or heterosis, providing guidelines for MRRT necessarily involves risk. Given Frankham et al.'s (2011) conclusion and the yet undetermined levels of relatedness within each of the broodstock source populations, we recommend mixing these populations when they are brought into the hatchery, thereby prioritizing the risks of inbreeding as more severe than risks of outbreeding, if the genetic analyses suggest low possibility of close relatedness. Although an ideal situation would involve restricting the number of outcrosses, the development of MRRT broodstock is likely to be limited by low numbers of wild fish, and differential maturation times of these fish when brought into the hatchery. As such, we expect it will be necessary to breed whichever fish are available, and may not be able to select ideal outcrosses. Given these limitations, accurate recording of all fish as they are brought into the hatchery, and of all mating pairs used, will be

essential for assessing the consequences of this outcrossing and for adapting the breeding strategy if necessary. See Section 7 for full details.

C. Genetic Diversity

Although we have discussed genetic diversity in the context of both Ne and inbreeding, there has been ample research into methods specifically aimed at the preservation of genetic diversity in captive populations (Ballou and Lacy 1995; Montgomery et al. 1997; Saura et al. 2008; Sonneso and Meuwissen 2000; Wang et al. 2002; Yokota et al. 2003). The general principle that has been recommended through both empirical and theoretical testing is the minimization of mean kinship (MK) (Ballou and Lacy 1995; Caballero and Toro 2002; Falconer and Mackay 1996; Fisch et al. 2012; Fraser 2008). Kinship (*f*) between two individuals is the probability that two alleles at a given locus, one chosen at random from each individual, are identical by descent (Falconer and Mackay 1996). An individual's mean kinship *mk* is the average kinship between that individual and all other individuals in the population, including itself. Kinship can be calculated from pedigree data when it is available, or approximated using molecular markers (Doyle et al. 2001). Breeding schemes designed to minimize overall *mk* have the effect of maximizing Ne and ensuring that individuals with rare genotypes are represented in offspring (Caballero and Toro 2002). MK methods have been shown to be most effective when individuals with similar *mk* values are mated together (Ivy and Lacy 2012).

After the initial period that will focus on mixing the different source populations, we recommend the Hatchery adopt the "ranked MK" breeding pair selection methods outlined by Ivy and Lacy (2012), which they showed to be adept at retaining genetic diversity. This method first calculates *mk* for each individual in the population. The individual with the highest *mk* is removed and placed in a sex-specific list. Each individual's *mk* is then recalculated and the individual of the opposite sex with the highest *mk* is removed and placed in a second list. This process is repeated, with males of decreasing *mk* placed in one list and females in the other, with the lists populated from bottom to top. The two individuals at the top of their respective lists are therefore those with the lowest *mk*, and should be paired for breeding.

This strategy is based on the premise that those individuals with lower *mk* are more "valuable," since their genetic information is not duplicated in other individuals. Their contribution to the subsequent generation, therefore, is more important than the contribution of

an individual with many close relatives, who can also pass on its genetic material. As such, this mating scheme is designed to ensure that valuable individuals in one population will be paired with valuable individuals in the other population. Thus, their value will not be "diluted" by being paired with a very common genotype from the other population.

D. Pedigree Construction

All fish that are collected for the hatchery, as well as all offspring produced in the hatchery, will be genotyped, either using existing microsatellite markers or SNP markers currently in development for use with MRRT and other rainbow trout subspecies. Individual genotypes of both parents and all of their offspring will allow for parent pairs to be assigned to all offspring. This can be done using software such as CERVUS (Kalinowski et al. 2007; Marshall et al. 1998), which uses a likelihood approach to assign parents, and can calculate confidence intervals for each assignment. Although the first several generations of breeding pairs will be based on molecular estimates of relatedness instead of pedigree information, the pedigree will eventually provide much more accurate information for this process. Once a pedigree has been constructed, this information can be imported into software specifically designed for the management of captive populations and pedigrees, such as PMx software for pedigree analysis and management (Ballou et al. 2010; Lacy et al. 2012). Such software provides a single platform that can manage the pedigree, as well as calculating demographic and genetic data about the broodstock. Maintaining this pedigree information over time will allow managers to assess the retention of genetic diversity, and track the mixing of broodstock source populations.

An essential element of building the pedigree and for the development of the broodstock in general, is the keeping of detailed, accurate records of all fish brought into the hatchery and their offspring. The ability to trace an individual fish to its population or populations of origin is important for achieving the best management of the hatchery, and allowing practices to adapt to new information.

7. BREEDING SCHEME

The following is the recommended protocol for the incorporation of wild fish into a hatchery broodstock, and for the on-going maintenance of that broodstock. Ideally, equal numbers of males and females will be collected from each population, although we

acknowledge that this may not be always possible. The general protocol is outlined in Figure 3.

A. Wild stock (F0)

1. Upon being brought to the Hatchery, wild fish should be:

a. Kept in population-specific tanks to avoid mixing and to avoid potential disease transfer

b. Ad-clipped, with the tissue sample used to genotype each fish. Genotyping will be performed using the panel of microsatellite markers used for analysis in this project, or other suitable genetic markers if they become available.

c. Marked with a unique tag.

2. When these fish are ready to breed (presumably spring of the following year if adults are population as follows:

a. All fish should be outcrossed, meaning they are paired with only fish from other source populations. Ideally, fish will be collected from all recommended source populations, and each fish can be paired with a mate from each of the other population for a total of three collected, or longer if juvenile fish or eggs are collected, but this timing may be variable), each fish should be paired for mating with at least 3, and not more than 5, fish from another matings per fish.

b. This breeding should be carried out such that individuals with low mean kinship within one population should be paired with individuals with low mean kinship within the other populations. Because the pairings involve mating two distinct populations, relatedness among paired individuals is not a concern. Additionally, the matings will be factorial, so the more complex kinship minimization (MK) process that we recommend for later generations need not be used.

3. In addition to these protocols, we recommend:

a. Mean kinship (*mk*) should be calculated for each individual within each of the broodstock source populations. We recommend using the estimators RRL, RQG and Wang's RXY. For added precision of *mk* estimates, the three estimators should be tested on simulated individuals of known relations using the procedure in Fisch et al. (2011). Kinship calculations can be

DRAFT

performed using SPAGeDi [Spatial Pattern Analysis of Genetic Diversity, developed by Hardy and Vekemans (2002)] or comparable software. Males and females from each population should be placed in lists according to this mean kinship calculation, with the lowest *mk* fish at the "top" and the highest *mk* fish at the "bottom."

b. The individual of each gender at the top of the list for each source population should be paired with the individuals of the opposite gender at the top of the list for each of the other 3 source populations. In this way, the individuals with the lowest *mk* from each population will be paired with the lowest *mk* individuals in the other populations. Correspondingly, the #2-ranked individuals from all populations should be paired, and so on. Although the goal will be to collect equal numbers of males and females from each population, this is unlikely. In addition, wild fish are likely to mature at different times, meaning that only a subset of the fish collected will be available for mating. In such instances, there will be individuals for which the above mating scheme will not be possible, since there will not be enough corresponding fish. In such cases, the remaining fish should be paired with individuals from other populations that have not been mated, or have been mated less than 3 times if possible. Otherwise, pairs should be chosen such the individuals with low *mk* values are used preferentially, but that no single fish is mated more than 5 times. The result will be that some individuals are mated with more than 1 individual from a single source population, and have no mates from other source populations. The primary goal of this mating is to ensure both outcrossing and the contribution of every individual to the broodstock. As such, completely even crossing of all populations is not necessary. Due to the unpredictability of maturation times, collection numbers, and sex ratios, we reiterate that accurate documentation of the fish collected and the pairs that are mated is essential for tracking the creation of the broodstock, since it is likely to deviate from a strict plan.

4. We recommend these practical hatchery measures to optimize conformance with the above-detailed genetic protocols.

a. The batch of fertilized eggs that result from each pairing should be incubated separately until hatching.

b. At hatching, each batch of eggs should be equalized to the size of the smallest batch. In the case that certain pairings produce fewer than 50 eggs that reach hatching, other families should be equalized to 50, but not less. Upon equalizing family size, offspring can be kept in separate

DRAFT

half-sib groups or pooled together, depending on available facilities.

c. When large enough, each offspring should be ad-clipped, with the fin kept for genetic analysis, and marked with a unique tag for identification.

d. Each offspring should be genotyped and assigned to a parent pair. Such assignments will be possible because all possible parents will have been genotyped. The software CERVUS, or other comparable software, should be used for parentage assignments, such that confidence estimates can be made for each assignment. In this way, a complete pedigree of the broodstock will be recorded.

e. This protocol should be followed annually as new wild fish are brought in from each of the source populations.

B. First generation of hatchery-bred fish (F1)

1. After the first round of mating, there will be up to 6 groups of fish, representing all possible pairings of the 4 source populations. At this point, however, population of origin will not be explicitly involved in mate selection. Subsequent mate pairing should follow the "ranked MK" method. Also described in Section 6C (Genetic Diversity, Page 20), this method begins by calculating *mk* for all individuals. The individual with the lowest *mk* is then removed and placed in a list and *mk* is calculated again for the remaining individuals. The individual of the opposite sex with the lowest *mk* is then removed and placed in a second list, and *mk* is calculated again for remaining individuals. This procedure is repeated until there is a male list and a female list, at which point they are paired corresponding to their location in the list, so that the first male is paired with the first female. These matings will not be factorial, so each individual will only be paired with one other individual.
2. To limit the incidence of inbreeding, the kinship value between each breeding pair should be calculated after all pairs have been selected. If this value is higher than the overall average kinship for the population, this pair should not be bred (as recommended by Ivy and Lacy 2012).
3. For the resulting offspring, follow the procedures detailed in Section 7 [Breeding Scheme A Wild Stock (F0) Section 4a – e], Page 23. As a result, family sizes will be equalized, and each remaining offspring will be genotyped and tagged. Genotypes should again be used to assign

parents to each offspring.

C. Subsequent generations

Breeding should continue to use the ranked MK method for mate pair selection. As the pedigree grows, *mk* should be calculated based on the pedigree rather than on molecular marker estimates, which will provide more accurate information on relatedness.

D. Integrating wild fish collections

As described earlier, collections from each of the broodstock source populations should continue until the target number is reached (75 –100 from each). Ideally, each annual collection will contribute new genetic diversity to the broodstock. Itis possible, however, that fish collected will be close relatives of fish collected in previous years. It is therefore recommended that new collections are kept separate from existing broodstock when initially brought into the hatchery. In addition, it is vital that each wild fish have the opportunity to contribute to the broodstock if possible, so that factorial mating of wild fish should be practiced. Therefore, wild fish breeding should follow that established for the initial broodstock in Section 7A (Page 24 *et seq*.). After this initial round of factorial breeding, these fish can be incorporated into the existing broodstock collection, and have mates selected using the ranked MK scheme. This method will both increase the chance of genetic contribution of each wild fish by having one round of factorial mating, while also incorporating new wild diversity into the existing broodstock as quickly as possible.

8. MINIMIZING ADAPTATION TO CAPTIVITY

Captive-born populations have been shown to exhibit lower fitness in the wild compared to wild-born individuals (Araki et al. 2007; Araki et al. 2008). This difference is thought to be due at least in part to individuals becoming adapted to a very different environment in captivity (Frankham 2005). A certain degree of adaptation is almost certain to occur, regardless of measures taken, but the rate at which it occurs and its severity can be addressed by a number of strategies (Flagg and Nash 1999; Frankham 2008; Williams and Hoffman 2009). Not all of these strategies are logical or practical for MRRT. For example, lengthening the generation time and minimizing the number of generations spent in captivity have both been shown to be among the most effective methods for reducing adaptation

DRAFT

(Frankham 2008; Williams and Hoffman 2009). These choices are not viable for a hatchery that also aims to generate fish for planting.

There are options or alternatives suitable for the conservation of wild MRRT genetic integrity in hatchery stocks that have been shown to be effective. As mentioned earlier, Allendorf (1993) demonstrated that equalizing family size could slow the rate of adaptation to captivity, even when the population is large enough to avoid significant genetic drift. Flagg and Nash (1999), among others, have also recommended the use of enriched tanks, which are aimed at reducing the difference in forces between captive and wild environments. Enriched tanks include features such as overhead cover, in-stream structures, underwater feed delivery systems, and variable water flow, which provides hatchery fish with more exercise and more accurately reflects a natural system. The use of these features with Pacific salmonids has been shown to increase survival of smolts upon release (Maynard et al. 1996 – in Berejikian 2005), increase competitive ability of juveniles (Berejikian et al. 2000) and improve juvenile dorsal fin quality (Berejikian 2005).

Not all of the features of enriched tanks may be feasible at the Merced River Hatchery (MeRH). Nonetheless, we recommend adopting as many as possible. Possible modification of the non-working Chinook salmon spawning channel at MeRH for MRRT rearing as an "enriched" tank should be evaluated. These features will work to decrease adaptation to captivity by more closely mimicking the natural environment, as well as increasing post-release survival of captive-reared fish. Both of these characteristics will make restoring MRRT more effective, and allow for harvestable fish as well as increasing the likelihood that the captive-reared fish will survive to create naturally reproducing populations.

9. PLANTING

There is now widespread recognition that hatchery fish can negatively affect wild populations (Agostinho et al. 2010; Araki et al. 2007; Laikre et al. 2010; Pearsons 2008). Such negative interactions often result from non-native hatchery fish being planted in the same location as wild native fish, either to supplement the wild population or to generate harvestable fish. The planting of MRRT, however, is likely to be of a different nature, and will therefore avoid these dangers to a certain degree. While the distributions of native and non-native populations of a large portion of the upper Merced River are not known at the time of this report, the planting distributions will be sensitive to, and designed for, pure strain, native MRRT populations. As a

DRAFT

result, planting MRRT in these locations will, ideally, help to remove a non-native species from the ecosystem. We assume that effects from hatchery stocking to species other than trout have already occurred during the long history of stocking, and that continued planting will not exacerbate them.

Those locations that still contain MRRT populations with low amounts or no introgression should receive no hatchery fish under any circumstance, which likely include those populations from isolated tributaries of the upper Merced River (above El Portal) and the upper South Fork, Merced River (near Wawona). This restriction should also include locations close enough to MRRT populations that hatchery fish are likely to migrate into them, even without being directly planted there. Although the hatchery fish are derived from MRRT, they are still hatchery fish, and will pose unnecessary risks to wild MRRT populations. These interactions, therefore, should be avoided, and planting should continue only in those areas containing trout that are currently heavily introgressed with non-native hatchery trout, most likely below El Portal and Wawona. An evaluation, analysis, and consideration of stocking impoundments with MRRT should be conducted.[16]

Lastly, we recommend that planting of the stock currently produced at the other San Joaquin Hatcheries[17] (non-MRRT stock) be stopped immediately and not continued. This measure will increase the likelihood of survival of the new MRRT stock by reducing potential competition and predation by larger, previously planted trout. We expect that the duration between the last planting of non-MRRT stock and the beginning of the planting of MRRT will positively correlate with survival and success of the planted MRRT stock. Consideration of increased limits (removal) of non-native trout species (brown trout) should also be evaluated and considered for reduction of competition with MRRT populations.

10. MONITORING

Monitoring is a critical component of this project, and will provide essential information for optimal management of the Hatchery, as well as providing data for other research. Monitoring must include both genetic and non-genetic methods, and focus on both wild populations as well as fish at the Hatchery.

[16] Lake McClure, Lake McSwain, Merced Falls Reservoir, and below Crocker Huffman Diversion.
[17] Moccasin Hatchery, San Joaquin Hatchery

A. Wild populations

Monitoring the remaining populations of wild MRRT is important for ensuring that the Hatchery broodstock represents native MRRT as accurately as possible. The wild populations of MRRT can be broadly divided into three categories, similar to those used by Alves et al. (2004; 2008) in developing a conservation and species status plan for Rio Grande cutthroat trout.

1. MRRT populations that show no introgression, which currently includes only those four populations recommended as broodstock sources, should be monitored on a regular basis to ensure that broodstock collection is not negatively affecting their persistence. Genetic monitoring ofthese populations will be possible since fish collected for broodstock will be genotyped after being brought to the Hatchery. Genotyping will allow verification of a lack of introgression, as well as for the tracking of genetic diversity changes over time.

2. MRRT populations that show less than 15% introgression with coastal rainbow trout should be considered to have conservation value, and should be monitored accordingly. Genetic monitoring of these populations would ideally take place at least once every five years. This schedule would allow managers to track changes to introgression levels. If introgression is shown to be very low at any of these, it may warrant consideration as a broodstock source in the future, particularly because it likely contains MRRT genetic diversity not present in the current broodstock. Increased knowledge about gene flow and how it relates to the spread of introgression with non-native trout in the system will also be beneficial to managers.

3. Locations that are currently heavily introgressed will be the focus of MRRT plantings and should be monitored at least every three years. Monitoring these populations will be of particular importance for gauging the effects of the Hatchery suppleinmentation. Unlike with anadromous salmonids, for which migratory returns allow for relatively straightforward assessment of survival, hatchery-reared MRRT populations will have to be monitored by in-stream sampling. Genetic samples collected from these populations will allow managers to assess whether or not Hatchery-introduced MRRT are surviving, and more importantly, if they are successfully reproducing in the wild. In addition to measuring the presence or absence of hatchery-reared MRRT, genetic monitoring of these populations will provide invaluable insight into whether, and how quickly, these individuals hybridize with the non-native (previously introduced) hatchery trout populations that currently exist. Effective, regular monitoring of these

DRAFT

populations has the potential to show the dynamics of introgression (in this case, of a fish back into its native range) as it proceeds. If monitoring of these populations fails to show MRRT hatchery-origin fish surviving, it will indicate that changes are necessary. Such changes may include rearing to a larger size, release in different locations, or incorporating more genetic diversity into the broodstock.

DFW's South Fork Merced River Wild Trout Management Plan (California DFG, 1979; see Figure 4) has several commendable recommendations regarding baseline and subsequent frequent monitoring of populations of trout in the South Fork of the Merced (near Wawona) that should be considered as a component of the monitoring effort.

B. Broodstock Population Monitoring

1. *Genetic monitoring* of hatchery fish will provide critical information regarding the genetic integrity of the bloodstock (MRRT Hatchery) population:

a. Evaluation of inbreeding in the broodstock.

b. Assessment of retention of genetic diversity through time. Both of these metrics can be calculated simultaneously with the generation of the pedigree after each breeding event, and will allow managers to decide whether current broodstock levels and sources are adequate to achieve the goals of the MRRT Hatchery. If inbreeding is high, perhaps due to low diversity in the source populations, low fish availability, or other factors, this will identify a need for additional wild stock to be introduced into the breeding program. If genetic diversity of the founders is not being retained at a sufficient level, inspection of breeding protocols and facilities may be required to ensure that pairing and breeding is being carried out appropriately.

2. *Non-genetic monitoring* in three stages should include assessing survival in the hatchery as well as post-release:

a. *Post-hatch mortalities* at levels which negate the equalizing of family sizes and would dictate that full-sibling families are kept separate for a longer period, and equalized after the period of elevated mortality has passed.

b. *Multiple generation survival* and fitness may give insight into possible effects of outbreeding. If, for example, fitness declines after the first two generations, and inbreeding is not indicated, this may signal that outbreeding depression is occurring. Although there are a number of factors that might contribute to such a fitness decline, this scenario may indicate the need to

DRAFT

limit outcrossing. In such a case, it may be advisable to only outcross wild fish with a single other population when being brought into the hatchery, and then keeping these outcrosses separate so that they are not outcrossed a second time.

c. *Survival after release* should be monitored. This component may be the most difficult. We suggest that one or more telemetry or similar studies (Meyer et al. 2012) be performed on post-release hatchery individuals and wild resident populations. The resulting data would provide insight into the movements, behaviors, and survival of hatchery individuals, which can be used to evaluate hatchery methods. Because recapture of individual fish may be difficult, telemetry may provide the best means to assess the performance of hatchery-reared MRRT upon release.

11. GENERAL GUIDELINES

1. No more than 10% of the estimated size of a wild population should be collected for use as broodstock.
2. A goal of 25 fish per population per year should be collected for of 4 years, for a total of 100 fish per populations. If fewer fish must be collected, collection should occur over a longer period until the goal of 75-100 fish is met for each source population.
3. Detailed records must be kept of all fish that enter the hatchery, including the source population from which they were collected. Such records are essential for tracking and evaluating the development of the broodstock.
4. All wild fish should initially be crossed with fish from another population.
5. Wild individuals should be mated factorially (with more than one mate) to ensure their contribution to the broodstock.
6. The family size of each mating should be equalized.
7. Inbreeding should be avoided to the maximum extent possible.
8. Fish should be kept in family groups to make parentage inferences easier. Tanks can include more than one family group, but offspring of a given pair should not be spread over multiple tanks.
9. Enriched tanks, including appropriately placed feeders, structure, over-head cover and variable

DRAFT

water flow should be used whenever possible.

LITERATURE CITED

Agostinho AA, Pelicice FM, Gomes LC, Júlio Jr.HF (2010) Reservoir fish stocking: When one plus one may be less than two. Natureza & Conservação 08:103–111.

Allendorf FW (1993) Delay of adaptation to captive breeding by equalizing family size. Conservation Biology 7:416–419.

Allendorf F, England PR, Luikart G, Ritchie PA, Ryman N (2008) Genetic effects of harvest on wild animal populations. Trends in Ecology and Evolution 23:327–337.

Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids: setting conservation guidelines. Trends in Ecology & Evolution 16(11):613-622.

Allendorf FW, Ryman N (1987) Genetic management of hatchery stocks. In: Ryman N, Utter F (eds) Population Genetics and Fishery Management. University of Washington Press, Seattle, WA, pp 141–159.

Alves JE, Patten KA, Brauch DE, Jones PM (2008) Range-Wide Status of Rio Grande Cutthroat Trout ((*Oncorhynchus clarki virginalis*): 2008. Colorado Parks and Wildlife, Rio Grande Cutthroat Trout Conservation Team Report, April, 2008. 145 pp. Accessed at: http://cpw.state.co.us/Documents/Research/Aquatic/CutthroatTrout/RGCTStatusAssessment2008.pdf on 11/1/2015.

Alves J, Krieger D, Nesler T (2004) Conservation plan for Rio Grande cutthroat trout (*Oncorhynchus clarki virginalis*) in Colorado. Colorado Division of Wildlife.

Araguas RM, Sanz N, Fernández R, Utter FM, Pla C, Marin JLG (2009) Role of genetic refuges in the restoration of native gene pools of brown trout. Conservation Biology 23:871–878.

Araki H, Berejikian BA, Ford MJ, Blouin MS (2008) Fitness of hatchery-reared salmonids in the wild. Evolutionary Applications 1:342–355.

Araki H, Cooper B, Blouin MS (2007) Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science 318:100–103.

Baerwald MR, Stephens MR, Bork KS, Meek MH, Tomalty K, May BP (2011) Spring-run Chinook salmon genetic management plan—San Joaquin River Restoration Program. Davis (CA): Genomic Variation Lab University of California, Davis. 124 p. Available from: *http://dx.doi.org/10.6084/m9.figshare.801104*

Bagley MJ, Gall GAE (1998) Mitochondrial and nuclear DNA sequence variability among populations of rainbow trout (Oncorhynchus mykiss). Molecular Ecology 7 (8): 945–961.

Ballou, JD, Lacy RC, Pollak JP (2010) PMx: software for demographic and genetic analysis and management of pedigreed populations. Chicago Zoological Society, Brookfield, Illinois, USA. Accessed at: http://www.vortex10.org/PMx.aspx on October 30, 2015.

Ballou, Jonathan D. and Lacy, R. C. 1995. Identifying genetically important individuals for management of genetic diversity in captive populations. In: Ballou, Jonathan D., Gilpin, M. and Foose, T., *Population Management for Survival and Recovery*. Columbia: Columbia University Press, pp.76-11.

Beck S (1995) Yosemite Trout Fishing Guide. Frank Amato Publications, Inc. Portland OR.

DRAFT

Berejikian BA (2005) Rearing in enriched hatchery tanks improves dorsal fin quality of juvenile steelhead. North American Journal of Aquaculture 67:289–293.

Berejikian BA, Tezak EP, Flagg TA, LaRae EL, Kummerow E, Mahnken CVW (2000) Social dominance, growth, and habitat use of age-0 steelhead (*Oncorhynchus mykiss*) grown in enriched and conventional hatchery rearing environments. Can. J. Fish. Aquat. Sci. **57**(3): 628-636.

Brannon EL, Amend DF, M. A. Cronin MA, Lannan JE, LaPatra S, McNeil WJ, Noble RE, Smith CE, Talbot AJ, Wedemeyer GA, Westers H (2004) The controversy about salmon hatcheries. Fisheries 29(9): 12-31.

Boecklen WJ, Howard DJ (1997) Genetic analysis of hybrid zones: numbers of markers and power of resolution. Ecology 78:2611–2616.

Bork KS, Adelizi PD (2010) Hatchery and Genetic Management Plan: San Joaquin River Salmon Conservation and Research Facility. California Department of Fish and Game.

Caballero A, Toro MA (2002) Analysis of genetic diversity for the management of conserved subdivided populations. Conservation Genetics 3:289–299.

Caton, JD (1869) Trout fishing in the Yosemite Valley. American Naturalist 3: 519-522.

California Department of Fish and Game (Department of Fish and Wildlife) [California DFG] (1979) South Fork Merced River Wild Trout Management Plan. Report by Inland Fisheries Branch, Dingell-Johnson Project California F-10-R, Inland Fisheries Branch, Sacramento. 34 p.

Clark G (1857) Column in *Mariposa Gazette* on Indians Fishing at Wawona. Mariposa Gazette, Mariposa, CA 08/24/1857 p2/col3.

Coulson TN, Pemberton JM, Albon SD, Beaumont M, Marshall TC, Slate J, Guinness FE, Clutton-Brock TH (1998) Microsatellites reveal heterosis in red deer. Proceedings of the Royal Society of London B 265:489–495.

Crow JF, Denniston C (1988) Inbreeding and variance effective population numbers. Evolution 42:482–495.

Deiner K, Garza JC, Coey R, Girman DJ (2006) Population structure and genetic diversity of trout (*Oncorhynchus mykiss*) above and below natural and man-made barriers in the Russian River, California. Conservation Genetics 8:437–454.

Doyle RW, Perez-Enriquez R, Takagi M, Taniguchi N (2001) Selective recovery of founder genetic diversity aquacultural broodstocks and captive, endangered fish populations. Genetica 111:291– 304.

Edmands S (2007) Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Molecular Ecology 16:463–75.

Emlen JM (1991) Heterosis and outbreeding depression: a multi-locus model and an application to salmon production. Fisheries Research 12:187– 212.

Evans WA, Wallis OL (1949) Fishes of Yosemite National Park-2nd Edition. Yosemite Nature Notes 23(1):1-32.

Falconer D, Mackay T (1996) Introduction to quantitative genetics, 4th ed. Longman Scientific & Technical, Burnt Mill, Harlow, England.

Fisch KM (2011) Conservation genetics of the endangered delta smelt (*Hypomesus transpacificus*) in the San Francisco estuary. Ph.D. Dissertation. University of California Davis. 112 p.

Fisch KM, Ivy JA, Burton RS, May B (2012) Evaluating the performance of captive breeding techniques for conservation hatcheries: A case study of the delta smelt captive breeding program. The Journal of Heredity

DRAFT

104:92–104.

Fisch KM, Henderson J, Burton R, May B (2011) Population genetics and conservation implications for the endangered delta smelt in the San Francisco Bay-Delta. Conservation Genetics 12(6): 1421-1434.

Fiumera AC, Porter BA, Looney G, Assmussen MA, Avise JC (2004) Maximizing offspring production while maintaining genetic diversity in supplemental breeding programs of highly fecund managed species. Conservation Biology 18:94–101.

Flagg TA, Nash CE (eds) (1999) A conceptual framework for conservation hatchery strategies for Pacific salmonids. U.S. Dep. Commer., NOAA Tech. Memo.NMFS -NWFSC-38. Fisheries Science.

Frankel OH, Soule ME (1981) Conservation and evolution. Cambridge University Press, Cambridge, England.

Frankham R (2008) Genetic adaptation to captivity in species conservation programs. Molecular Ecology 17:325–33.

Frankham R (2005) Stress and adaptation in conservation genetics. Journal of Evolutionary Biology 18:750–5.

Frankham R (1996) Relationship of genetic variation to population size in wildlife. Conservation Biology 10:1500–1508.

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge.

Frankham R, Ballou JD, Eldridge MDB, Lacy RC, Ralls K, Dudash MR, Fenster CB (2011) Predicting the probability of outbreeding depression. Conservation Biology 25:465–75.

Fraser DJ (2008) How well can captive breeding programs conserve biodiversity? A review of salmonids. Evolutionary Applications 1:535–586.

Fraser DJ, Weir LK, Bernatchez L, Hansen MM, Taylor EB (2011) Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. Heredity 106:404–420.

Garza JC, Pearse DE (2008) Population genetic structure of *Oncorhynchus mykiss* in the California Central Valley. Final report for California Department of Fish and Game Contract # PO485303 with University of California, Santa Cruz and NOAA Southwest Fisheries Science Center, Santa Cruz, CA. Manuscript Report. 54 p.

Good TP, Waples RS, Adams PB (2005) Updated status of federally listed ESUs of west coast salmon and steelhead. NOAA Technical Memo. NMFSNWFSC-66. Seattle (WA) and Santa Cruz (CA)**:** U.S. Dept. of Commerce. 598 p.

Gow JL,Tamkee P, Heggenes J, Wilson GA, Taylor EB (2011) Little impact of hatchery supplementation that uses native broodstock on the genetic structure and diversity of steelhead trout revealed by a large-scale spatio-temporal microsatellite survey. Evolutionary Applications 4 (2011): 763–782.

Hansen MM, Ruzzante DE, Nielsen EE, Menseberg KLD (2000) Microsatellite and mitochondrial DNA polymorphism reveals life-history dependent interbreeding be-tween hatchery and wild brown trout (*Salmo trutta* L.).Mol Ecol 9:583–594.

Hardy OJ, Vekemans X (1999) Isolation by distance in a continuous population: reconciliation between spatial autocorrelation analysis and population genetics models. Heredity 83:145–54.

Hardy OJ,Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2:618–620.

Hedrick PW, Dowling TE, Minckley WL, Tibbets CA, DeMarais BD, Marsh PC (2000) Establishing a captive

broodstock for the endangered bonytail chub (*Gila elegans*). Journal of Heredity 91:35–39.

Hubbs CL, Wallis OL (1948) The Native Fish Fauna of Yosemite National Park and Its Preservation. Yosemite Nature Notes 27(12):131-144.

Huff DD, Miller LM, Chizinski CJ, Vondracek B (2011) Mixed-source reintroductions lead to outbreeding depression in second-generation descendants of a native North American fish. Mol Ecol 20(20):4246–4258.

Hutchings JM (1886) Re-published 1990. In the heart of the Sierras. Yo Semite Valley and the Big Tree Groves Browning P (ed.), Great West Books, Lafayette, California. 505 pp.

Ingvarsson PK (2001) Restoration of genetic variation lost -the genetic rescue hypothesis. Trends in Ecology & Evolution 16:62– 63.

Ivy JA, Lacy RC (2012) A comparison of strategies for selecting breeding pairs to maximize genetic diversity retention in managed populations. The Journal of Heredity 103:186–96.

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099–106.

Keeley ER, Parkinson EA, Taylor EB (2006) The origins of ecotypic variation of rainbow trout: a test of environmental vs. genetically based differences in morphology. Journal of Evolutionary Biology 20:725–36.

Kozfkay CC, Campbell MR, Heindel JA, Baker DJ, Kline P, Powell MS, Flagg T (2008) A genetic evaluation of relatedness for broodstock management of captive, endangered Snake River sockeye salmon, *Oncorhynchus nerka*. Conservation Genetics 9:1421–1430.

Lacy RC (1994) Managing genetic diversity in captive populations of animals, in: Restoration of Endangered Species. Cambridge University Press, Cambridge. pp. 63–89.

Lacy RC, Ballou JD, Pollak JP (2012) PMx: software package for demographic and genetic analysis and management of pedigreed populations. Methods in Ecology and Evolution 3:433–437.

Laikre L, Schwartz MK, Waples RS, Ryman N (2010) Compromising genetic diversity in the wild: unmonitored large scale release of plants and animals. Trends in Ecology and Evolution 25:520–9.

Lawrence JH (1884) Discovery of the Nevada Fall. Overland Monthly (second series). Vol. 4, No. 22 (October 1884): 360-371.

Leitritz E (1970) A History of California's Fish Hatcheries 1870-1960. California Department of Fish and Game, Fish Bulletin 150, 92 p., 55 figs.

Lindley ST, Schick RS, Agrawal A, Goslin M, Pearson T, Mora E, Anderson JJ, May B, Greene S, Hanson C, Low A, McEwan D, MacFarlane RB, Swanson C, Williams JG (2006) Historical Population Structure of Central Valley Steelhead and its Alteration by Dams. San Francisco Estuary and Watershed Science 4(1)(3):1-19. February 2006. http://repositories.cdlib.org/jmie/sfews/vol4/iss1/art3

Lynch M, Ritland K (1999) Estimation of pair wise relatedness with molecular markers. Genetics 152:1753–66.

Marshall T, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7:639–655.

McClelland EK, Naish KA (2007) What is the fitness outcome of crossing unrelated fish populations? A metaanalysis and an evaluation of future research directions. Conservation Genetics 8:397–416.

McCracken GF, Parker CR, Guffey SZ (1993) Genetic Differentiation and Hybridization between Stocked Hatchery

DRAFT

and Native Brook Trout in Great Smoky Mountains National Park. Transactions American Fisheries Society 122(4):533-542.

Meyer KA, High B, Elle FS (2012) Effects of Stocking Catchable-Sized Hatchery Rainbow Trout on Wild Rainbow Trout Abundance, Survival, Growth, and Recruitment. 141(1): 224-237.

Miller LM, Kapuscinski AR (2003) Genetic guidelines for hatchery supplementation programs. In: Hallerman EM (ed) Population Genetics: Principles and Practices for Fisheries Scientists. American Fisheries Society, Bethesda MD, pp 329–355. Transactions American Fisheries Society

Meek MH, Stephens MR, Tomalty KM, May B, Baerwald MR (2014) Genetic Considerations for Sourcing Steelhead Reintroductions: Investigating Possibilities for the San Joaquin River. San Francisco Estuary & Watershed Science 12(10):1-26.

Mobrand LE, Barr J, Blankenship L, Campton DE, Evelyn TTP, Flagg TA, Mahken CVW, Seeb LW, Seidel PR, Smoker WW (2005) Hatchery reform in Washington State. Fisheries 30:11–23.

Montgomery ME, Ballou JD, Nurthen RK, England PR, Briscoe DA, Frankham R (1997) Minimizing kinship in captive breeding programs. Zoo Biology 16 (5):377– 389.

Moyle PB, Yoshiyama RM, Knapp RA (1996) Status of fish and fisheries. Sierra Nevada Ecosystem Project: final report to Congress. Vol II. Davis (CA): Centers for Water and Wildland Resources, University of California, Davis.

Moyle PB, Yoshiyama RM, Williams JE, Wikramanayake ED (1995) Fish Species of Special Concern in California. CA Department of Fish and Game, Inland Fisheries Division. Rancho Cordova. Final Report for Contract No. 2128IF with UC Davis. 277 p.

Nakadate M, Shikano T, Taniguchi N (2003) Inbreeding depression and heterosis in various quantitative traits of the guppy, *Poecilia reticulata*. Aquaculture 220:219– 226.

National Marine Fisheries Service [NMFS] (2014) Recovery Plan for the Evolutionarily Significant Units of Sacramento River Winter-run Chinook Salmon and Central Valley Spring-run Chinook Salmon and the Distinct Population Segment of California Central Valley Steelhead. California Central Valley Area Office. July 2014.

Pearse DE, Martinez E, Garza JC (2011) Disruption of historical patterns of isolation by distance in coastal steelhead. Conserv Gen 12:691–700.

Pearse DE & Campbell MA (2018). Ancestry and adaptation of rainbow trout in Yosemite National Park. Fisheries, 43(10), 472-484.

Pearsons TN (2008) Misconception, reality, and uncertainty about ecological interactions and risks between hatchery and wild salmonids. Fisheries 33:278–290.

Pister EP (2001) Wilderness fish stocking: History and perspective. Ecosystems 4:279–286.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258–275.

Ralls K, Ballou J (1986) Captive breeding programs for populations with a small number of founders. Trends in Ecology and Evolution 1:19–22.

Reed DH, Lowe EH, Briscoe DA, Frankham R (2003) Inbreeding and extinction: Effects of rate of inbreeding. Conservation Genetics 4:405–410.

Reisenbichler RR (2004) Uncertainty and research needs for supplementing wild populations of anadromous Pacific salmon. In: Nickum MJ, Mazik PM, Nickum JG, MacKinlay DD, Mudrak V, eds. Propagated Fish in Resource

DRAFT

Management, pp.263–276. American Fisheries Society Symposium 44, Bethesda, MD.

Rudnick JA, Lacy RC (2008) The impact of assumptions about founder relationships on the effectiveness of captive breeding strategies. Conservation Genetics 9:1439–1450.

Satterthwaite WH, Beakes MP, Collins EM, Swank DR, Merz JE, Titus RG, Sogard SM, Mangel M (2009) Steelhead life history on California's central coast: insights from a state-dependent model. Transactions American Fisheries Society 138:532–548.

Satterthwaite WH, Beakes MP, Collins EM, Swank DR, Merz JE, Titus RG, Sogard SM, Mangel M (2010) State-dependent life history models in a changing (and regulated) environment: steelhead in the California Central Valley. Evolutionary Applications 3:221–243.

Saura M, Pérez-Figueroa A, Fernández J, Toro MA, Caballero A (2008) Preserving population allele frequencies in ex situ conservation programs. Conservation Biology 22:1277–87.

Schwartz MK, Luikart G, Waples RS. 2007. Genetic monitoring as a promising tool for conservation and management. Trends in Ecology and Evolution 22:25–33.

Seiler MB, Keeley ER (2009) Intraspecific taxonomy and ecology characterize morphological divergence among cutthroat trout (*Oncorhynchus clarkii ssp. Richardson*) populations. Biological Journal of the Linnaean Society 96:266–281.

Sekino M, Sugaya T, Hara M, Taniguchi N (2004) Relatedness inferred from microsatellite genotypes as a tool for broodstock management of Japanese flounder *Paralichthys olivaceus*. Aquaculture 233:163– 172.

Simmons RE, Lavretsky P, May B (2009) Introgressive Hybridization of Redband Trout in the Upper McCloud River Watershed. Transactions American Fisheries Society 139:201–213.

Sonesso AK, Meuwissen THE (2000) Mating schemes for optimum contribution selection with constrained rates of inbreeding. Genetics, Selection, Evolution 32:231–248.

Shebley WH (1927) History of fish planting in California. California Fish and Game 13: 163-173.

Sriphairoj K, Kamonrat W, Na-Nakorn U (2007) Genetic aspect in broodstock management of the critically endangered Mekong giant catfish, *Pangasianodon gigas* in Thailand. Aquaculture 264:36–46.

Snyder JB (Historian, Yosemite National Park, National Park Service) (1993) Memorandum to Park Superintendent Mike Finley: "Did salmon reach Yosemite Valley or Hetch Hetchy?" 9 May 1993 manuscript. P.O. Box 577, Yosemite National Park. 8 p.

Tallmon DA, Luikart G, Waples RS (2004) The alluring simplicity and complex reality of genetic rescue. Trends in Ecology and Evolution 19:489–96.

Thrower FP, Hard JJ (2009) Effects of a single event of close inbreeding on growth and survival in steelhead. Conservation Genetics 10:1299–1307.

United States Commission of Fish and Fisheries [USFC] (1876) Correspondence relating to the San Joaquin River and its fishes. Report of the Commissioner for 1873-4 and 1874-5. Part XXIII, pp. 481-483. Washington, D.C.

United States Fish and Wildlife Service [USFWS] and National Oceanic and Atmospheric Administration [NOAA] (1996) Endangered and threated wildlife and plants: proposed policy and proposed rule on the treatment of intercrosses and intercross progeny (the issue of "hybridization"). 61 FR 4710.

Van Doornik DM, Waples RS, M. Baird MC, Moran P, and Berntson EA (2011) Genetic monitoring reveals genetic stability within and among threatened Chinook salmon populations in the Salmon River, Idaho. North American

DRAFT

Journal of Fisheries Management 31:96–105.

Wang J (2002) An estimator for pairwise relatedness using molecular markers. Genetics 160:1203–1215.

Wang S, Hard JJ, UtterF (2002) Salmonid inbreeding:a review. Reviews in Fish Biology and Fisheries 11:301–319.

Waples RS (1999) Dispelling Some Myths about Hatcheries. Fisheries 24:12–21.

Waples RS, Do C (1994) Genetic risk associated with supplementation of Pacific salmonids: Captive broodstock programs. Canadian Journal of Fisheries and Aquatic Sciences 51:310–329.

Williams SE, Hoffman EA (2009) Minimizing genetic adaptation in captive breeding programs:A review. Biological Conservation 142:2388–2400.

Williams RN, Leary RF, Currens KP (1997) Localized Genetic Effects of a Long-Term Hatchery Stocking Program on Resident Rainbow Trout in the Metolius River, Oregon. North American Journal of Fisheries Management 17(4): 1079-1093.

Yokota M, Harada Y, Iizuka M (2003) Genetic drift in a hatchery and the maintenance of genetic diversity in hatchery-wild systems. Fisheries Science 69:101–109.

Yoshiyama RM, Gerstung ER, Fisher FW, Moyle PB (2001) Historic and present distribution of chinook salmon in the Central Valley drainage of California. In: Brown RL, editor. Fish Bulletin 179. Contributions to the biology of Central Valley salmonids. Vol. 1. Sacramento (CA): California Department of Fish and Game. p 71–176.

DRAFT

FIGURES

DRAFT

Figure 1. Map of the Merced River watershed.

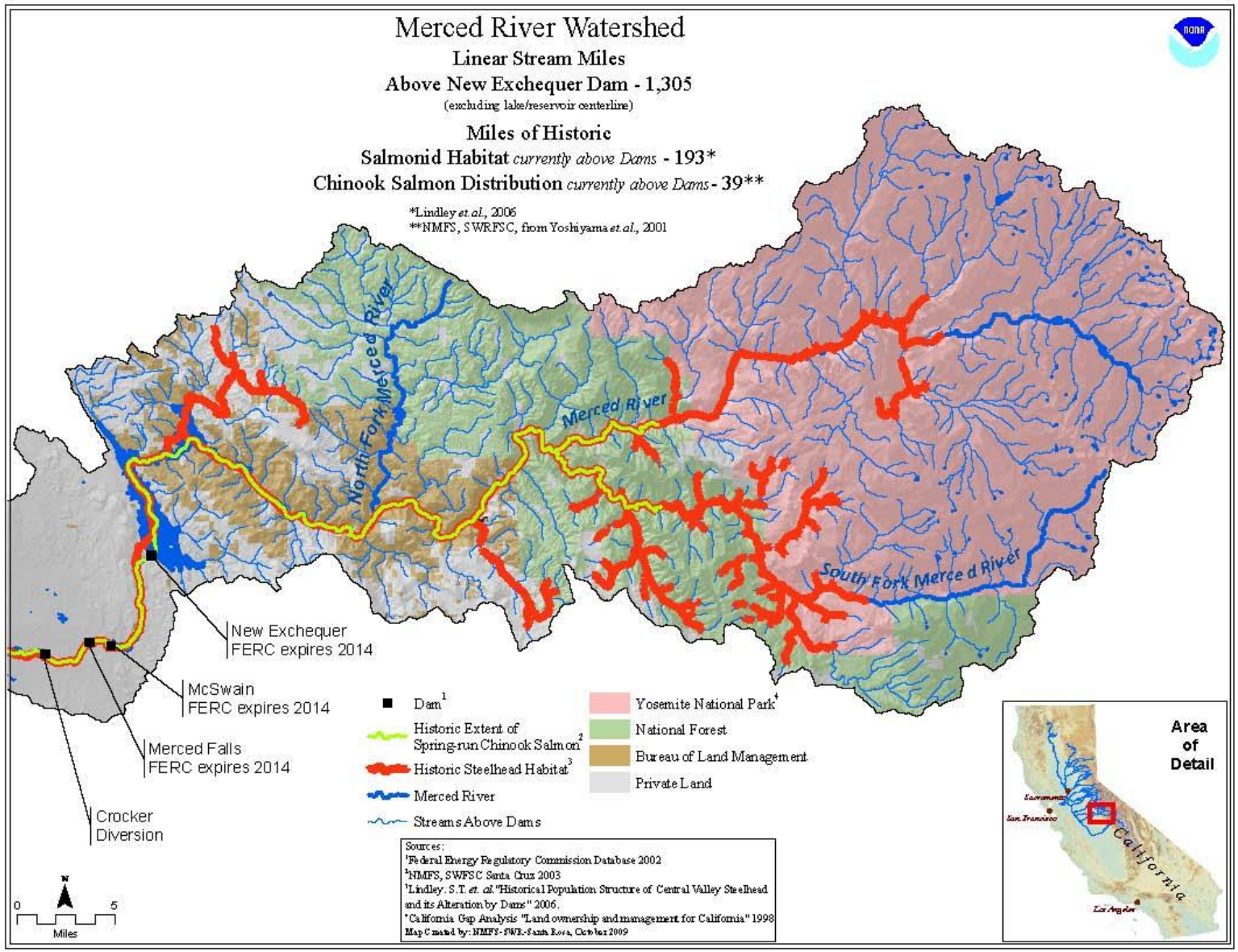
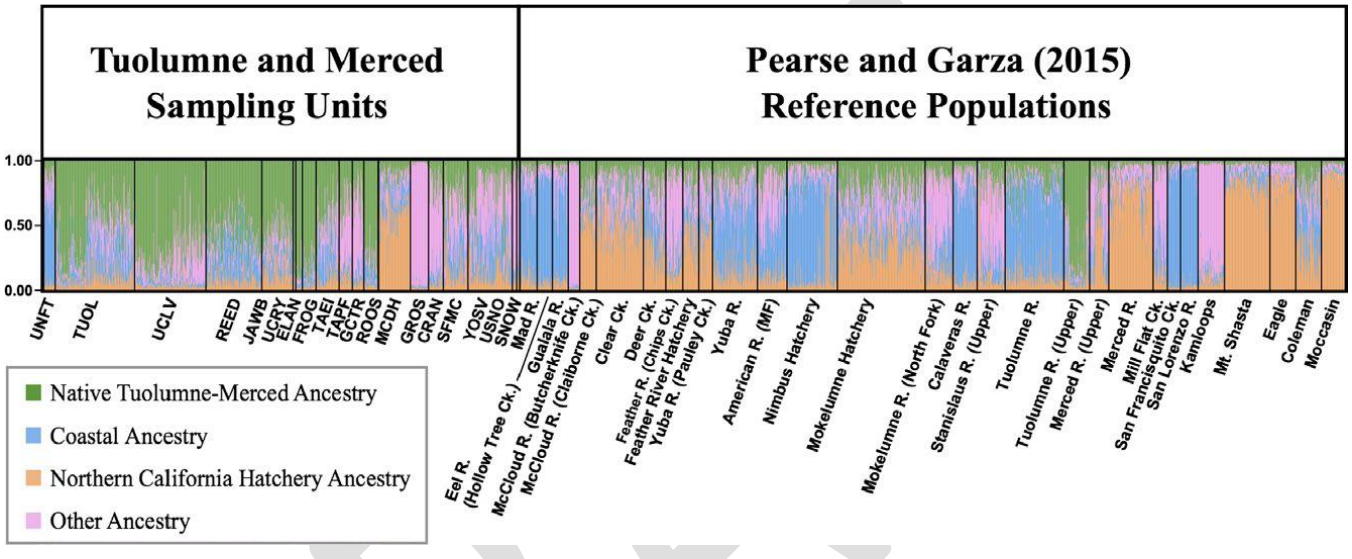


Figure 2. Individual-based plot of fractional ancestry from a hypothesized number of distinct genetic groups (K = 4) for *Onchorhynchus mykiss.* Sampling units and reference populations are described in Pearse and Campbell (2018). Each individual is represented by a vertical line, and the proportion of estimated ancestry from each of the hypothetical groups is represented by the proportionate amount of color within the vertical column (inferred ancestry: green = Yosemite National Park ancestry; blue = coastal ancestry; orange = northern Central Valley hatchery ancestry; pink = other ancestry).



DRAFT

Figure 3. Flowchart showing general steps for initiating and maintaining the broodstock, from fish collected at the source locations. See text for details.

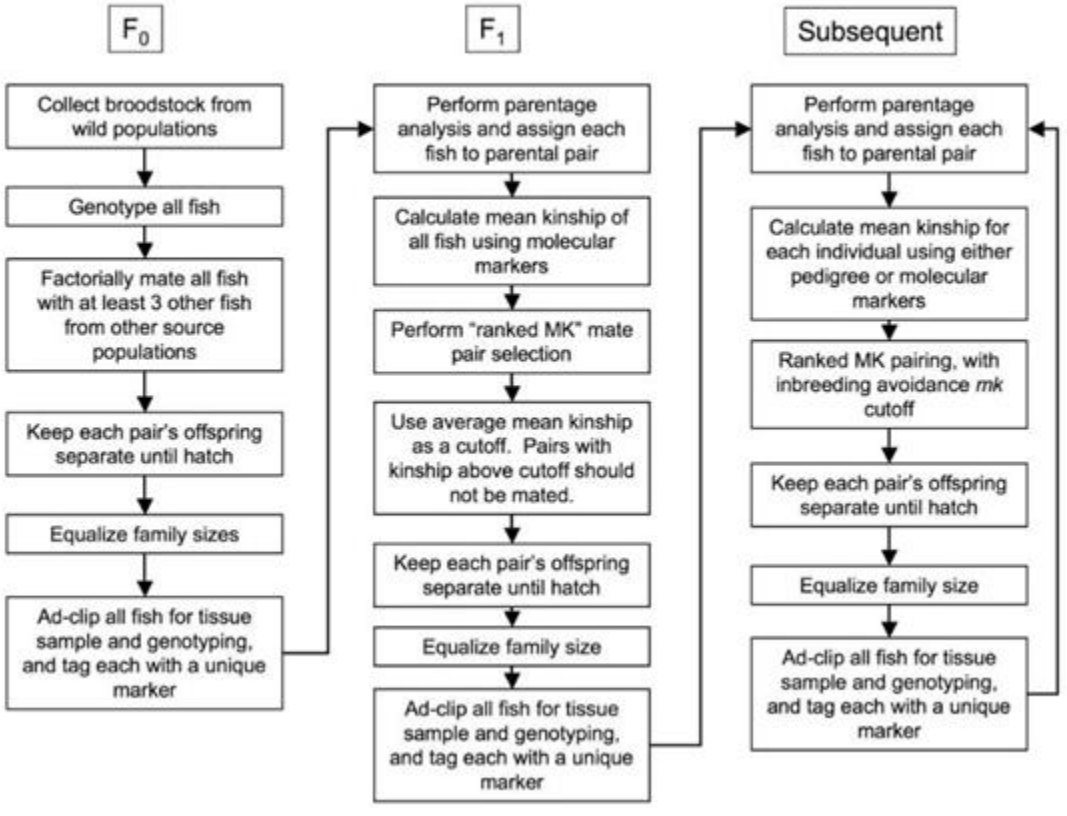


Figure 4. Trout population monitoring plan from South Fork Merced River Wild Trout Management Plan (California DFG 1979).

TABLE 1. Fishery Management Activities for South Fork Merced River

---

I. Obtain baseline data.

A. Define dynamics of the fishery (1980, 1981, and 1982)[a/]

1. Establish three transects in the Bishop Creek area.

2. Sample using standard electrofishing techniques to obtain:

a. population estimate (mark and recapture or diminishing return)

i. per surface area

ii. per mile

b. land/weight relationship

c. age/growth relationship

d. size distribution

e. age class distribution

f. species composition, both game and nongame

3. Tag at least 100 rainbow trout (>6 inches long).

II. Monitor fishery.

A. Repeat tagging and transect evaluation every 5 years (beginning in 1987).

TABLES

DRAFT

Table 1: Sample data and summary statistics for selected San Joaquin River Basin tributary trout genotyped by Garza and Pearse (2008). Population samples are classified by whether samples were taken above or below known barriers to anadromy. Exp. Hz is expected heterozygosity. Obs. Hz is observed heterozygosity. Na is observed number of alleles. Ar is allelic richness. LD is linkage (gametic phase) disequilibrium estimated as the proportion of locus pairs with significant non-random associations.

Sub-basin Population/Strain	Age	Sample size	Exp. Hz	Obs. Hz	Na	Ar	LD
Tuolumne Below-Main	Mixed age	127	0.6980	0.6545	10.44	7.01	30.1
Tuolumne Above-Main, Cherry Creek	Mixed age residents	47	0.7170	0.6379	10.94	7.76	5.9
Kings Above-Deer Cove/Mill Flat Creeks	Mixed age residents	59	0.6686	0.6434	9.83	7.22	66.7
American American River Hatchery Eagle	Juveniles	50	0.5957	0.5904	5.24	4.51	13.3
American AmericanRH/HotCreekH Coleman	Juveniles	85	0.6050	0.5860	7.22	5.31	7.5
American American River Hatchery Moccasin	Juveniles	55	0.6122	0.5820	5.65	4.89	11.8
American American RH/Mt.ShastaH Mt.Shasta	Juveniles	120	0.5978	0.5648	6.28	4.51	7.5
Hot Creek Hot Creek Hatchery Kamloops	Juveniles	50	0.6112	0.5872	7.59	5.88	6.7

DRAFT