TECHNICAL REPORT ◦ FEBRUARY 2024

Rescue, Reintroduction, and Genetic Conservation for Southern California Steelhead – Evaluation and Guidance

PREPARED FOR

Resource Conservation District of the
Santa Monica Mountains
4505 Las Virgenes Rd., Suite 215
Calabasas, CA 91302

PREPARED BY

Stillwater Sciences
996 S. Seaward Ave., Suite 102
Ventura, CA 93001

Stillwater Sciences

Suggested citation:
Stillwater Sciences. 2024. Rescue, Reintroduction, and Genetic Conservation for Southern California Steelhead – Evaluation and Guidance. Prepared by Stillwater Sciences, Ventura, California for Resource Conservation District of the Santa Monica Mountains, Calabasas, California.

Cover photos: Steelhead and steelhead habitat photos provided by RCDSMM Stream Team and Stillwater Sciences. Photos taken from Topanga Creek (upper left and right), Arroyo Sequit (lower left), and Matilija Creek (lower right).

Disclaimer:
Drafts of this document were reviewed by a Technical Advisory Committee (TAC) made up of stakeholders with diverse backgrounds, professional experience, and philosophical views. Although the TAC provided comments that ultimately helped shape the document, the TAC's involvement in the process does not necessarily indicate members of the TAC support the recommendations herein.

Table of Contents

Tables

Figures

Appendices

GLOSSARY OF TERMS

Term	Definition
Adfluvial	Refer to a life cycle trait of fish in which adults migrate from lakes to reproduce in rivers.
Allee effects	A decline in individual fitness at low population size or density, that can result in critical population thresholds below which populations crash to extinction
Anadromous	Individuals: Refer to a life cycle trait of fish in which adults migrate from salt water to reproduce in fresh water and juveniles migrate from fresh water to mature in salt water. Waters: Refer to reaches that are potentially accessible to adult steelhead migrants (i.e., not blocked by a complete barrier to migration).
Assisted colonization	Organisms that are translocated to habitat outside a species' indigenous range (IUCN and SSC 2013, Hayes and Banish 2017) (interchangeable with introduction); the movement of a species to a location outside of its existing or historical range into a new range where it should survive in future climate scenarios to avoid extinction (IUCN and SSC 2013).
Assisted migration	Organisms that are translocated around barriers when volitional upstream and/or downstream passage is not possible under current conditions; anthropogenic-assisted movement of salmonids around barriers for conservation
Biogeographic Population Group (BPG)	The division of southern California steelhead populations based on physical characteristics representing differing natural selective regimes for populations.
Conservation introduction	The intentional movement and release of an organism outside its indigenous range (IUCN and SSC 2013).
Conservation hatchery	A program that "conserves and propagates steelhead taken from the wild for conservation purposes, and returns the progeny to their native habitats to mature and reproduce naturally" (NMFS 2012).
Conservation translocation	The intentional movement and release of a living organism where the primary objective is a conservation benefit (IUCN and SSC 2013). Rescues, reintroductions, and reinforcements fall under this action.
Donor population	The "source" or "founder" population used in reintroductions.
Distinct Population Segment (DPS)	A population of species listed under the U.S. Endangered Species Act.
Founder effects	A reduction in genomic variability that occurs when a small group of individuals becomes separated from a larger population
Fry	Juvenile fish that has absorbed their yolk sac and can feed on their own.
Introduction	Fish that are translocated to habitat outside a species' indigenous range (IUCN and SSC 2013, Hayes and Banish 2017) (interchangeable with assisted colonization).
Isolated Rescues	Rescue action in response to a stochastic disturbance event or in response to isolated observations of stranded fish that was not previously established.
Juvenile *O. mykiss*	Immature *O. mykiss* who could either enter the anadromous or resident *O. mykiss* life history stage
Kelt	An adult steelhead that has successfully spawned and is returning to the ocean.
Natural colonization	The establishment or reestablishment of a population via natural mechanisms such as straying and dispersal.
Omy5	A large genomic region associated with anadromy in *O. mykiss.*

Term	Definition
O. mykiss	To refer to life stages that are indistinguishable as either steelhead or resident rainbow trout.
Population mining	Extracting individuals from their habitat.
Programmatic Rescues	Rescue of organisms in response to previously established biological and environmental triggers that are known to occur repeatedly.
Redd	The spawning location or nest of certain fishes.
Reinforcement	A type of conservation translocation: the intentional movement and release of an organism into an existing population of conspecifics.
Reintroduction	A type of conservation translocation: the intentional movement and release of an organism inside its indigenous range from which it has disappeared.
Rescues	A type of conservation translocation: movement of organisms out of habitat that is no longer suitable due to catastrophic event into suitable habitat.
Resident *O. mykiss*	*O. mykiss* individuals that are freshwater-resident (interchangeable with resident rainbow trout).
Resident rainbow trout	*O. mykiss* individuals that are freshwater-resident (interchangeable with resident *O. mykiss*).
Smolt	A juvenile salmonid that exhibits traits of physiological change in preparation for downstream migration and entering the ocean.
Steelhead	Individuals: *O. mykiss* that express anadromous life-history. Populations: Contain steelhead individuals and possibly resident *O. mykiss* individuals.
Stray	A life-history strategy where an individual spawns within a non-natal watershed.
Translocation	The human-mediated movement of living organisms from one area to release in another (IUCN and SSC 2013).
Young-of-the year	Juvenile fish that are less than a year old.

EXECUTIVE SUMMARY

The Southern California Steelhead Distinct Population Segment (DPS) is at the southern end of the species' range. The DPS is listed as endangered under the U.S. Endangered Species Act. Population declines across the DPS are attributed to numerous anthropogenic factors including presence of migration barriers, habitat loss, flow manipulations, and climate change. In southern California, the frequency and severity of droughts, wildfire, and debris flows are anticipated to increase under climate change predictions, which could further reduce population abundance, cause extirpations, and limit the effectiveness of recovery efforts. The National Marine Fisheries Service (NMFS) (2012) Recovery Plan and subsequent 5-year status reviews (NMFS 2016, 2023) recommend actions that address the fundamental issues underlying population declines, and in addition, recommend consideration of alternative actions that could be used to prevent population extirpation and expand the demographic variability of the Southern California Steelhead DPS.

Following these recommendations, this Evaluation and Guidance document considers two specific conservation actions, rescues and reintroductions, that could be used in the short-term to prevent population extirpation and increase demographic variability. These actions could ultimately reduce the risk of extinction and increase the effectiveness of recovery actions focused on providing additional habitat that are being implemented over longer time frames. In addition, genetic conservation strategies are evaluated that could be used to preserve unique genetic adaptations of southern steelhead to extreme conditions (e.g., temperature, intermittent flows). Conservation of these adaptations could aid long-term viability of more northern populations in the face of climate change. The risks, benefits, and constraints of rescue, reintroduction, and genetic conservation actions are evaluated both broadly as conservation measures for salmonids (within this Evaluation and Guidance document), as well as on a watershed-specific basis (within watershed-specific plans that are available upon request). Specific guidelines for implementation of rescue and reintroduction actions are also presented.

Rescue actions, which fall under the category of "conservation translocations," in response to ecological disturbances (e.g., drought, wildfire, and debris flows) could be used to maintain population sizes and reduce the risk of extirpation, as well as maintain genetic diversity and prevent genetic bottlenecks. Potential risks to consider include spread of pathogens, density dependent effects at release sites, and disruption of natural selection processes. To address risks, a set of biological (fish densities) and environmental (flows, wildfire severity) triggers were developed to reduce risks, and factors were considered that could be used to prioritize release sites. Generally, rescues would be implemented when flows create intermittent habitat during low flow periods and when fish densities at release sites support additional stocking. Rescues in response to wildfires would be implemented after a wildfire event that burns large portions of the watershed (including headwaters) at moderate to severe intensity and that eliminates a large proportion of the canopy cover coupled with an imminent risk of extirpation from debris flows. Relocation sites would be prioritized within the rescue watershed to reduce risks, but relocation to external watersheds could be considered if watershed conditions are not suitable for release due to limited unimpacted or poor habitat or the potential for density dependent effects at release sites. If an external release site was required, adjacent watersheds that share similar environmental conditions and have suitable habitat, but where steelhead are no longer present due to extirpation, would be prioritized. The potential application of temporary holding facilities within the context of rescue and reintroduction programs is also considered.

Reintroduction actions, which also fall under the category of "conservation translocations," could result in increased species distribution, promote population redundancy, increase genetic/phenotypic/life-history diversity, and restore ecosystem function. Potential risks to consider include stress and mortality on individuals used for reintroductions, disease transmission, deleterious effects on the donor population due to removal of individuals (i.e., population mining), genetic founder effects, increased straying rates, and reduced fitness of wild populations due to outbreeding depression. Human-mediated reintroductions can also disrupt natural recolonization processes, but natural recolonization rates have likely been severely disrupted and may not be able to ensure population persistence under a rapidly changing climate.

To address risks, guidelines are presented for selecting reintroduction sites and donor (or source) populations. Reintroduction sites would be prioritized where steelhead had been extirpated and based on habitat suitability, the likelihood of natural recolonization (i.e., the distance to extant populations), the ability to provide diverse life-history expression, and the degree to which limiting factors have been addressed. Donor (or source) populations for reintroductions would only include wild steelhead of native coastal ancestry (or with evidence of limited hatchery introgression), with high genetic diversity, and with the anadromous Omy5 haplotype. Due to the small size of existing anadromous populations, which are likely not capable of sustaining population mining, either fish collected from above barrier populations or fish collected during rescues actions within anadromous waters would be used for reintroductions. Based on the proposed guidelines, large numbers of fish reintroduced over multiple generations are not recommended to prevent negative effects from straying and outbreeding depression.

Following rescue or reintroduction actions, monitoring at release sites and within the donor population is essential for understanding the action's efficacy. Monitoring would assess population abundance, growth rates, dispersal, movements, habitat use, fitness, genetic and life history diversity, as well as ecological impacts. Monitoring should align with California's Coastal Monitoring Plan (CMP) outlined in Fish Bulletin 182 (Boughton et al. 2022). Results from monitoring can be used for decision-making under an adaptive management framework, and we recommend convening a technical advisory committee to assist with decision-making regarding implementation of rescues and reintroductions.

A key step toward potential implementation of rescue or reintroduction actions is determining which watershed(s) would be suitable for either action. Within appendices to this report, a decision framework is described that can be used to prioritize watersheds for implementation of rescue and reintroduction actions based on watershed-specific characteristics including steelhead presence, population size, distribution, and potential for natural recolonization. Watershed-specific information is also compiled and proactively summarized in accordance with existing California Department of Fish and Wildlife (CDFW) policies and guidelines regarding rescues and reintroductions (actions which fall under 'translocations' as defined in CDFW Bulletin 2017-05). These watershed-specific summaries will not be publicly available due to concerns related to poaching but can be made available upon request by contacting the Resource Conservation District of the Santa Monica Mountains (RCDSMM).

In addition to considering rescues and reintroductions, a set of genetic conservation approaches were reviewed to evaluate suitability for preserving unique genetic traits and increasing genetic diversity of southern California steelhead that build upon CDFW guidelines (CDFW Bulletin 2017-04, discussed further in Section 1.4). Assisted migration, assisted colonization, conservation hatcheries, streamside incubators, and cryopreservation were defined and assessed as potential genetic conservation approaches.

Assisted migration, defined as the human-mediated movement of fish around barriers, could increase genetic diversity between fragmented populations and promote variable life history expression. Low collection efficiencies of downstream migrants would limit the numbers of migrants that could be moved downstream, but reduced collection efficiency would ensure the above barrier population continues to express variable life-history strategies that could help maintain genetics associated with anadromy. It was determined that assisted migration should be used as an interim strategy while volitional passage (or barrier removals) is implemented or when volitional passage was not feasible. In particular, assisted downstream migration could promote anadromous life history expression and increase numbers of returning adults across the DPS.

Assisted colonization, defined as the introduction of fish outside their native range, would not meet the goals of conserving local genetic diversity, although movement of the DPS northward would provide opportunities for genetic mixing with more northern populations, both a potential benefit and a risk.

Conservation hatcheries that use wild broodstock (and not hatchery or crosses between hatchery and wild fish) could be used to increase the effectiveness of reintroductions by providing increased numbers of individuals for release, which reduces the risks of founder effects. However, many risks are associated with this strategy, mostly related to reduced genetic diversity and domestication selection. Increased straying rates of broodstock progeny could also result in mixing between hatchery offspring and wild populations in adjacent watersheds, potentially reducing the fitness of wild populations. Ultimately, conservation hatcheries are an extreme form of human intervention that come with greater risks than other conservation actions recommended herein (e.g., rescues and reintroductions).

Streamside incubators, a form of captive breeding where gametes from wild fish are fertilized and incubated at the release site using the local water source, reduces many of the risks related to conservation hatcheries by providing additional time for imprinting on natal cues and more favorable conditions for natural selection. We recommend experimental implementation of streamside incubators for reintroduction of *O. mykiss* into extirpated watershed to evaluate the effectiveness and constraints.

Finally, cryopreservation could be a tool for storing genetic material as a last resort conservation strategy and as an “insurance policy” to preserve variable genetics associated with local adaptations expressed among different populations in the DPS. However, the efficacy of cryopreservation is questionable and would likely come with high costs, and thus, cryopreservation is not recommended until this technique has been further developed.

Based on our review of the various conservation actions and our scientific judgement, we specifically recommend the following:

- Continue convening a Technical Advisory Committee (TAC) to aid decision-making regarding implementation and to evaluate success of actions.
- Implementing rescues in response to specific environmental and biological triggers on an individual watershed basis, as well as in response to stochastic environmental disturbances, such as being done by CDFW and NMFS.
- Implementing an experimental reintroduction into an extirpated watershed using direct release of wild fish from a source population or from wild rescued fish.
- Implementing an experimental reintroduction into an extirpated watershed using progeny from wild fish broodstock using streamside incubators.
- Developing a temporary holding facility to aid rescue and reintroductions.
- Implementation of assisted migration to aid downstream movements of above-barrier populations when volitional passage or barrier removals are not possible.

- Increased monitoring including implementation of the CMP.
- Identification of current and future drought refugia that will be resilient to climate change.
- Research on habitat suitability, genetics, metapopulation dynamics, and adaptations of southern California steelhead.

In summary, there is concern whether steelhead populations with adequate life history, spatial, and genetic diversity will be available in the future to respond to environmental variability and to recolonize habitat naturally following implementation of recovery actions that could take decades to implement. Given the current state of steelhead in southern California, short-term conservation strategies including rescues and reintroductions, as well as genetic conservation strategies could protect existing populations, increase demographic stability, and conserve genetic diversity while long-term recovery actions are implemented. The actions recommended in the NMFS (2012) Recovery Plan are designed to address the underlying causes of declines, and we believe the implementation of these measures (e.g., removing dams, increasing instream flows, removing invasive predators) are the only means to achieve long-term viability. However, as described in NMFS (2023), without short-term bold and decisive action, the current rate of extirpation is threatening the opportunity for long-term recovery. Thus, although there are risks associated with our recommended conservation actions, there are also risks to the status quo. The actions recommended herein are designed to minimize risks, to mimic natural life-history processes, and ultimately, to ensure more intensive human interventions such as conservation hatcheries are not needed in the future. Implementation of rescue and reintroduction strategies should be carefully considered on a watershed-by-watershed basis before widespread implementation. The watershed-specific information provided within appendices to this document will aid decision-making regarding where and when it is appropriate for implementation. Experimental implementation of some of these approaches is recommended followed by monitoring to further explore efficacy.

1 PART 1 — INTRODUCTION

Steelhead (*Oncorhynchus mykiss*) in southern California are at the southern edge of the species' range where habitat has been most severely impacted by anthropogenic stressors (National Research Council 1996, Gustafson et al. 2007). Artificial barriers, urbanization, and changes in land and water use over the last half century have resulted in habitat loss and fragmentation, which, in turn, have decreased steelhead populations within southern California, where it is estimated that steelhead now occupy between 37 and 43% of its historical watersheds (NMFS 2012). As a result of declining numbers, the Southern California Steelhead Distinct Population Segment (DPS) was listed as endangered under the Endangered Species Act (ESA) in 1997 (NMFS 2012). Decreases in southern California steelhead populations is of particular concern because, in addition to being of ecological and cultural importance, these fish may possess important traits that allow them to persist under variable conditions (e.g., high temperatures and intermittent flows), and these traits could be important for creating resilient populations in northern areas in the face of climate change.

Steelhead in southern California evolved and persisted for thousands if not millions of years in an environment characterized by extremes (Nielsen 1990, Waples et al. 2008). Drought, wildfire, and other ecological disturbances are ubiquitous features in southern California. However, elevated water temperatures and more frequent and severe drought and wildfire events associated with human-caused climate change have increasingly contributed to population declines and extirpation and threatened recovery efforts. These disturbances are expected to become more severe and frequent due to climate and land use change, and statewide climate change models predict "fewer wet days, wetter winters, drier springs and autumns, and an increase in dry years as well as maximum precipitation in a single day" (Pierce et al. 2018). In addition to erratic weather patterns, these climatic changes have the potential to increase the frequency and magnitude of wildfires and associated debris flows; increase the frequency, severity, and duration of droughts; result in the acidification and rapid rise of temperatures in oceans; increase surface water temperatures; disrupt vegetative cover; and increase the risk of pathogens, all detrimental effects to already threatened populations of fish (Luers and Moser 2006, NMFS 2012, CEC 2020, Cheng et al. 2021). These threats are exacerbated for steelhead due to the use of different habitats (oceans, estuaries/lagoons, and freshwater) during its life history cycle.

In response to steelhead population declines and ESA listing, the National Marine Fisheries Service (NMFS) released the Southern California Steelhead Recovery Plan (Recovery Plan) in 2012 (NMFS 2012). The Recovery Plan outlines recovery actions, including barrier removals, habitat restoration, and flow management, that address fundamental causes of declines and are essential for the recovery and long-term viability of the population. Following issuance of the Recovery Plan, NMFS issued a status review of the DPS in 2016 and 2023, outlining the current status of population trends and implementation of recovery actions. As described in the latest status review in 2023, there has been substantial progress toward implementation of the Recovery Plan over the last decade, but there is no evidence at this point that recovery actions have improved steelhead viability and population declines have continued during this same period (NMFS 2023); although as noted in NMFS (2023), sufficient monitoring is lacking within the region. A major factor contributing to declines and preventing potential recovery is an extended drought that has been affecting southern California since 2014 and concurrent elevated ocean temperatures (Cheng et al. 2021). In addition, wildfire and associated debris flows have also directly extirpated populations throughout the DPS. Persistent loss of habitat availability and connectivity due to the presence of barriers and other human factors have made populations more susceptible to these disturbances. Ultimately, there are no existing *O. mykiss* populations that are considered viable in the long term (NMFS 2012, 2016, 2023).

Recently, the rate of population extirpation appears to be alarmingly higher than the rate at which natural recolonization is occurring within the region, as well as higher than the rate of recovery actions, such as barrier removals, can be implemented. This discrepancy between rates of extirpation and recovery is a result of rapid shifts in climate combined with smaller, fragmented populations, and the social, financial, and logistical challenges of implementing recovery actions. As an example, steps toward the removal of the Matilija Dam in the Ventura River watershed began in 1998, and dam removal is unlikely until at least 2030. During this period, a severe wildfire, the 2017 Thomas Fire, burned large portions of the watershed and extirpated steelhead from a number of tributaries upstream and downstream of barriers, as well as populations from other coastal watersheds. However, as evidence of the resiliency of steelhead, reaches of the mainstem Ventura River where steelhead were extirpated following the 2017 Thomas Fire have been recolonized by upstream populations that survived the wildfire and debris flows (NMFS 2023), highlighting the importance of having broadly distributed populations across variable habitats. Also, the recent drought has reduced opportunities for anadromy, thereby reducing the probability of recolonization.

FOCUS ON TWO STEELHEAD CONSERVATION TRANSLOCATION ACTIONS:

RESCUE: movement of individuals out of habitat that is no longer suitable due to catastrophic event into suitable habitat

REINTRODUCTION: the intentional movement and release of individuals inside its indigenous range from which it has disappeared.

Historically, life history strategies such as straying (i.e., dispersal) allowed recolonization, increased genetic diversity, and sustained steelhead populations in California (Clemento et al. 2009, Pearse et al. 2009, Donohoe et al. 2021). Indeed, population expansion and retreat of steelhead in this region occurred in response to changing climates over millennia, which is only accomplished through straying. However, the current accelerated rate of climate change is unprecedented, and when combined with the loss of life history and habitat variability and fewer, spatially discrete populations across both southern and central California, it is uncertain whether natural recolonization rates will be able to keep up with increased extirpation rates. Simply put, within the concept of metapopulation dynamics, there are increasingly fewer, smaller, fragmented populations, which are more susceptible to demographic and environmental fluctuations and the loss of genetic diversity. The loss of genetic diversity further compromises steelhead population viability by making the population more susceptible to deleterious genetic effects from inbreeding depression and genetic drift.

In summary, there is concern whether steelhead populations with adequate life history, spatial, and genetic diversity will be available in the future to respond to environmental variability and to recolonize habitat naturally following implementation of recovery actions that could take decades to implement. Because of the current state of steelhead in southern California, there is a need to consider additional short-term conservation actions that could protect existing populations and increase demographic stability while long-term recovery actions are implemented. We recognize the comprehensive depth of the NMFS (2012) Recovery Plan and believe that implementation of the bold measures described therein (e.g., removing dams, increasing instream flows, removing invasive predators) will dramatically increase the probability of the species long-term viability. However, as described in NMFS (2023), without short-term bold and decisive action, the current rate of extirpation is threatening the opportunity for long-term recovery.

Within this document (hereafter “Evaluation and Guidance document”), we evaluate two specific conservation translocation actions—rescues and reintroductions—that could be implemented in the near term to prevent extirpation and increase demographic variability, respectively. The evaluation of these conservation actions addresses two recommendations within the NMFS (2023) 5-year status review: (1) "Explore other means of conserving individual populations of *O. mykiss* that may face the risk of extirpation"; and (2) "Coordinate and implement relocation activities and plans (including post relocation monitoring) for rescued *O. mykiss* within all Southern California Steelhead DPS [Biogeographic Population Groups, (BPGs)]" (see Section 4.2 in NMFS 2023). In addition, these conservation actions have the potential to support and enhance the effectiveness of long-term recovery actions presented in the NMFS (2012) Recovery Plan by addressing viable salmon population parameters (abundance, population growth rate, population spatial structure, and diversity) as described in McElhany et al. (2000).

This Evaluation and Guidance document goes further by proactively summarizing watershed-specific information that can be used to inform watershed-specific rescue and relocation coordination and planning and provide required information needed for execution as outlined in the California Department of Fish and Wildlife (CDFW) Bulletins 2013-04 and 2017-05 (CDFW 2013, 2017a). This Evaluation and Guidance document is not intended to replace or compete with existing recovery plans, but rather, to explore additional alternative conservation actions that could ensure the population remains intact with enough spatial and genetic diversity to enhance the success of long-term habitat restoration actions. Both CDFW and NMFS stress collaboration with partners and stakeholders to conserve and rebuild populations, and it is in that spirit that these actions are explored.

The following document is divided into four parts and accompanying appendices. Part 1 (this section) summarizes background information on southern California steelhead, provides justification for considering rescues and reintroductions as conservation actions, and discusses how these actions relate to existing recovery plans and policies. Part 2 evaluates and presents guidelines for rescues and reintroduction conservation actions. The information presented in Part 2 is used to help prioritize and develop watershed-specific guidance documents under a decision framework, which is presented in Appendix A. Part 3 then reviews genetic conservation approaches. Part 4 provides specific recommendations and steps for near- and long-term implementation of conservation actions. Prior to discussing conservation strategies specifically, we first outline the goals and objectives of this Evaluation and Guidance document.

1.1 Goals and Objectives

The overall goal of this Evaluation and Guidance document is to evaluate conservation actions that can be implemented in the short-term to maintain existing steelhead populations, expand demographic variability, and ultimately reduce extinction risk and aid in the recovery and long-term viability of the Southern California Steelhead DPS. Specific objectives include the following:

- Evaluation of a set of short-term conservation actions that could be used to maintain existing steelhead populations and expand demographic variability;
- Development of decision-making guidelines to assign and prioritize appropriate short-term actions for watersheds across the Southern California Steelhead DPS;
- Compilation of watershed-specific information that can be used to inform appropriate short-term actions;
- Identification of data gaps needed for directing conservation actions; and
- Evaluation of approaches for conserving genetic diversity.

1.2 Overview of Conservation Actions

Numerous conservation actions are commonly applied to aid in the conservation and management of anadromous salmonids. Many of these strategies, including barrier removals, habitat restoration, and flow management, have been thoroughly considered within the NMFS (2012) Recovery Plan. These actions are a necessity for ensuring a self-sustaining and viable long-term Southern California Steelhead DPS by addressing the underlying causes of population declines, including habitat fragmentation and loss. However, large-scale dam removal and habitat restoration can take years, if not decades, to implement due to financial, technical, and social challenges. Meanwhile, the effects of human actions and climate change will continue to reduce population abundance and life-history and genetic diversity, further reducing the adaptive capacity and long-term viability of southern California steelhead. In contrast to recovery actions that address underlying causes of declines, more intensive human interventions, such as the use of conservation hatcheries and assisted migration, have also been considered (including within the NMFS [2012] Recovery Plan) as additional approaches for conserving anadromous salmonid populations. Indeed, conservation actions occur across a spectrum from natural—with the goal of creating natural, free flowing, rivers (e.g., habitat restoration, barrier removals) to intensive human interventions—with the goal of supplementing populations (e.g., conservation hatcheries).

A useful framework for considering conservation strategies in an anthropogenically altered world is the resist-accept-direct (RAD) framework (Thompson et al. 2021, Kocik et al. 2022). Kocik et al. (2022) applied the RAD framework to conservation strategies for anadromous salmonids, highlighting three options for managers to consider: (1) *accept* changes that have occurred and focus on conserving other species, (2) *resist* changes by restoring habitat and connectivity, and (3) *direct* conservation strategies by creating/using novel or artificial habitats. As it relates to southern California steelhead, *accept* could mean that population declines and extirpations are inevitable. As mentioned previously, extirpation can be a natural event, but the rate of extirpation has greatly accelerated beyond natural levels due to direct human influences and climate change. Moreover, the *accept* option disregards the fact that existing populations have demonstrated resilience to environmental variability over recent time and therefore may have important traits (genetic, physiological, or behavioral) that warrant protection. For these reasons, the *accept* option is not considered further in this Evaluation and Guidance document. There is strong enough resistance within the steelhead management community to the *direct* options (e.g., novel habitats outside of identified natural range, conservation hatcheries) that considering these options more than conceptually will be a disservice to goals of this Evaluation and Guidance document. Therefore, the actions considered herein fall under the *resist* option, which is proportionate to the risk faced by the DPS at this time.

The recovery actions presented in the NMFS (2012) Recovery Plan focus on addressing fundamental causes of population declines and fall under the *resist* option. Herein, we consider a category of more human interventionist conservation actions known as conservation translocations, which also fall under the *resist* option. Conservation translocations are defined as the intentional movement and release of a living organism (or gametes, propagules, or reproductively viable plant parts) where the primary objective is a conservation benefit (IUCN and SSC 2013, CDFW 2017b). We specifically consider the following two classes of conservation translocation techniques:

- Fish rescues—an individual or group of *O. mykiss* are moved out of habitat impacted by a catastrophic event (i.e., habitat that is no longer suitable) into suitable habitat to prevent the direct mortality of individual fish (falls under “translocation” as defined by CDFW Bulletin 2017-05).

- Targeted reintroduction (hereafter "reintroduction")—a group of *O. mykiss* are moved from an extant population to an area within the indigenous range from which *O. mykiss* have disappeared, with the goal of populating the region (falls under "translocation" as defined by CDFW Bulletin 2017-05).

These techniques (fish rescues and reintroduction) were evaluated and developed in detail within this Evaluation and Guidance document for the following reasons:

- Rescues and reintroductions meet our objectives of being short-term conservation actions that can be used to maintain existing populations and expanding demographic variability, respectively.
- These actions have been widely applied as conservation measures for at-risk fish populations.
- Because these actions have been widely applied, there are informed guidelines that can be followed to increase success and minimize risks.
- Fish rescues are already conducted by CDFW across the Southern California Steelhead DPS region, albeit reactively rather than proactively.
- Reintroductions are conducted by CDFW as part of rescue operations for *O. mykiss* within southern California, although without a specific intention of reintroducing extirpated populations (CDFW Bulletin 2017-05).
- Fish rescues and reintroductions align with objectives outlined in the NMFS (2012) Recovery Plan and address recommendations from the NMFS (2023) 5-Year Status Review (specific details in Section 1.4 below).
- Actions such as barrier removal, habitat restoration, and flow management (including functional flows) are thoroughly covered in other recovery plans (NMFS 2012, 2016, 2023) and would be implemented over longer time scales.
- Other actions, such as conservation hatcheries and assisted migration, would either be implemented over the long term or have many risks/uncertainties that are not easily overcome (see Part 3 for additional discussion).
- Rescues and reintroductions could be more or less immediately implemented as conservation measures (i.e., it will take less time to implement than many restoration and barrier removal projects).

While we consider rescues and reintroductions in detail herein, we emphasize that we are not recommending their widespread implementation within the DPS. There are numerous considerations that inform the appropriateness of either strategy, which are reviewed in more detail within Part 2. Essentially, we evaluate these strategies in detail to consider their justification and to inform when, where, why, and how these conservation actions could be implemented. Decisions on implementation will ultimately be made by regulatory agencies, and we hope this document, along with watershed-specific information compiled within the appendices, will provide the background information needed to inform decision-making.

In addition to rescues and reintroductions, other strategies that could be used to conserve genetic diversity are reviewed separately in Part 3 of this report. Immediately below, we briefly review southern California steelhead status and life-history and provide an overview of existing recovery plans and policies.

1.3 Southern California Steelhead Summary

Steelhead are native to the Pacific coast of North America (NMFS 2012). The Southern California Steelhead DPS is ecologically and genetically discrete from other *O. mykiss* regional groups, encompassing steelhead populations from the Santa Maria River to the Tijuana River (NMFS 2012). This DPS was listed as "endangered" under the federal ESA in 1997 (considered an evolutionary significant unit at the time of listing and subsequently relisted in 2006). Most anadromous southern California steelhead populations have been extirpated, especially in the southern end of their range (NMFS 2012). Anadromous steelhead historically inhabited 46 watersheds in the Southern California Steelhead DPS range and now occupy between 37 and 43% of these watersheds; adult steelhead runs have declined dramatically in recent decades. For example, only 177 individual adult steelhead were observed between 1994 and 2018 (Dagit et al. 2020). The effectiveness (and depth) of adult monitoring is extremely limited in southern California due to high flows and sediment loads that are characteristic of conditions that would facilitate migration. Thus, the reported numbers of returning anadromous adults are likely underestimated, but the actual number is certainly low (both from historical and viability perspective) as evidenced by the current population trends (NMFS 2023). Wildfires, drought, and debris flows are among the most common and most severe threats to steelhead within the Southern California Steelhead DPS, and these events are expected to become more frequent and severe in worsening climate change conditions.

Steelhead have complex and varied life histories. Steelhead are the anadromous form of *O. mykiss* that migrate to the ocean, whereas resident rainbow trout are *O. mykiss* that remain in freshwater over their entire life cycle. An anadromous steelhead can produce both anadromous and resident offspring, and vice versa (Donohoe et al. 2021, Boughton et al. 2022). Due to these complexities and uncertainties regarding life-history types, this document uses the term *O. mykiss* to refer to life stages that are indistinguishable as either steelhead or resident rainbow trout. Steelhead exhibit greater variation in the timing and location of each life-history stage than any other Pacific salmonid species in the genus *Oncorhynchus*. Rearing in freshwater can take 1–3 years, and subsequent maturing in the ocean can take 1–4 years. Ocean migrants grow larger and produce more eggs than freshwater residents (NMFS 2012).

Southern California steelhead are considered a "winter-run" type, meaning they enter rivers from the ocean in the winter and spawn shortly thereafter. Winter-run adult steelhead along the California coast can enter rivers as early as October and as late as June when hydrologic conditions allow, but most adult steelhead enter rivers between January and April (Shapovalov and Taft 1954). River entry and upstream migration of steelhead in many southern California watersheds is dependent on high-flow events that breach sandbars in the lagoon to provide upstream passage. These high-flow events occur during the winter and spring and follow periods of substantial precipitation. The life-history types described above are only some of the predominant life histories, and many other life-history forms, such as lagoon anadromous, can be important to populations in southern populations (Kendall et al. 2014). The overall flexibility and diversity of life-history types is what makes *O. mykiss* capable of occupying variable habitats and persisting within the extreme environments in southern California.

Similar to anadromous Pacific salmonids, most steelhead return to natal rivers or streams to reproduce, but some stray to non-natal watersheds. Straying is an important evolutionary life-history strategy that supports colonization or recolonization of new or previously occupied habitat, respectively, and is also a mechanism to enhance genetic diversity across populations (Keefer and Caudill 2014). Straying is thought to be particularly important for supporting southern California steelhead, especially small coastal populations that may be too small to be viable (NMFS 2012). Documentation of straying is limited in California, but a study on the Santa Ynez River found 6 of the 16 (38%) anadromous adult steelhead sampled in 2008 were strays (COMB 2013, NMFS 2016, COMB 2021), a much higher straying rate compared to estimates from more northern populations or winter-run steelhead (4–14%; Keefer and Caudill 2014). A more recent study from central California showed strays in the watershed can be from distances as far as 680 kilometers apart (Donohoe et al. 2021). These studies indicate that straying is an important life history strategy in steelhead and that steelhead can stray from distant watersheds. The influences of straying on metapopulation dynamics of steelhead warrants additional study, especially in southern California.

The studies described above are examples of straying into watersheds where *O. mykiss* were already present. Like other anadromous salmonids, steelhead locate spawning grounds by following natal olfactory cues imprinted during early life stages (Hasler and Scholz 1983, Dittman and Quinn 1996), or in the absence of natal olfactory cues, it is thought that anadromous salmonids rely on olfactory cues from conspecifics (Bett and Hinch 2016). The ability to use cues from conspecifics, whether co-migrating adults, outmigrating smolts, or rearing juveniles, would increase the likelihood of locating suitable habitat and mates. These navigational mechanisms would be more important for semelparous species of Pacific salmon compared to iteroparous steelhead, which have more than one opportunity to spawn within a lifetime. Straying into habitats where homing cues are absent provides a mechanism to recolonize formerly occupied habitat or colonize new habitat and may be especially important in a high disturbance regime like southern California and for smaller, coastal watersheds that tend to be more prone to extirpation events. Following an extirpation event in smaller watersheds, it is thought that nearby, larger watersheds that retain populations will recolonize extirpated habitat through straying. Documentation of this phenomena is rare, but in the San Mateo River in Orange and San Diego counties, a steelhead population was established by anadromous strays in 1999 after a more than 50-year absence (Hovey 2004). In Topanga Creek, steelhead recolonized in the late 1990s following extirpation in the 1980s (Bell et al. 2011). In the late 1990s, steelhead were found to be extirpated in the Santa Margarita watershed, then were observed to be present in 2009 (Becker et al. 2010, Dagit et al. 2020). We found no other documentation of recolonization events that have occurred in the last two decades, but once again, limited monitoring is conducted throughout the region.

While there are few examples of recolonization of extirpated watersheds, there are numerous examples of recolonization of extirpated habitat from other occupied habitat within a watershed (i.e., within watershed dispersal). For example, reaches of the mainstem Ventura River, Murrieta Creek, and Matilija Creek where steelhead were extirpated following the 2017 Thomas Fire have been recolonized, likely by upstream populations that survived the wildfire and debris flows (NMFS 2023). In the Santa Ynez watershed, *O. mykiss* were extirpated from large sections of upper El Jaro Creek in 2015 due to a drought resulting in no flow; however, an adult was observed in 2020, indicating recolonization (COMB 2022).

There are also examples of recolonization of upstream habitat following barrier removal. Famously, steelhead returned to upstream habitat in the Elwah River following dam removal, although it is believed the returning steelhead originated from the existing resident rainbow trout population that persisted above the dam. Steelhead also recolonized habitat in Mill Creek, California, less than a year following dam removal. In another example, two Arizona-type crossings (one of which limited passage over approximately 95% of the flow range and was located 0.75 mile upstream from the ocean, Becker et al. 2010) were removed in the lower Arroyo Sequit Creek in 2015, opening up habitat to migrating steelhead. Two steelhead were observed in the creek just 2 years later in 2017 (Dagit et al. 2020), but despite steelhead being observed, no population currently exists in Arroyo Sequit Creek, presumably due to low flows and poor habitat quality following the 2018 Woolsey Fire. These examples highlight the importance of having broadly distributed populations across variable habitats and also demonstrate the ability of populations to respond quickly to large-scale restoration actions.

Population expansion and contraction of steelhead is a natural feature within dynamic landscapes affected by dry and wet seasons and more long-term fluctuations in environmental conditions such as drought. For example, based on research from the Carmel River, during periods of drought, steelhead populations retract into more stable, drought refugia habitats, but then expand into less stable habitats once conditions improve (Boughton et al. 2020, Boughton and Ohms 2022; NFMS 2023). Increased variability in life history expression would be expected when environmental conditions create more abundant and widely distributed populations. Thus, the recent drought affecting southern California would be associated with reduced rates of anadromy from both a population abundance and migration opportunity perspective. Reduced rates of anadromy would, in turn, reduce the probability of natural recolonization. As conditions improve, we would expect to see increased numbers of anadromous adults, increased genetic mixing among populations through straying, and potentially increased rates of recolonization of previously extirpated habitat.

However, a fundamentally changed environment will present many challenges to these historically successful life history dynamics that operated at natural reoccurrence intervals for disturbances. For example, increases in freshwater temperature associated with climate change may reduce juvenile growth rates, an intrinsic biological factor associated with anadromy (Hayes 2008, Kendall et al. 2014), but only if temperature increases beyond optimal conditions for growth. In addition, climate change predictions for the region indicate more frequent and severe droughts, as well as potentially increased amounts of precipitation across fewer days (i.e., the annual rainfall with be encompassed within fewer, heavier storm events; Modrick and Georgakakos 2015, NMFS 2023). Finally, steelhead ocean migration appears to be largely driven by temperature (Miller 2020), and increased ocean temperatures could drive ocean migrations further northward, making it less energetically feasible for anadromous migrants to return to southern California streams. These conditions could further reduce anadromous rates and opportunities. Overall, it is difficult to predict how these dynamics will play out in the future, which is a primary reason for considering all available conservation options for southern California steelhead.

All *O. mykiss* downstream of barriers to anadromy are federally protected and are considered candidate species for California Endangered Species Act (CESA) listing (currently in review as of October 2022). Resident *O. mykiss* upstream of barriers to anadromy are not federally protected despite being genetically similar to anadromous populations downstream of barriers. Indeed, Clemento et al. (2009) and Garza and Clemento (2007) showed that resident populations above barriers are more genetically similar to anadromous fish below barriers of the same watershed compared to fish populations in a different watershed. Resident *O. mykiss* that currently exist upstream of barriers are considered by NMFS as having potential to contribute to the recovery of the Southern California Steelhead DPS by adding population spatial structure and diversity (NMFS 2023).

1.4 Overview of Existing Recovery Plans and Policies

The NMFS (2012) Recovery Plan and 5-year status reviews (NMFS 2016, 2023) provide extensive information on the biology and ecology of steelhead trout and the Southern California

Steelhead DPS, threats to the DPS, recovery goals, strategy, and actions, as well as information on the BPGs, adaptive management, and implementation of the Recovery Plan. Of note, this Evaluation and Guidance document described herein is not intended to reproduce or refine materials presented in the Recovery Plan; rather, it is intended to support the Recovery Plan. For example, as part of a summary of watershed-specific information, this Evaluation and Guidance document identifies known locations with drought refugia, which is a key factor for determining core populations where recovery action should be focused, as described in the NMFS (2016) 5-year status review. Furthermore, the actions considered herein will increase the likelihood of success of the NMFS (2012) Recovery Plan by ensuring that adequate numbers of fish/populations remain in the DPS to benefit from recovery actions.

Of note, the conservation actions considered herein addresses three of the six recovery objectives from the NMFS (2012) Recovery Plan, including the following:

- “Prevent steelhead extinction by protecting existing populations and their habitats.”
- “Maintain current distribution of steelhead and restore distribution to some previously occupied areas.”
- “Conserve existing genetic diversity and provide opportunities for interchange of genetic material between and within viable populations.”

Recovery actions recommended by NMFS to address these objectives are focused on resolving the fundamental causes of population declines, mainly decreased habitat availability and connectivity. In this way, the NMFS-recommended recovery actions differ from rescues and reintroductions, which do not address fundamental causes of population level declines. However, NFMS (2012) also states: “Opportunistically, other recovery actions may be implemented prior to these actions.” Furthermore, the recent NMFS (2023) 5-year status review outlined specific recommendations for preventing local extirpation of *O. mykiss*. The following were among recommendations in the NMFS (2023) 5-year status review and are supported by the information presented on rescues and reintroductions presented herein:

- “Explore other means of conserving individual populations of *O. mykiss* that may face the risk of extirpation (e.g., using other existing facilities at academic institutions or museums, or natural refugia habitats); and
- Coordinate and implement relocation activities and plans (including post relocation monitoring) for rescued *O. mykiss* within all Southern California Steelhead DPS BPGs” (see Section 4.2 in NMFS 2023).

In addition to the NMFS Recovery Plan and 5-year status reviews, additional federal and state strategy and policy documents relevant to conservation actions are considered herein.

The California Interagency Anadromous Fish Rescue Strategy 2021 to 2026 (NMFS and CDFW 2021) describes the goals of fish rescue efforts, the roles and responsibilities of NMFS and CDFW, fish rescue approval and authorizations, general guidance on fish rescue implementation, and notification, reporting, and conflict resolution processes. This Evaluation and Guidance document uses specific guidelines from this rescue strategy to develop watershed-specific guidelines.

CDFW Bulletin 2013-04 fish rescue policy (CDFW 2013) defines fish rescue actions and describes the Fish and Game Code and Commission policies that apply to fish rescues, including Fish and Game Code sections 1001 and 1700 and Fish and Game Commission policies on Anadromous Rainbow Trout (Steelhead) and Salmon. This document also describes the procedure for the appropriate Regional Manager to make decisions on fish rescue actions. This Evaluation and Guidance document uses specific guidelines from this rescue policy to develop watershed-specific guidance documents and to ensure all conservation actions described herein adhere to existing policies and procedures.

CDFW Bulletin 2017-04 on propagation (CDFW 2017a) provides guidance in captive propagation programs for conservation and education. This document states that propagation usually requires “take” of the species being propagated, which therefore requires proper “take” authorizations. This document also lists considerations for using captive populations for conservation purposes, including the need for intervention, overall management strategy for the species, resources required for the action, and potential risks of the action. The implementation section details the processes of initial assessment and approvals, plan preparation and evaluation, and examples of captive propagation plans that are available for reference. This Evaluation and Guidance document does not specifically recommend propagation activities, but the guidelines provided were considered when evaluating potential conservation strategies.

CDFW Bulletin 2017-05 on conservation translocations (CDFW 2017b) defines conservation translocation actions and the challenges associated with them. This document provides guidance for conservation translocations, and although it does not cover other types of translocations such as rescues, the same principles would apply in other situations. This document also describes the implementation of translocations and provides a decision matrix for these actions, with considerations such as current threats to the donor and receiving populations, potential risks, potential effectiveness, urgency, feasibility, and resources available. The Recommended Metadata section lists the information to track throughout the translocation process, including information on the collection, release, decision determination, and evaluation of success or failure. This Evaluation and Guidance document used information and guidance presented in CDFW (2017b) to inform decisions regarding conservation actions and for development of watershed-specific guidance documents.

CDFW Fish Bulletin 182 on monitoring and management (Boughton et al. 2022) provides guidance for monitoring methods for *O. mykiss* based on Adams et al. (2011) that are updated and expanded upon to include nuances to the southern California flow regime as well as *O. mykiss* distributions and life history phases that differ from the more northern California populations. This document describes the efficiencies and practicalities to California’s Coastal Monitoring Plan (CMP) for the Southern California Steelhead DPS. This Evaluation and Guidance document used information and guidance presented in Boughton et al. (2022) to determine guidelines for monitoring and management for rescues and reintroductions.

In addition to the recovery plans and policies described above, we followed translocation guidelines and recommendations presented in the International Union for Conservation of Nature (IUCN) and the Species Survival Commission’s *Guidelines for Reintroductions and Other Conservation Translocations* (IUCN and SSC 2013) and NOAA’s *Anadromous Salmonid Reintroductions: General Planning Principles for Long-Term Viability and Recovery* (NOAA 2018). Additional publications within the peer-reviewed literature were also reviewed and incorporated into this Evaluation and Guidance document.

2 PART 2 — RESCUE AND REINTRODUCTION EVALUATION AND GUIDELINES

Rescues and reintroductions are forms of conservation translocations. A conservation translocation is defined as a human-mediated movement of living organisms from one location to another where the primary objective is a quantifiable conservation benefit (George et al. 2009, IUCN and SSC 2013, CDFW 2017b). Fish that are translocated (or sometimes referred to as "transplanted") can be moved to (a) habitat where the species are present (termed "reinforcement"), (b) habitat that the species historically occupied but where they are no longer present (termed "reintroduction"), or (c) to habitat outside a species' indigenous range (termed "introduction" or "assisted colonization") (IUCN and SSC 2013, Hayes and Banish 2017).

We emphasize that rescues and reintroduction conservation actions described herein are not intended to work independently of other recovery actions, such as habitat restoration or dam removal. The tools discussed below are intended to protect current steelhead populations, slow the decline of steelhead, and prevent further extirpation, and the recovery of southern California steelhead will only occur through implementation of a host of recovery actions outlined in detail within NMFS (2012).

A concern expressed by some stakeholders is that implementing these rescues and reintroductions would distract from the recovery actions recommended in the NMFS (2012) Recovery Plan, which are needed to address the fundamental causes of population declines. The concern is that society is less likely to take actions that are needed to address fundamental causes of population declines if they perceive threats have either been diminished or that actions can be taken in lieu of restoration (in addition, funding and resources could be diverted away from other recovery actions). The best example of this type of distraction is from large scale salmon production hatcheries in the Pacific Northwest that have artificially inflated numbers of salmon available for harvest, effectively masking (while also contributing) to the severity of declines of naturally produced salmon. However, there are also examples when this scenario does not hold true. In the Carmel River, the San Clemente Dam was removed to support the recovery of steelhead, despite very developed fish rescue and rearing programs and the removal of several other large dams, including the Elwha, Marmot, Condit, and most recently the initiation of removal of four dams on the Klamath River, to support the recovery of salmonid populations that were being augmented by hatcheries.

2.1 Rescue Guidelines

2.1.1 Background

For the purposes of this Evaluation and Guidance document, rescue is a management strategy with the goal of preventing direct mortality or loss of genetic traits due to detrimental environmental conditions. Rescue requires the movement (translocation) of fish from habitats that have become unsuitable for survival to habitats better suited for individual survival and population sustainability. Salvage is a term used interchangeably with rescue, indicating the relocation of individuals from habitat that is degraded to refuges as a risk-spreading strategy (Peacock et al. 2010). Fish rescues are conducted for a suite of reasons, the most common in southern California streams being seasonal low flows that strand fish in isolated pools with poor water quality. Low to moderate streamflow years or partial barriers can also force adults to spawn in habitat that becomes unsuitable for rearing in the summer/fall, which leads to high mortalities of YOY/fry. In addition, deleterious conditions caused by drought or wildfire can result in rescues. Rescue operations can also occur coincident with the operations of dams and diversions, such as during dewatering of fish ladders for maintenance.

A review of the most recent rescue efforts led by CDFW indicated 10 rescues of 514 individuals across five watersheds occurred in the Southern California Steelhead DPS region in 2022, with the majority being attributed to low flows creating isolated habitat with poor water quality (e.g., low dissolved oxygen [DO] levels, elevated temperatures, pollutants) where fish had become stranded and were likely to die if no action were taken. Over the last decade (since 2012), a total of 1,642 *O. mykiss* have been rescued and relocated by CDFW (K. Evans, CDFW, pers. comm., 25 January 2023). The reported numbers of rescued individuals were all within anadromous waters in Santa Barbara, Ventura, and Los Angeles counties. Additional rescues have been conducted in non-anadromous waters (i.e., reaches upstream of impassable barriers) over the same period, but the numbers of rescued fish from non-anadromous waters were not included in the summary.

Historically, rescues were also frequently conducted by the California Department of Fish and Game (CDFG). In the 1940s, for example, prior to construction of Bradbury Dam, CDFW rescued fish stranded in drying pools in the lower Santa Ynez River during drought years. The CDFG translocated these rescued fish to perennial habitat within the Santa Ynez River system and to other rivers, such as the Santa Maria River. The number of rescued fish translocated during the CDFG program numbered in the millions, which could have legacy effects on the genetic composition of steelhead throughout southern California.

Ultimately, the goal of rescue is to prevent mortality of individual fish, and rescues typically occur within populations at risk of extirpation. Rescue as a conservation action could help maintain population sizes, reduce the risk of extirpation, maintain genetic diversity, and prevent genetic bottlenecks and inbreeding depression. Rescues also have the potential to increase population connectivity, augment populations with low abundance, and provide new genetic material depending on release location of rescued individuals (Anderson et al. 2014). Indeed, the introduction of even five adults was predicted to dramatically increase the heterozygosity of an isolated, depressed population of cutthroat trout (*Oncorhynchus clarkii*) based on theoretical models (Kovach et al. 2022).

The largest risks associated with rescues result from overstocking that could increase competition at release locations (i.e., create density-dependent effects) and increase risk of disease transmission from rescued fish that have been stressed, thereby creating conditions in which fish are more susceptible to pathogens. There is also a risk to non-target species in the streams, if pathogens for other species of fish or invertebrates are inadvertently moved along with the rescues. In addition, rescue is a form of artificial selection because rescued fish (and their traits) could have perished without human intervention—i.e., they would have been selected against. Rescued fish that exhibited potential maladaptive traits could then compete and breed with fish at the release site, reducing the fitness of the population. Similarly, rescued fish that are released into non-natal watersheds could also reduce fitness of wild populations in non-target, adjacent watersheds through increased straying that could introduce maladaptive genetic traits and increase competition. Conversely increased straying could benefit wild populations through increased genetic diversity and genetic rescue. Ultimately, while there is a lot of uncertainty regarding the genetic effects of rescued fish mixing with wild populations, it is likely that most southern California steelhead in need of rescue were prevented from expressing important life history variability by anthropogenic factors such as water use and climate change (i.e., their impending mortality was also a form of artificial selection). Additional consideration for rescues specific to southern California steelhead is discussed in the following sections.

Two separate types of rescues actions are described below. The first type of rescue action is considered programmatic and includes rescues in response to previously established biological and environmental triggers that are known to occur repeatedly (hereafter "Programmatic Rescues"). The second type of rescue action is in response to a stochastic disturbance event or in response to isolated observations of stranded fish that were not previously established (hereafter "Isolated Rescues"). The specific details of either type of rescue, Programmatic and Isolated, will be specific to the watershed and disturbance event, but general details are provided below as guidelines. For the Programmatic Rescues, we describe an approach for developing biological and environmental triggers for rescue actions. For Isolated Rescues, we describe general disturbance triggers in sufficient detail to inform implementation of rescue actions.

2.1.2 Programmatic Rescue

Programmatic Rescues described herein were modeled after the Carmel River Steelhead Rescue and Rearing Management Program (RRMP) that is used by the Monterey Peninsula Water Management District (MPWMD 2018). MPWMD rescue and rear wild juvenile *O. mykiss* annually to mitigate impacts of domestic water supply activities that often result in drying of the lower Carmel River, potentially leading to fish mortalities without the rescue program. The rescue program has been in place since 1989. Rescued fish are removed from the drying reaches, transported into perennial reaches or into a temporary holding facility (located on a flood terrace adjacent to the Carmel River), and then released back into the Carmel River once conditions are suitable. Rescues are triggered based on flow declines that are known to lead to impaired juvenile passage in lower Carmel River. Since it began in 1990, the Carmel River RRMP has rescued more than 769,000 individuals, substantially contributing to the maintenance of the Carmel River steelhead population over time (Boughton and Ohms 2022). Anywhere between approximately 1,000 to 100,000 individuals are brought into the holding facility annually (MPWMD 2018).

Programmatic Rescues in response to biological and environmental triggers will only occur in summer and fall when environmental conditions (e.g., intermittent surface water, elevated temperature, decreased DO, absence of prey) can be limiting to *O. mykiss* rearing within southern California streams. Typically, Programmatic Rescues would be implemented within middle and lower reaches of watersheds that are more prone to fish stranding due to the low flows and pool isolation that occur in the summer and fall that create suboptimal conditions (elevated temperature and low DO) for steelhead survival and growth. The best candidate locations for implementing a Programmatic Rescue would have the following conditions:

- Reaches with intermittent or dry summer/fall conditions downstream from perennial habitat;
- Frequently observed fish stranding in isolated pools or habitats;
- Rescues that are routinely implemented by CDFW in response to stranding, but not within a structured or strategic framework;
- Low quantities of *O. mykiss* within anadromous reaches of the watershed, meaning the rescue of even a few individuals is a substantial proportion of the population; and
- Existing and ongoing *O. mykiss* monitoring to establish population distribution and densities.

Programmatic Rescues would be implemented only once biological and environmental triggers are met. Biological triggers for implementing fish rescues within reaches would be based on fish density and abundance estimates from upstream reaches where rescued fish could be relocated.

O. mykiss densities in streams vary considerably across time due to environmental conditions and density-dependent processes. Thus, the stocking capacity of a particular reach (or habitat unit) would be expected to vary annually and seasonally. To determine stocking capacity, surveys in the early summer (i.e., before conditions in the lower watershed deteriorate) could be used to determine fish densities in upper reaches and to form a predictive relationship between physical habitat measures (e.g., pool depths, pool area, pool-riffle ratios) and fish densities across age classes of fish. Reaches or habitat units with below-average densities for the given conditions would be identified as available for stocking. Ideally, over the long term, data collected on habitat and fish densities could be used to model habitat suitability for specific streams and more broadly across southern California. Alternatively, the 5-year status review of southern California steelhead (NMFS 2023) determined a population density viability criterion below 0.30 fish/square meter $(m)^2$ during the summer low-flow season buffers against density dependent effects. Thus, 0.30 fish/m^2 during sampling could be used to identify suitable habitat for stocking in the absence of site-specific data. Overstocking may not be a major concern, however, due to most of the effects of trout density on growth occurring at the lowest densities (Jenkins et al. 1999). In addition, steelhead/trout can move fairly freely among stream sections in the absence of barriers when winter flows are high, so natural dispersal mechanisms may alleviate ethe effects of competition at a local scale.

Environmental triggers for conducting fish rescues would be based on stream flow. Rescue operations would be triggered when stream flow is low enough to create semi-isolated or isolated habitat in the summer and fall. The specific flows that create intermittent conditions would need to be identified based on measured flow and field observations or could be modeled. A secondary environmental trigger could rely on water temperature and DO within isolated pools. The water temperature trigger could be met when temperature exceeds the thermal optimum for growth or when it approaches lethal levels. However, suboptimal and lethal temperatures are not well defined for *O. mykiss* in southern California, and the use of existing temperature standards, which are derived from more northern populations, may be overly conservative. For example, steelhead were present and feeding in a southern California stream at temperatures up to 28 degrees Celsius (°C) (Sloat and Osterback 2013), a temperature that is at or above the critical thermal maximum for more northern populations (McKenzie et al. 2020). The uncertainties associated with defining a specific temperature threshold for *O. mykiss* in southern California is why we recommend basing the environmental rescue trigger primarily using flow with the assumption that temperature and DO conditions will deteriorate and become stressful or lethal within isolated habitat in most southern California streams in the summer and fall. However, flow and temperature are not necessarily related in all locations because groundwater inputs into isolated pool habitat can provide thermal refuge (Nielsen et al. 1994). Thus, we recommend basing the environmental trigger initially using flow and using temperature and DO as a secondary measure to confirm conditions warrant rescues.

In addition to biological and environmental triggers, operational triggers associated with dams, diversions, and other anthropogenic water use activities should also be considered. For example, rescues in response to maintenance and dewatering of fish passage infrastructure at diversion dams are routinely conducted at both the Freeman and Robles diversions in the Santa Clara and Ventura rivers, respectively.

Based on the guidelines presented above, annual fish surveys would likely be a part of a Programmatic Rescue program. Substantial resources and time are required for fish habitat surveys and for the execution of Programmatic Rescues. Hence, watersheds would need to be prioritized for Programmatic Rescues based on numerous factors including watershed conditions, accessibility, and availability of resources, to name a few. A watershed prioritization framework is discussed in Appendix A.

After a rescue is triggered, all wetted habitat within the identified rescue reach/es would be surveyed using dipnets or backpack electrofishing combined with seining. The rescue reaches would be identified within watershed-specific guidance documents. Any captured *O. mykiss* would be relocated to suitable upstream habitat (or habitat downstream that is connected to the estuary), unless there is evidence of smolting, in which case, *O. mykiss* showing smolting characteristics could be released into the lagoon, depending on the watershed and time of the year.

If no suitable upstream habitat is present within the watershed or if overstocking is a concern, rescues could still be implemented, but a release site external to the watershed would be needed. External release sites could include a temporary holding facility (see Section 2.3 for additional details about holding facilities) or a separate watershed. Release into a separate watershed effectively becomes either a reinforcement translocation if *O. mykiss* were already present or a reintroduction translocation if *O. mykiss* are not present (see Section 2.2). Benefits to reinforcement translocation could include increasing genetic diversity and numbers of effective spawners; however, many risks are associated with reinforcement translocations, including introduction of maladaptive genetics or pathogens, as well as overstocking. Reintroductions reduce risks of genetic mixing and overstocking. A temporary holding facility could be preferable over an external watershed because it simplifies decision-making (e.g., there is no need to evaluate risks associated with release into an external watershed), and it provides the easiest route for reintroducing fish back into the natal watershed after conditions have improved (see Section 2.3 for a more detailed discussion of temporary holding facilities).

2.1.3 Isolated Rescues

Isolated Rescues would be designed for a stochastic disturbance event or could be undertaken in response to isolated observations of stranded *O. mykiss* that are not covered by the Programmatic Rescues described above. Isolated rescues in response to unpredictable operations of dams, diversions, and other water use infrastructure, such as unplanned dewatering for maintenance, could also occur. General descriptions of triggers in response to different types and severities of disturbances are discussed below. Drought and wildfires, which are the most common disturbances that would result in a need for fish rescues, are the focus of the ensuing discussion. In the case of a drought, which leads to watershed conditions that are somewhat predictable because they occur over longer periods, the rescue action would be modeled after the Programmatic Rescues described above (i.e., they would be triggered based on biological and environmental conditions).

If the disturbance led to conditions in the watershed that are unpredictable, as is the case with wildfires, sudden unexpected water extractions, or pollution events, a variety of factors or triggers, including location, timing, and severity of the event, would need to be considered prior to conducting a rescue event. Wildfires and associated debris flows have been the most ubiquitous, acute source of extirpation within the region over the last decade, and these events are expected to increase in frequency and severity in the future. Thus, Isolated Rescues in response to wildfires are considered in more detail hereafter.

Any rescue in response to a wildfire would occur after the wildfire, but before potential storm events that could create high sediment erosion rates, transport, and deposition that may cause *O. mykiss* extirpation. The timing of the rescues after a wildfire is due to the uncertainties related to wildfire movement across landscapes and, most importantly, safety concerns for a rescue team. The rescue should occur before potential debris flows to avoid extirpation; however, it is difficult to predict the occurrence and severity of a post-fire debris flow event because it is related to the location and severity of the wildfire, the proportion of the watershed and riparian burned, and the intensity, magnitude, and frequency of subsequent rain events (USFS 2018, Cooper et al. 2021). Based on the literature, the following conditions are likely to cause severe debris flows and could trigger an isolated rescue in response to a wildfire: (a) the wildfire burns large portions of the watershed (e.g., 75% or more) at medium to high severity; (b) the wildfire extent includes the upper watershed where there is potential refuge habitat; and (c) there is moderate to severe burning of the riparian habitat (>50% of riparian habitat is burned). If these conditions are met, a rescue should be implemented following a wildfire once conditions are safe for field crews to enter and prior to a predicted significant rainfall event.

Although these conditions are associated with higher probability of debris flows and thus extirpation of *O. mykiss*, other conditions could also be considered. The existence of *O. mykiss* in tributaries that are not impacted by a wildfire could provide a means of natural recolonization of extirpated habitat following a wildfire given barriers are not present. In addition, a wildfire that only burns small proportions of the watershed or only affects the lower watershed or wildfires followed by light or no rains would not trigger rescues or would only trigger local rescues.

Remarkably, there are instances when *O. mykiss* persist in a watershed after severe debris flows following a wildfire. For example, following the 2017 Thomas Fire and subsequent, significant debris flows, resident populations of *O. mykiss* persisted and repopulated extirpated reaches in the Ventura River watershed (NMFS 2023). Alternatively, resident *O. mykiss* that were known to be present in Carpinteria and Arroyo Hondo creeks are thought to be extirpated from the watershed after the 2017 Thomas and 2021 Alisal fires, respectively, created deleterious conditions downstream of the burned reaches (NMFS 2023). An Isolated Rescue could have saved the Carpinteria and Arroyo Hondo creek populations by removing fish from the watersheds prior to the severe debris flows that likely extirpated *O. mykiss* from each watershed.

Determining how many fish to target for removal could be based on numerous considerations, including population size, accessibility, and availability of habitat or facilities for relocation. For larger populations, the absolute numbers of fish rescued could be high, but the relative proportion of the population could be low compared to a smaller population where fewer individuals would be rescued but the number could be a larger proportion of the total population. It should be noted that habitat restoration, including lagoon restoration over the long term, could provide opportunities for fish to locate refuge from debris flows, reducing the need for rescues.

As outlined above, numerous factors must be considered when determining the need for an Isolated Rescue, and we are unable to predict and account for all potential scenarios. Importantly, decisions regarding rescues need to be made in real time, and in some cases, these decisions must be made quickly, which is one reason the watershed-specific guidance documents were developed. We also recommend convening a technical advisory committee to support decision-making regarding implementation of rescues, including decisions about where (optional relocation sites are a major consideration) and when to relocate fish.

2.1.4 Release Site Selection

Several factors will dictate where rescued fish can be relocated including the quality of upstream habitat (e.g., water quality parameters, presence of cover, food availability, predators, and perennial habitat) and the severity and location of the disturbance event (e.g., if there are any unburned areas and reaches above a fire). If a wildfire or other large-scale disturbances affect the entire watershed, it may be necessary to consider release sites outside the watershed. Important considerations for selecting among release sites are discussed below. Critically, release sites should be prioritized that contain refuge habitat from low flows and high temperatures over sites with suitable spawning substrates because it's assumed that fish released into refuges from drought will redistribute to find suitable spawning habitat when flows are higher.

2.1.4.1 Within watershed release

Optimally, rescued fish will be relocated to habitat that is normally accessible to fish within the watershed where the rescues occurred, reducing handling/transport times and risks from pathogen spread and genetic mixing. Typically, in southern California streams, the lower and uppermost reaches of the watershed are more prone to drying and stranding, whereas intermediate reaches in steep terrain consist of more perennial habitat. However, intermittent reaches can occur far upstream within watersheds, depending on hydrogeomorphology. Fish and fish habitat surveys should be conducted within potential release sites to determine habitat suitability prior to release following the protocols described above for Programmatic Rescue releases (Section 2.1.2). If surveys are not possible (due to safety or timing) or there are no suitable release locations within the watershed where fish are rescued, an external site, such as an external watershed or temporary holding facility, should be considered. A temporary holding facility offers many benefits over release into an external watershed, as discussed below.

Estuaries/lagoons could be another release location in watersheds where lagoons contain suitable oversummer rearing habitat but are typically disconnected from the rest of the watershed due to dewatering, disconnected subsurface flow in mainstem habitat, or due to poor conditions in mainstem rivers. Seasonal lagoons may contain conditions that promote growth but could also come with risks related to increase predation. Release in estuaries/lagoons would mimic natural movement patterns in undisturbed watersheds and promote diverse life-history expression, which are key goals of conservation actions.

Another potential release location within a watershed could be in habitat that is upstream of barriers to anadromy. Historically, the highest-quality habitat exists upstream of barriers to anadromy within the Southern California Steelhead DPS (NMFS 2012), and these habitats could serve as refuge habitat for rescued fish, but habitat suitability upstream of these barriers would need to be confirmed. The presence of resident *O. mykiss* in the upstream barrier habitat is a good indication of suitable habitat, but if *O. mykiss* were present, the protocols described above for Programmatic Rescue releases (Section 2.1.2) should be followed to reduce the potential for overstocking. The genetic lineages of the resident *O. mykiss* population upstream of the barrier and risks tied to pathogens spread and invasive species should also be considered. Genetic structure of many populations upstream of the barrier has been evaluated (Aguilar and Garza 2006; Pearse et al. 2007, 2014; Clemento et al. 2009; Garza et al. 2014; Pearse and Garza 2015; Abadía-Cardoso 2016), and information from these studies can be used to facilitate decision-making regarding genetic mixing. Of note, resident *O. mykiss* populations upstream of barriers have been shown to have genetic divergence in alleles associated with anadromy (Pearse et al. 2014, Apgar et al. 2017, Pearse et al. 2019), but they have also been shown to be more genetically similar to populations downstream of barriers within the same watershed compared to neighboring populations (Clemento et al. 2009; see Part 3 for more discussion). Indeed, frequent downstream movements of fish from above barrier populations occurs through spills.

2.1.4.2 Temporary holding facility

A controlled temporary holding facility, such as that used near the Carmel River, could be highly effective for temporarily holding fish that will be reintroduced into their natal watersheds following a disturbance. A temporary holding facility could also be used for holding fish while decisions are being made regarding an appropriate release location if conditions in the natal watershed are not expected to recover in a timely manner. The use of temporary holding facility would “buy time” for decision-making in the face of uncertainty. A major concern with the use of a temporary holding facility is imposing artificial selection and rewarding fish that exhibited sub-optimal strategies with increased growth. A temporary holding facility that is able to hold listed steelhead is not currently available within the Southern California Steelhead DPS, but Filmore Fish Hatchery is currently being considered as a temporary holding site for southern California steelhead (R. Burg, CDFW, pers. comm., 28 February 2024). A more detailed discussion of temporary holding facilities is provided in Section 2.3 below.

2.1.4.3 External watershed

Release into an external watershed is a viable option only if fish cannot be released into other reaches within the watershed or if a temporary holding facility is unavailable. The best candidate external watersheds would be those within the same BPG as the watershed where fish were rescued, those that share similar habitat characteristics to the rescued site before it was disturbed, and those that have habitat capable of supporting *O. mykiss* year-round. Selecting a nearby watershed within the BPG would also reduce transport times. However, if the disturbance event is extensive enough to impact the entire BPG, or if no other watersheds have suitable habitat, it may be necessary to consider relocating fish to a watershed outside the BPG. In such cases, it would be preferable to reintroduce *O. mykiss* into a watershed that does not currently, but historically did support *O. mykiss* because this would reduce risks associated with increased competition and genetic mixing between populations while promoting demographic expansion. In this case, the release of rescued fish effectively becomes a reintroduction translocation and would follow guidelines presented within Section 2.2. Release sites in external watersheds could be in anadromous waters or within habitat in upstream barriers to anadromy. If an *O. mykiss* population was present in the release site, whether upstream or downstream of barriers to anadromy, risks associated with genetic mixing, overstocking, and disease spread should be considered as discussed in the preceding sections. Increased straying of fish released into non-natal watersheds and the risks this poses to wild populations should also be considered. However, straying of a small number of rescued fish into other wild populations is not expected to have a large negative genetic impact (this is more of a concern for large scale hatchery releases), and instead, could provide some genetic benefits to small populations that are experiencing genetic drift and inbreeding depression.

2.1.5 Release Protocols

Optimally, rescued fish that are moved to an external site would eventually be released back into their natal watershed as feasible, except as described below. The timing and circumstances that would support release back into natal watersheds is case specific. As mentioned previously, fish that are held in a temporary holding facility would be released back into their natal watershed once conditions are suitable to support *O. mykiss* for the long term. For example, following a wildfire, fish should not be released back into their natal watershed until after rainfall events to avoid potential exposure to high sediment loads, unless the wildfire was low in extent and severity (e.g., the wildfire did not burn large portions of the watershed and the riparian vegetation remained relatively intact). For fish rescued during a drought, release back into their natal watershed would typically occur after flows in the wet season provide adequate connectivity and water quality. Similar to the Programmatic Rescues, environmental triggers could be developed to determine appropriate conditions for reintroduction. For example, once flows increase beyond a threshold after a date when risks of high temperatures are minimized (e.g., after November 1), fish would be reintroduced.

Determining whether to keep fish in the release watershed would occur when stream conditions do not improve or are not anticipated to improve within the rescue watershed. For example, biological, physical, and chemical properties of streams can take years to recover following an extreme wildfire event (Cooper et al. 2021). Recovery can be prolonged by severe debris flows (Verkaik et al. 2013). In the case of a wildfire, habitat conditions within the watershed would need to be surveyed prior to releasing fish back into their natal watershed to confirm conditions will support reintroduction. Key factors to evaluate would include the condition of riparian vegetation, pool-to-riffle ratios, pool depths, availability of spawning gravels and in-stream cover, percent substrate embeddedness, and DO and temperature levels (see CDFW Stream Channel Type Work Sheet by Flosi et al. 2010). Surveys can help confirm habitat is suitable for release back into natal watersheds, but decisions will likely rely on some expert judgement by local, state, and federal biologists. Ultimately, if conditions are not suitable for releasing fish back into natal watershed, the rescued fish would need to be either held for longer periods in a holding facility or reintroduced to another watershed, in which case, reintroduction guidelines would be followed (see below).

2.2 Reintroduction Guidelines

2.2.1 Background

This section focuses on reintroductions via direct human actions rather than natural reintroductions that could occur following recovery actions. Additional consideration of the need for human-mediated reintroductions compared to waiting for natural recolonization are discussed in more detail throughout this section. We also focus on reintroductions rather than reinforcements because fewer genetic and population level risks are tied to releasing fish into vacant habitat compared to occupied habitat. However, one recent study showed that reinforcement was more biologically and cost effective compared to another common conservation strategy, non-native fish removals (Yackulic et al. 2021). Rescues, as described above, can result in any of the translocation types depending on the release location.

Reintroductions are typically driven by objectives to expand population size and species distribution, promote population redundancy, increase genetic/phenotypic/life-history diversity, restore ecosystem function, and ultimately, decrease the chances of extinction. Thus, reintroductions can enhance the viability of salmon populations by addressing key parameters as described in McElhany et al. (2000), including abundance, productivity, spatial structure, and diversity. Moreover, reintroductions that expand the current amount of occupied habitat address a key parameter, occupied area, that has been found to be the most important predictor of extinction risk in the face of climate change (Pearse et al. 2014).

Many biological risks and constraints are associated with reintroductions. Biological risks include disease transmission, deleterious effects on the donor population due to removal of individuals (i.e., population mining), invasion by non-natives, genetic founder effects, and other unintended negative consequences to nontarget species or populations (McClure et al. 2018). The loss of locally adapted traits and the genetic homogenization of populations both within and across sites should also be considered (Anderson et al. 2014, Hayes and Banish 2017). Human-mediated reintroductions also disrupt natural recolonization processes (although these have already been disrupted). As mentioned previously, straying is an important evolutionary mechanism that allows steelhead to recolonize habitat following extirpation events. Natural recolonization would be more likely when there are more abundant steelhead populations that are more closely distributed and with fewer barriers. Reintroduced individuals could also be more likely to stray into adjacent wild populations than individuals that were not associated with human-mediated reintroductions. Straying into wild populations is a concern because it could increase competition, and reintroduced fish that have effectively been artificially selected could introduce deleterious traits into a wild population (i.e., outbreeding depression). However, the small numbers of individuals that would stray from a reintroduced population are not expected to have significant negative genetic impacts through outbreeding depression as long as the numbers of reintroduced fish remains relatively low and reintroductions do not occur consistently over multiple generations. Conversely, straying could also increase genetic diversity in wild populations and could improve metapopulation dynamics in the short-term by assisting natural recolonization rates, as long as reintroductions are not dependent upon for long-term recovery.

For successful reintroductions, constraints should be considered prior to developing a reintroduction plan (IUCN and SSC 2013) and will be highly watershed specific. The most common constraints for reintroductions of anadromous populations include the selection of an appropriate donor population and the availability of suitable habitat at the reintroduction site (i.e., one that lacks migration barriers, McClure et al. 2018). It is worth noting that conservation hatcheries could play a role in reintroductions by increasing early life stage survival and numbers of fish that can be subsequently reintroduced into habitat. However, early stages are abundant in many above-barrier populations that could serve an analogous role to conservation hatcheries but without the domestication effects. Part 3 discusses conservation hatcheries in more detail, but this section focuses on using naturally produced individuals (i.e., ‘wild’ fish) to support reintroductions.

Rescues in southern California are often conducted when local populations are threatened by disturbances, such as wildfires, that pose an imminent risk to a local *O. mykiss* population (J. O’Brien, CDFW, pers. comm., 25 January 2023). Rescues that result in reintroductions are usually implemented within non-anadromous waters due to regulatory challenges and to prevent the spread of *O. mykiss* with unknown genetic lineages, with a few exceptions. Movement of rescued fish between watersheds is rare and has been associated with lack of available habitat in the rescue watershed (K. Evans, CDFW, pers. comm., 26 January 2023). Rescues to or between anadromous waters and non-anadromous waters have occurred at four locations since 2015 (K. Evans, CDFW, pers. comm., 26 January 2023). For example, in 2022, CDFW rescued individuals in Murrieta Creek (non-anadromous stream) and transported them to the North Fork Matilija Creek (anadromous stream) within the Ventura River watershed. As another example, in 2017, *O. mykiss* were rescued from Arroyo Sequit and translocated into Topanga Creek. CDFW in direct coordination with NMFS conducted these rescues/reintroductions within anadromous waters.

More broadly, reintroductions have been used as a conservation measure across numerous species and geographic locations with mixed results (see reviews by George et al. 2009, Anderson et al. 2014, Lusardi and Moyle 2017). In Oregon, cutthroat trout were successfully reintroduced into streams where trout had been extirpated, and genetic studies showed low genetic drift even with a low effective population size of the reintroduced population (Peacock et al. 2010). Reintroductions can also lead to negative effects on large-scale populations. For example, transfers of fish among watersheds in the Pacific Northwest has been detrimental to the overall population structure and local adaptation of salmonids in the region (Williamson and May 2005, Naish et al. 2007, McClure et al. 2008). As shown by this example, unsuccessful reintroductions can largely be attributed to failure to correctly identify and distinguish among local genotypes, species, or subspecies, which results in introgression of non-native, non-target, or maladaptive genes into a native population. However, advances in genetic mapping could reduce negative effects if rapid genetic testing could be done on rescued fish prior to reintroductions. Other causes of unsuccessful reintroductions (i.e., resulting in unintended or undesirable negative consequences for species or populations) are due to reintroducing fish into unsuitable habitat, insufficient numbers of reintroduced individuals, and the introduction of endemic pathogen strains (Anderson et al. 2014). Certainly, challenges and risks will be encountered when considering reintroductions as a conservation measure, but, overall, reintroductions can be an effective tool for species conservation when carefully planned.

From a broad conservation perspective, it will be necessary to determine when it would be appropriate to implement reintroductions and at what scale. These decisions cannot be easily addressed with the available information, and differences in philosophical views and professional judgment will influence decision-making among individuals. Several factors can be considered to objectively inform these decisions, such as the number of remaining watersheds with extant populations, causes of extirpation (human versus natural; although due to climate change, most extirpations could be considered human caused), use of a replacement rate (e.g., 1:1) for extirpations, among many others. As part of the NMFS (2012) Recovery Plan, biological criteria for viability includes targets for the minimum number of viable populations within a given BPG. For example, within the Santa Monica Mountains BPG, three viable populations are the target (currently, there is only one existing population). It is unlikely that reintroductions will, at least in the short term, result in a viable population, as defined within McElhany et al. (2000), but these population numbers could still be used as targets for reintroductions. If reintroductions are based on rescued fish, some of the decisions regarding timing and scale will be dictated by the circumstance of rescues.

The following section discusses the reintroduction framework and guidelines that include assessments of reintroduction site and donor population selection, reintroduction numbers, pre- and post-action surveys, and reintroduction timing. Generally, we followed reintroduction guidelines presented in IUCN and SSC (2013) and NOAA (2018), as well as guidelines for "minimizing biological risks" and the decision framework for selecting a "low-risk colonization strategy and source population" presented in Anderson et al. (2014). This guidance on reintroductions aligns with recent recommendations in the NMFS (2023) 5-year status review, which recommends: "Coordinate and implement relocation activities and plans (including post relocation monitoring) for rescued *O. mykiss* within all Southern California Steelhead DPS BPGs" (see Section 4.2 in NMFS 2023).

2.2.2 Reintroduction Framework

The framework for reintroductions used in this Evaluation and Guidance document is based on the guidelines that were recommended in *Anadromous Salmonid Reintroductions: General Planning Principles for Long-Term Viability and Recovery* (McClure et al. 2018) that aim to design programs to establish or expand populations of salmonids. This Evaluation and Guidance document recommends taking the following steps before and post reintroduction:

- Establish the major goal(s) of the reintroduction effort (e.g., reduce risk of extinction of and contribute to the long-term recovery of the DPS);
- Select reintroduction site (described more in Section 2.2.3);

- Confirm the absence of *O. mykiss* at the potential reintroduction site using environmental DNA (eDNA);
- Confirm the suitability of the potential reintroduction site by measuring physical, chemical and biological properties (using Surface Water Ambient Monitoring Program [SWAMP] protocols);
- Select the donor population (described more in Section 2.2.4);
- Develop objectives that are measurable, time-limited, specific, and scientifically based to assist in realizing the goal(s) (e.g., objectives specific to abundance, productivity, spatial structure, and/or diversity of the reintroduced population);
- Identify quantifiable actions that establish monitoring of the objectives and the temporal expectations (e.g., a seasonal or annual monitoring program used to collect data for each objective);
- Identify biological risks and constraints (e.g., disease transmission, source population mining, invasion, excessive straying);
- Develop an exit strategy that determines when and how to halt reintroductions;
- Monitor the reintroduced population (described more in Section 2.6); and
- Adaptive management (described more in Section 2.7).

2.2.3 Reintroduction Site Selection

The following guidelines were used to prioritize watersheds for development of reintroduction guidance documents:

- Prioritize watersheds where *O. mykiss* have been extirpated;
- Prioritize watersheds that have low potential for natural recolonization (e.g., that are greater distances from extant populations);
- Prioritize watersheds with high-quality, perennial habitat that is accessible to steelhead;
- Prioritize sites where limiting factors to habitat, such as migration barriers, have either been addressed or are planned to be addressed; and
- Prioritize locations with limited negative ecological, social, and economic impacts.

In the case of reintroductions described herein, we considered the priority locations for reintroductions to be watersheds where *O. mykiss* have been extirpated. Reintroducing fish to a location where *O. mykiss* have been extirpated reduces biological risks associated with increased competition, the spread of pathogens, and potential negative effects from genetic mixing between the donor and recipient populations. The absence of *O. mykiss* should be confirmed prior to a reintroduction using eDNA surveys.

A key factor to consider is whether locations where *O. mykiss* have been extirpated have the potential to be naturally recolonized. We assume there is a relatively high likelihood of natural recolonization if there are existing *O. mykiss* either in habitat upstream of barriers or within separate tributaries of a watershed with no major barriers that prevent dispersal among tributaries. Based on the principles of metapopulation dynamics, we would also prioritize locations that are greater distances away from watersheds with existing *O. mykiss* populations, such as populations in the Santa Catalina Gulf Coast BPG. Garza et al. (2014) also found genetic distance between populations was associated with geographic distance, indicating reduced rates of dispersal with increasing distances. The following paragraph expands on mechanisms for natural recolonization when no existing steelhead populations occur within the same watershed (i.e., no ability for fish to disperse from upstream barriers or from tributaries) and further discusses how these mechanisms have been impacted by the effects of humans and climate change.

As described in Section 1.3, straying is thought to be an important evolutionary mechanism that helped maintain southern California steelhead populations, especially small, coastal populations (NMFS 2012). However, the historical rates of natural recolonization functioned across scales of natural changes in climate (e.g., glaciation) and natural disturbance regimes. Current rates of climate change and the severity and frequency of disturbance events are unprecedented, and it is questionable whether historical rates of recolonization will sufficiently offset extirpation rates to prevent extinction. Numerous factors would contribute to lower chances of natural recolonization under a fundamentally altered steelhead metapopulation that extends from southern to central and even northern California. First, low numbers of returning anadromous adults across the entire DPS (NMFS 2012, Dagit et al. 2020) combined with reduced population sizes within the South-Central California Coast DPS, Central California Coast DPS, and Central California DPS means there are fewer anadromous adults that could stray (not to mention straying may be more likely from hatchery fish; Keefer and Caudill 2014). Small, fragmented populations can lead to depensatory processes (e.g., Allee effects). For example, for steelhead to naturally recolonize an extirpated stream, two anadromous adults would need to locate and enter the same watershed within a similar time frame. These fish would then have to locate suitable spawning habitat and each other. While this certainly occurred historically and there is recent evidence of this (e.g., San Mateo and Topanga creeks were both recolonized in the 1990s), the probability has been greatly diminished due to the existence of fewer, smaller, fragmented populations.

Compounding these issues are other human disturbances, such as increased frequency of severe wildfires that extirpates populations at increased rates compared to pre-human conditions. The ideal solution would be to reduce human-caused wildfires (e.g., by burying transmission lines), restore connectivity by removing barriers, improve management of flows, and increase suitable habitat among other recovery needs. These recovery actions, which are the focus of the NMFS (2012) Recovery Plan and are necessary for recovery of the DPS, will take time to implement. The current rate of extirpation is an immediate threat to the Southern California Steelhead DPS. Hence, why we evaluate reintroductions as an immediate response that may be appropriate to meet the severity of the threat.

It is also important to select watersheds with few constraints on reintroduction that could limit the establishment of a long-term, viable steelhead population. For example, a reintroduction site could be prioritized because it contains high-quality habitat that is accessible for releasing and monitoring fish. Further, any threat(s) that caused extirpation need to have been identified and corrected to the extent possible. There are likely few sites where threats have been fully addressed throughout the DPS, but selecting sites where threats have been largely addressed will increase the likelihood of success. Streams with no downstream barriers to anadromy could also be prioritized and could lead to re-establishing an anadromous *O. mykiss* population in these watersheds. Locations that are upstream of barriers to anadromy should still be considered as sites for reintroduction and may require fewer permits compared to reintroductions into anadromous waters. Additional considerations include existing monitoring, funding, and social/cultural aspects, prioritization of sites with existing monitoring programs, funding sources, and social/cultural support for reintroduction.

2.2.4 Donor Population Selection

A donor population (also referred to as "source" or "founder" population) should be selected in coordination with regulatory agencies (i.e., CDFW and NMFS). Tradeoffs and specific considerations apply to selecting donor populations:

- Location (e.g., within the BPG versus outside the BPG versus outside the DPS);
- Environmental conditions;
- Transport distance;
- Genetics (e.g., native coastal steelhead versus hatchery lineages, genetic diversity);
- Donor population size;
- Permitting requirements;
- Access; and
- Social, cultural, and regulatory aspects.

Figure 1 provides a decision framework for selecting a donor population and the trade-offs are discussed below. For a more comprehensive discussion of donor population sources, we recommend reviewing Meek et al. (2014), which discusses genetic considerations for sourcing steelhead for reintroductions in the San Joaquin River in Central California. Salmonids can exhibit local adaptation to their environment (Taylor 1991) and genetic distance among southern California steelhead watersheds increases with increasing distance (Garza et al. 2014). Thus, an *O. mykiss* population within the BPG would be prioritized based on the assumption that environmental selection pressures would be similar between donor and recipient habitats and that fish from the donor population would possess local adaptations that are compatible with environmental characteristics within the recipient location. However, tradeoffs could exist if a within-BPG donor population has lower genetic diversity and less evolutionary potential (Weeks et al. 2011). If an appropriate population does not exist within the BPG, an *O. mykiss* population outside the BPG could be considered, but priority would be placed on a watershed that has similar environmental characteristics (e.g., water temperatures, migration conditions) to the recipient site. Only a population that is composed of individuals reflecting a native coastal steelhead lineage (or with limited hatchery introgression) and is large enough to accommodate the removal of individuals would be selected.

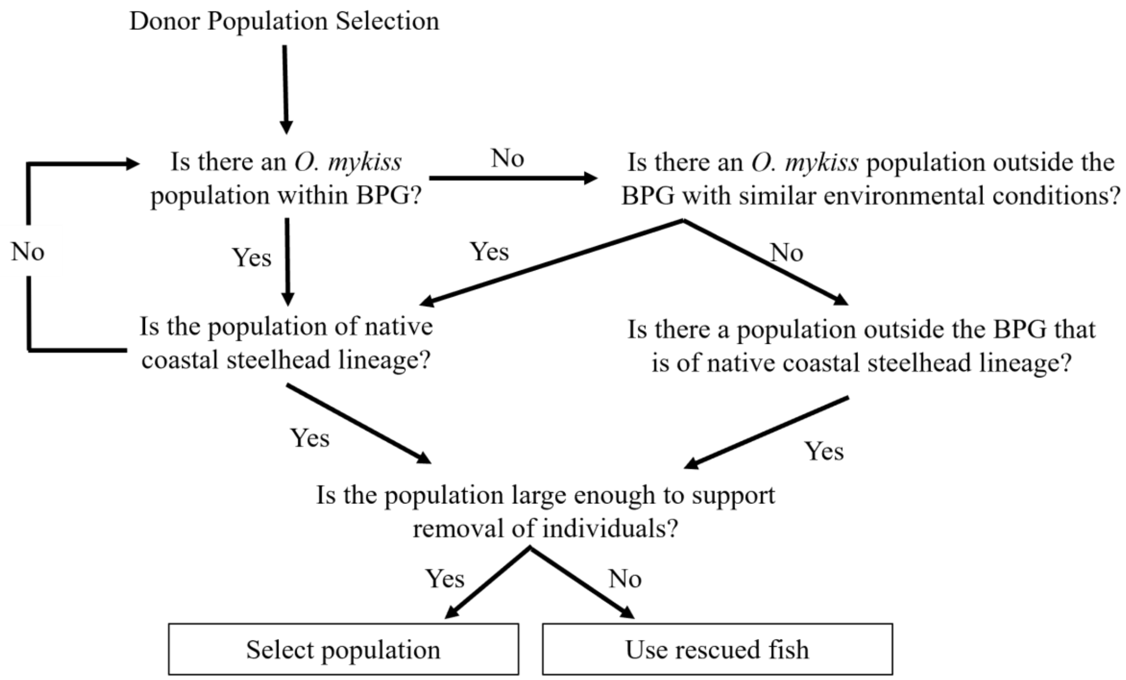


Figure 1. Decision framework for selecting a donor *O. mykiss* population for reintroductions. Boxes around text indicate decision endpoint. This figure was developed based on information presented in Anderson et al. (2014) and NOAA (2018).

It is likely that most existing populations in anadromous waters are not currently large enough to sustain removal of fish. A potential donor source from within a BPG could be from rescued fish. Fish rescued from a watershed would effectively have been lost from the population if not rescued; hence, rescuing the fish eliminates the risks of population mining other than what would have occurred without intervention. Rescue fish could come from Programmatic Rescues or Isolated Rescues when conditions are not suitable for releasing fish back into their natal watershed. Risks related to using rescued fish for reintroductions include disease/pathogen introduction and genetic founder effects. In addition, as mentioned previously, fish rescue is a form of artificial selection, and rescued fish could pass along potentially maladapted traits to the new population (i.e., they would be rewarded for a behavior that would have been selected against). However, fish in need of rescue are often being prevented from expressing life history variability or are being directly threatened by human actions, including water management practices, barriers, and climate-related factors. The risk of promoting maladaptive traits could be mediated by only using fish rescued from definitive circumstances when rescues were a result of human actions.

Figure 1 provides the basic decision framework, but other information should be considered when selecting a donor population including distance between donor and recipient sites, accessibility, and social, cultural, and regulatory factors. For example, a population would be prioritized in the same or closest neighboring watershed, when possible, to reduce transport time. Another consideration is whether the population is upstream or downstream of barriers to anadromy. Populations that are downstream of barriers to anadromy are listed under federal ESA and a candidate species under the state ESA, which could result in regulatory challenges, but these populations have access to the ocean and may express an anadromous life-history that is a specific focus of recovery.

Conversely, populations that are upstream of barriers to anadromy are not protected under either the state or federal ESA. These populations could have been subjected to strong selection against anadromy and could have increased genetic introgression from hatchery stocks (Pearse et al. 2014, NMFS 2023). However, genetic studies have shown that resident *O. mykiss* populations that upstream of barriers in many locations is southern California are largely of native coastal steelhead ancestry (Clemento et al. 2009), and these populations, while having reduced proportions of the haplotype associated with anadromy compared to populations that are downstream of barriers, still retain genetics associated with anadromy (Pearse et al. 2014, Pearse et al. 2019, Apgar et al. 2017). Finally, in southern California, *O. mykiss* populations that are upstream of barriers to anadromy tend to be larger than populations that are downstream of barriers because of better habitat conditions upstream of barriers. Therefore, a population that is upstream of barriers to anadromy could be capable of providing larger numbers of individuals for reintroductions compared to populations that exist downstream of barriers to anadromy, could reduce regulatory burdens, and ultimately would eliminate risks of mining small populations in anadromous waters.

The use of steelhead populations from outside the Southern California Steelhead DPS are not considered herein as a viable option due to numerous potential genetic risks, and due to uncertainty whether fish from more northern populations would be able to acclimation/adapt to conditions, such as high temperature, in southern California streams; although, rapid adaptation to local conditions is possible in salmonids (Hendry et al. 2000). We emphasize that all decisions regarding donor population selection would need to be in coordination with, and with support from, regulatory agencies.

2.2.5 Reintroduction Numbers

Determining the appropriate number of individuals to reintroduce is a challenge. Reintroduction numbers will invariably depend on the donor population source/s and size. It has been suggested that a minimum of 50 individuals of equal sex ratio is needed in a controlled setting (Williams et al. 1988), but it has been shown that smaller numbers of reintroduced fish can produce a viable population. *O. mykiss* have high fecundity and one female can produce 3,500 eggs or more (McEwan and Jackson 1996). Thus, successfully spawning of even a few individuals can seed large amounts of habitat. The main concern with the reintroduction of a small number of individuals is the potential for reduced genetic diversity, inbreeding depression, genetic drift, and founder effects that can by chance carry deleterious adaptations or may not provide sufficient genetic diversity for natural selection to act upon within the new environment. Larger numbers of reintroduced individuals would increase genetic diversity and increase the likelihood of long-term viability of the reintroduced population (Fischer and Lindenmayer 2000, Robert et al. 2007), but even under a natural recolonization, only a few individuals would be responsible for creating a new population. Once a reintroduction was implemented, reinforcement translocations over time could be used to increase genetic diversity and reduce risks of founder effects.

Another consideration for the number of individuals to reintroduce is the effect on the donor population. Generally, the goal should be to reintroduce as many fish as possible without having negative impacts on the donor population and while avoiding overstocking within the new habitat. Negative effects to a donor population could be reduced by taking advantage of natural population processes. For example, fry are typically over abundant during early summer and could be removed from the population with little impact to the demographics of donor populations because these fish would either not survive or would emigrate under natural conditions. Given the small *O. mykiss* population sizes within the Southern California Steelhead DPS, it may take multiple reintroduction events from different populations over multiple years to achieve successful reintroduction of a genetically diverse, productive, and viable population. If possible, the population of reintroduced fish should reflect the size and age class range from the donor population. The use of rescued fish for reintroduction would influence the number of fish available and effectively eliminate effects on a donor population. Overall, the number of fish to reintroduce will be specific to the situation and should be determined within future planning.

2.2.6 Pre-action Survey

Prior to a reintroduction, fish and fish habitat surveys at accessible recipient sites should be completed to confirm *O. mykiss* are absent (or are at very low densities to prevent over-seeding) and to identify release locations that are accessible and contain sufficient, suitable habitat (both quality and quantity) to support long-term viability of translocated *O. mykiss*. Defining "suitability" of habitat should be ideally based on physical, chemical, and biological measurements within the reintroduction sites; however, development of habitat suitability models for streams in southern California would provide more quantitative means of determining locations with suitable habitat. Prior to the availability of such models, it will be necessary to rely on more broadly available habitat suitability characteristics.

Prior to reintroduction, an additional survey/s should be conducted in the fall and spring to confirm these locations remain accessible and suitable for *O. mykiss*. Fall surveys provide information on rearing and resident habitat availability during low flows, a limiting time for *O. mykiss* populations due to decreased surface flow, whereas spring surveys can confirm suitable habitat availability following winter high-flow events that can reset stream morphology and physical conditions. Surveys should evaluate habitat suitability using the inventory methods described in the *California Salmonid Stream Habitat Restoration Manual* (Flosi et al. 2010) or another, appropriate alternative. Surveys may also be required to understand barriers and fish passage conditions in the receiving watershed. Fish surveys at the donor site also can be used to determine appropriate fish densities to establish at a similar recipient site and can confirm the numbers of fish that can be safely removed without harming the donor population.

2.2.7 Reintroduction Timing and Scale

Fish would be collected from donor populations (or from fish rescues) after all necessary permits have been acquired and pre-action surveys have been conducted to assess habitat suitability at the recipient site. If using rescued fish as the donor source, the timing of reintroductions would likely occur in the summer or fall when conditions are limiting at the rescue location. If using other donor sources, reintroductions could be implemented during the spring (May or June) when conditions (e.g., temperature and DO) are likely suitable for fish at both the donor and recipient sites. Although reintroduction in the spring could expose fish to a bottleneck during the dry season, habitat surveys could ensure year-around suitable habitat at the reintroduction location. An alternative strategy could be to release fish in the late fall when temperatures are more suitable, but there would likely be fewer individuals available within the donor population during this time because *O. mykiss* numbers tend to be highest in the spring owing to the presence of young-of-the-year (age 0) fish. High numbers of young fish in the spring will increase collection efficiency and reduce risks associated with removal of individuals from the donor population, but young-of-the-year fish may be more susceptible to stressors associated with capture, handling, and transport. This timing reflects a point in the life cycle when they are moving from the redd out into the stream to establish territories. Thus, moving them at this time to vacant habitat is more consistent with their natural life history. The protocols for fish collection, transport, and release are presented in Section 2.5.

2.3 Temporary Holding Facility

A temporary holding facility (facility) could be used to hold native *O. mykiss* with the objective of protecting existing *O. mykiss* populations and maintaining steelhead populations at viable levels across the Southern California Steelhead DPS. Such a facility could also serve as a temporary holding facility for fish prior to reintroduction to screen for diseases or assess viability. A facility could act as mitigation for fish stranding and for sustaining the DPS through small population and disturbance bottlenecks. Short-term (weeks to months) holding of rescued *O. mykiss* could increase the survival of individuals that could otherwise experience high mortality rates if there were no interventions. Fish held or reared in the facility would be released back into natal watersheds or streams where *O. mykiss* have been extirpated. A facility should not be conflated with a conservation hatchery program because there would be no propagation of fish within a temporary holding facility. A facility would not be used for long-term holding unless deemed necessary for research or other purposes.

An example of how a holding facility can be applied within the context of rescue is the Holy Fire that impacted Coldwater Canyon Creek (a tributary to the Santa Ana River). Following the Holy Fire, *O. mykiss* were rescued from Coldwater Canyon Creek prior to the first major storm and were moved to the Mojave Hatchery, and then to Marion Creek to rear (NMFS 2023). Eventually, the fish were returned to Coldwater Canyon Creek (NMFS 2023).

A facility could be modeled after the Sleepy Hollow Steelhead Rearing Facility that is used by the MPWMD as part of the RRMP (previously discussed in Section 2.1.2; MPWMD 2018). The MPWMD rears juvenile wild steelhead as recommended by NMFS for the management and operation of conservation hatcheries (Flagg and Nash 1999). The Sleepy Hollow Steelhead Rearing Facility is not a hatchery because it does not propagate fish. Instead, it simply rears fish rescued from native habitat for brief periods. Individuals are released back into the watershed once conditions are suitable (MPWMD 2018). Since it began in 1990, the Carmel River RRMP has rescued more than 769,000 individuals, substantially contributing to the maintenance of the Carmel River steelhead population over time (Boughton and Ohms 2022). Anywhere between approximately 1,000 to 100,000 individuals are brought into the holding facility annually (MPWMD 2018).

The goal of the MPWMD RRMP is to match or exceed survival, condition, and growth rates of wild fish reared in the Carmel River and has likely done so in all but 2 years between 1994 and 2012 (MPWMD 2018). Annual survival rates have trended upward due to facility upgrades and updated protocols (MPWMD 2018). The Carmel River RRMP outlines specific compliance "Performance Standards" that must be met (MPWMD 2018). A facility in southern California could follow similar "Performance Standards" as described within MPWMD (2018).

A holding facility could act as a straightforward, temporary solution to hold population(s) of southern California steelhead if a watershed has unsuitable conditions for fish. This option could lead to rescues of populations that may otherwise be extirpated due to inaction. Rescued populations could be held in a controlled setting, which could make screening for diseases and genetics feasible and easier than in a wild setting. In addition, capturing, holding, and moving the population back to the wild would be feasible after suitable habitats are identified, whether it is reintroducing the fish back into the habitat from which they were rescued or into a new watershed.

The major risk to a temporary holding facility is the potential for domestication selection from rearing in non-wild conditions (Naise et al. 2007, Larsen et al. 2019). Essentially, natural selection is relaxed within a controlled environment, and fish that were exhibiting poor traits (e.g., fish in in need of rescue may possess maladaptive behavioral traits) can be rewarded through increased growth. Fish that are held and released could then compete and breed with wild fish, potentially reducing population-level fitness if reared fish pass on maladaptive traits. In addition, developmental plasticity due to rearing environment can affect metabolism, growth, and behavioral life history traits of held fish that may not be beneficial in the wild environment (Gavery et al. 2019). The potential for domestication selection is similar to what could occur because of a conservation hatchery program, which is discussed in more detail in Section 3.6, but would be much less intense since it only involves one life-stage of the fish and no mate selection.

Held fish may become stressed or even exposed to conditions that may lead to mortality in a holding facility. To avoid these risks, fish held in facilities should be introduced or reintroduced back to the wild as soon as possible. Infectious diseases could affect held populations, and the diseases that occur within facilities generally have a higher prevalence or intensity than within wild environments potentially due to higher densities, levels of stress, and poorer water quality (Naish et al. 2007). In addition, the inflow of water into a facility may contain pathogens that could infect fish that would otherwise not be exposed (Naish et al. 2007). Infectious diseases within effluent could be introduced into the receiving waters and could affect wild fish that are present (Naish et al. 2007). Within small watersheds, large facilities could alter the receiving water (e.g., temperature, phosphorous, and organic water discharged), which could also negatively affect wild fish populations. However, many of these risks can be mitigated through careful planning and proper management of operations.

Currently, no facility in southern California is used for or can meet the objectives outlined above with one notable exception. The Riverside-Corona Resource Conservation District (RCRCD) operates two facilities, Greenbelt and Glenwood facilities, with the goal of providing mitigation for impacts to native fish habitat, including *O. mykiss*. These facilities are used to hold, rear, and study native fishes, as well as provide holding for rescued fish that are extirpated from their native habitat due to catastrophic events. These facilities are designed to mimic habitat conditions in streams where native fish will eventually be released, and the facilities have been used in numerous reintroduction projects for native fish since 2000. A few additional options for temporary holding facilities to support *O. mykiss* rescues and reintroductions are described hereafter.

The first option would be to upgrade an existing facility. Hatchery facilities are currently used in southern California for various reasons, but these hatcheries would need to be upgraded and converted to a rearing facility capable of holding rescued *O. mykiss* under natural conditions. The Fillmore Fish Hatchery is located along the Santa Clara River; however, this facility is designed to rear hatchery strain rainbow trout and nonnative brown trout (*Salmo trutta*) that will ultimately be planted in non-anadromous waters for recreational fishing (CDFW 2023a). This facility is currently in the planning/permitting stages for being able to accept/hold southern California steelhead (R. Burg. CDFW, pers. comm., 28 February 2024). This option would likely be expensive and time intensive to construct, and it could be difficult to permit. Alternatively, a holding facility, such as a rearing channel located adjacent to a perennial section of the watershed, could be designed to be minimally invasive within the watershed. However, this alternative may also be difficult to permit and expensive to construct. To reduce transport distances and increase rearing capacity, multiple temporary holding facilities across southern California may be required to meet demands. Similar to the holding facility used on the Carmel

River, mitigation from water users could be source of funding for these facilities in southern California.

2.4 Permitting

Coordination with local stakeholders, biologists, regulators, and managers is a critical step prior to initiating a plan for the rescue or reintroduction of steelhead and well before submitting any permit applications. At a minimum, conservation plan development and implementation requires coordination with NMFS and CDFW, but coordination with the U.S. Department of the Interior, Fish and Wildlife Service (USFWS) and the U.S. Department of Agriculture, Forest Service (USFS) may also be needed, depending on the location and the presence of federally listed non-anadromous species. We outline the various permits that may be required or that should be considered during the development of a rescue or reintroduction plan in Table 1. Prior to permit applications, a final rescue or reintroduction plan developed in close coordination with appropriate agencies needs to be developed. The permitting process for these actions (especially for reintroductions) is expected to be lengthy, highlighting the importance of inter-agency collaboration.

Table 1. Potential permitting strategy to support activities related to this Evaluation and Guidance document.

Regulation or permit	Agency	Agency type	Trigger for regulation	Process and requirements	Estimated time from application to permit, approval or issuance	Fee payment
Scientific Collecting Permit	CDFW	State	Required when take[1] or possession of fish and wildlife occurs for research, educational, or propagation purposes.	Scientific Collecting Permits (ca.gov)	Scientific Collecting Permits (ca.gov)	Scientific Collecting Permits (ca.gov)
CESA Permit	CDFW	State	If a species is listed under both the federal Endangered Species Act and the California Endangered Species Act (CESA).	Fish and Game Code Section 2080.1 allows an applicant who has obtained a federal incidental take statement (federal Section 7 consultation) or a federal incidental take permit (federal Section 10(a)(1)(B)) to request that the Director of CDFW find the federal documents consistent with CESA. If the federal documents are found to be consistent with CESA, a consistency determination (CD) is issued, and no further authorization or approval is necessary under CESA. However, a minimum 30-day wait will often be too long of a process when trying to rescue stressed or stranded fish.	30-day timeline	FileHandler.ashx (ca.gov)
USFWS Biological Opinion	USFWS	Federal	Potential impacts to USFWS federally listed species or critical habitat.	1. Informal consultation 2. Review 3. Determination 4. Formal consultation 5. Conclusion of BO 6. ESA Section 7 Consultation | U.S. Fish & Wildlife Service (fws.gov)		
NMFS ESA	NMFS	Federal	Required for any “take” of an endangered or threatened species incidental to, and not the purpose of, an otherwise lawful activity.	Take permit		
USFS special use permit	USFS	Federal	Needed to occupy, use, or build on National Forest System land for personal or business purposes, whether the duration is temporary or long term.	How Do I Apply For A Special-use Permit | US Forest Service (usda.gov)		

[1] Take is defined in Section 86, California Fish and Game Code as “hunt, pursue, catch, capture, or kill, or attempt to hunt, pursue, catch, capture, or kill.” Additionally, Section 650, Title 14, California Code of Regulations clarifies that takes also includes “capturing, marking, and releasing any animal.”

2.5 Fish Collection/Transport/Release Protocols

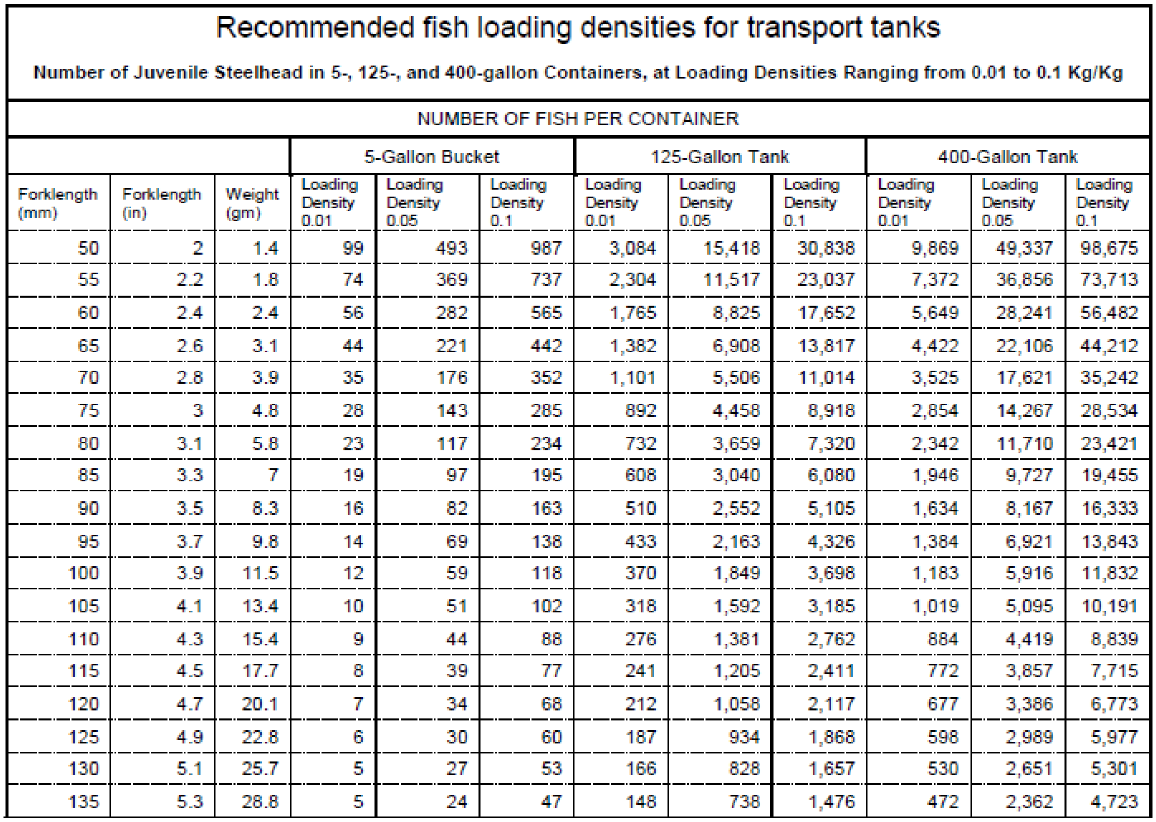
A general discussion of fish collection, transport, and release protocols is provided in this report and should be refined based on location, timing, and level of effort. Briefly, backpack electrofishing, seines, and/or in-stream traps would be used to collect fish from rescue or donor sites. Electrofishing should be conducted adhering to the recommendations in the NMFS (2000) electrofishing guidelines and the modifications for Southern California streams as written in the Programmatic Consultation on Funding and Permitting Restoration Projects within watersheds of San Luis Obispo, Santa Barbara, Ventura, Los Angeles, Orange and San Diego Counties, California (NMFS 2015) and may involve multiple passes within the same reach. To increase the efficiency of fish capture, electrofishing can be combined with seining. The target number of fish to remove for a reintroduction will be site specific and determined as outlined in Section 2.2.

Fish would be collected from the reach and immediately placed in a 5-gallon bucket (or larger) with battery-operated aerators to maintain adequate DO. All fish >65 millimeter (mm) could be injected with a Passive Integrated Transponder (PIT) tag to identify individuals and track survival, movements, and growth following release. However, if fish are observed or assumed to be in a state of stress (e.g., the individual is discolored, shows signs of infection, surface water temperature was >68°F at the rescue site, DO was <6 milligrams per liter (mg/L) at the rescue site), PIT tagging would not be conducted. The in-stream water would be tested for water quality. If adequate, the buckets would be filled with water from the river at the rescue location. Temperature and DO requirements would be maintained by refreshing water if needed to prevent sublethal or lethal effects (optimum range between 50–60°F and 6–8 mg/L). Data for collected fish would include location, quantity of fish, weight and fork length of captured individuals, density, number of mortalities, and potentially other pertinent information, such as environmental measurements at the collection site. Scale and fin (caudal) tissue samples would be collected for use in age and genetic analyses, respectively. Genetic analyses could include ancestry (native versus hatchery) identification, presence of the Omy5 haplotypes, heterozygosity, and parentage studies.

Following collection and data collection, fish would be transported to either a release site or a temporary holding facility. Release locations would be selected ahead of time per Sections 2.1 and 2.2. Table 2 lists suggested fish loading densities for transport tanks, whose size would be determined by the number of fish and distance transported (Table 2 [Table 6-2 from MPWMD 2018]). In addition, fish would be separated by size to prevent predation. The fish transport vehicle would travel from the collection site to the release site as efficiently as possible. Temperature and DO would be monitored and recorded before, during, and after transport.

Fish would be exposed to temperature and salinity levels similar to the recipient site prior to release per MPWMD (2018) recommendations. Fish would be transferred to holding pens within the stream at release locations and allowed to recover (e.g., normal ventilation rates, orientation, and reflexes) and released at night to reduce predation risk. If the fish were released in a stream in the same watershed from which it was collected, the release should occur on the same day. If different ages of fish are collected, the largest fish could be released in the downstream-most habitats. Before release, each fish would be visually examined for life-threatening wounds or disease and culled if it suffers from these maladies.

Table 2. Recommended fish loading densities for transport tanks. Source: Table 6-2 from MPWMD (2018).

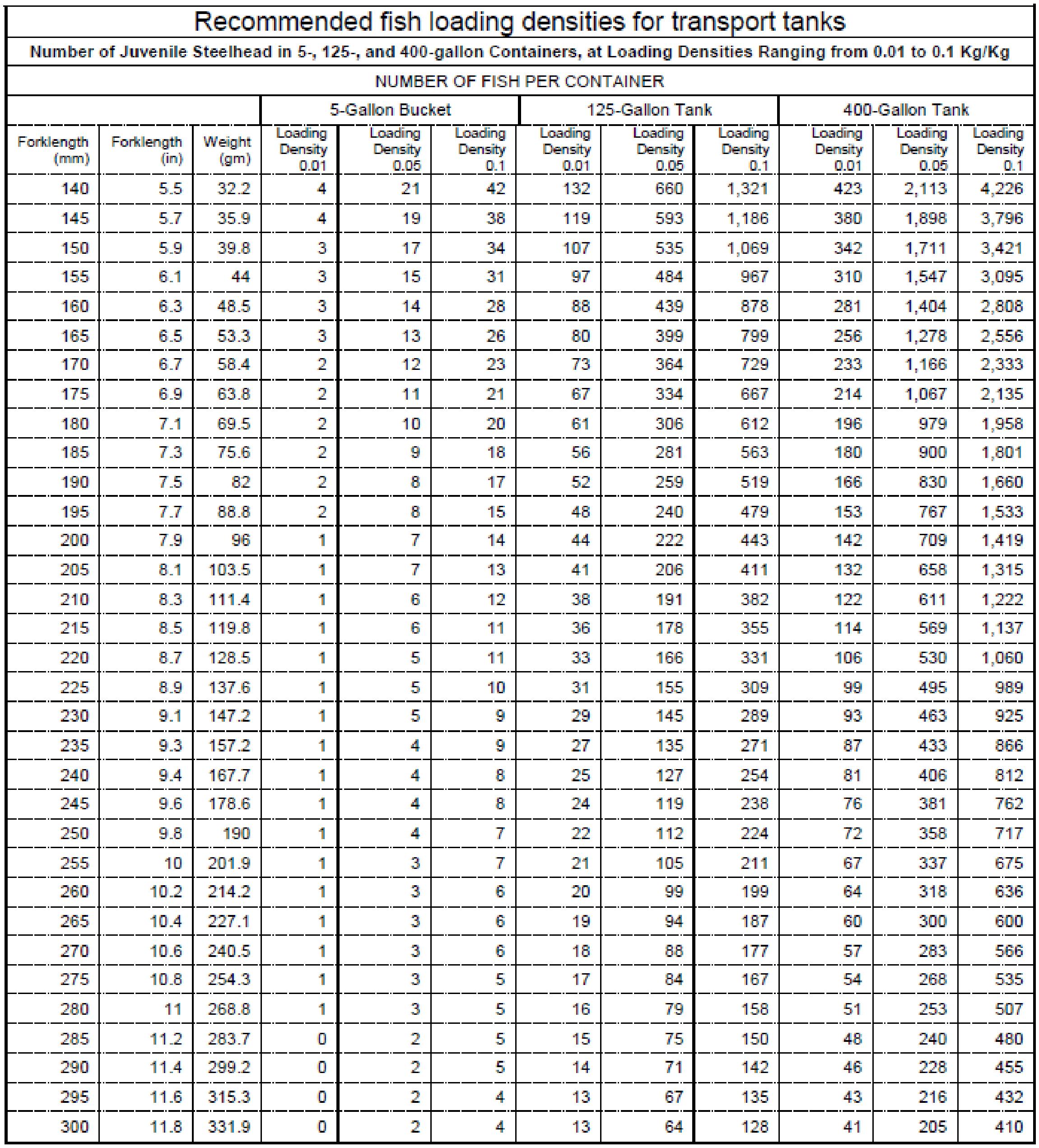


Recommended fish loading densities for transport tanks

Number of Juvenile Steelhead in 5-, 125-, and 400-gallon Containers, at Loading Densities Ranging from 0.01 to 0.1 Kg/Kg

NUMBER OF FISH PER CONTAINER

			5-Gallon Bucket			125-Gallon Tank			400-Gallon Tank		
Forklength (mm)	Forklength (in)	Weight (gm)	Loading Density 0.01	Loading Density 0.05	Loading Density 0.1	Loading Density 0.01	Loading Density 0.05	Loading Density 0.1	Loading Density 0.01	Loading Density 0.05	Loading Density 0.1
50	2	1.4	99	493	987	3,084	15,418	30,838	9,869	49,337	98,675
55	2.2	1.8	74	369	737	2,304	11,517	23,037	7,372	36,856	73,713
60	2.4	2.4	56	282	565	1,765	8,825	17,652	5,649	28,241	56,482
65	2.6	3.1	44	221	442	1,382	6,908	13,817	4,422	22,106	44,212
70	2.8	3.9	35	176	352	1,101	5,506	11,014	3,525	17,621	35,242
75	3	4.8	28	143	285	892	4,458	8,918	2,854	14,267	28,534
80	3.1	5.8	23	117	234	732	3,659	7,320	2,342	11,710	23,421
85	3.3	7	19	97	195	608	3,040	6,080	1,946	9,727	19,455
90	3.5	8.3	16	82	163	510	2,552	5,105	1,634	8,167	16,333
95	3.7	9.8	14	69	138	433	2,163	4,326	1,384	6,921	13,843
100	3.9	11.5	12	59	118	370	1,849	3,698	1,183	5,916	11,832
105	4.1	13.4	10	51	102	318	1,592	3,185	1,019	5,095	10,191
110	4.3	15.4	9	44	88	276	1,381	2,762	884	4,419	8,839
115	4.5	17.7	8	39	77	241	1,205	2,411	772	3,857	7,715
120	4.7	20.1	7	34	68	212	1,058	2,117	677	3,386	6,773
125	4.9	22.8	6	30	60	187	934	1,868	598	2,989	5,977
130	5.1	25.7	5	27	53	166	828	1,657	530	2,651	5,301
135	5.3	28.8	5	24	47	148	738	1,476	472	2,362	4,723



Recommended fish loading densities for transport tanks

Number of Juvenile Steelhead in 5-, 125-, and 400-gallon Containers, at Loading Densities Ranging from 0.01 to 0.1 Kg/Kg

NUMBER OF FISH PER CONTAINER

			5-Gallon Bucket			125-Gallon Tank			400-Gallon Tank		
Forklength (mm)	Forklength (in)	Weight (gm)	Loading Density 0.01	Loading Density 0.05	Loading Density 0.1	Loading Density 0.01	Loading Density 0.05	Loading Density 0.1	Loading Density 0.01	Loading Density 0.05	Loading Density 0.1
140	5.5	32.2	4	21	42	132	660	1,321	423	2,113	4,226
145	5.7	35.9	4	19	38	119	593	1,186	380	1,898	3,796
150	5.9	39.8	3	17	34	107	535	1,069	342	1,711	3,421
155	6.1	44	3	15	31	97	484	967	310	1,547	3,095
160	6.3	48.5	3	14	28	88	439	878	281	1,404	2,808
165	6.5	53.3	3	13	26	80	399	799	256	1,278	2,556
170	6.7	58.4	2	12	23	73	364	729	233	1,166	2,333
175	6.9	63.8	2	11	21	67	334	667	214	1,067	2,135
180	7.1	69.5	2	10	20	61	306	612	196	979	1,958
185	7.3	75.6	2	9	18	56	281	563	180	900	1,801
190	7.5	82	2	8	17	52	259	519	166	830	1,660
195	7.7	88.8	2	8	15	48	240	479	153	767	1,533
200	7.9	96	1	7	14	44	222	443	142	709	1,419
205	8.1	103.5	1	7	13	41	206	411	132	658	1,315
210	8.3	111.4	1	6	12	38	191	382	122	611	1,222
215	8.5	119.8	1	6	11	36	178	355	114	569	1,137
220	8.7	128.5	1	5	11	33	166	331	106	530	1,060
225	8.9	137.6	1	5	10	31	155	309	99	495	989
230	9.1	147.2	1	5	9	29	145	289	93	463	925
235	9.3	157.2	1	4	9	27	135	271	87	433	866
240	9.4	167.7	1	4	8	25	127	254	81	406	812
245	9.6	178.6	1	4	8	24	119	238	76	381	762
250	9.8	190	1	4	7	22	112	224	72	358	717
255	10	201.9	1	3	7	21	105	211	67	337	675
260	10.2	214.2	1	3	6	20	99	199	64	318	636
265	10.4	227.1	1	3	6	19	94	187	60	300	600
270	10.6	240.5	1	3	6	18	88	177	57	283	566
275	10.8	254.3	1	3	5	17	84	167	54	268	535
280	11	268.8	1	3	5	16	79	158	51	253	507
285	11.2	283.7	0	2	5	15	75	150	48	240	480
290	11.4	299.2	0	2	5	14	71	142	46	228	455
295	11.6	315.3	0	2	4	13	67	135	43	216	432
300	11.8	331.9	0	2	4	13	64	128	41	205	410

2.6 Effectiveness Monitoring

Monitoring is critical to evaluate the effectiveness of the conservation actions described herein. Monitoring should be used to evaluate survival, movements, growth, and abundance of rescued or reintroduced fish after their release. Invariably, monitoring approaches and goals will differ between rescue and reintroductions and will be highly site specific due to logistical and financial constraints. Generally, monitoring for survival, movements, growth, and abundance of *O. mykiss* should occur within the release locations. Monitoring the effects of fish removal on donor populations is also essential. Different monitoring approaches are described in the following section.

2.6.1 Monitoring Approaches

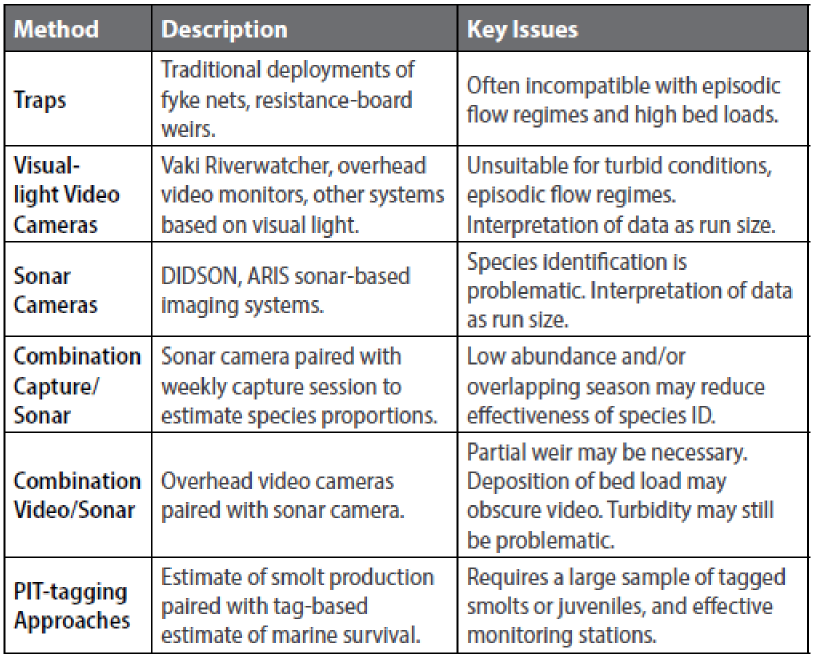
Various monitoring methods may be used to evaluate the effectiveness of the action based on project goals and are discussed further below. Generally, monitoring should include quantitative methods to determine population abundance, growth rates, dispersal, habitat use, fitness, genetic diversity, and ecological impacts (George et al. 2009). Monitoring should align with California's CMP outlined in Fish Bulletin 182 (Boughton et al. 2022) so that collected data and estimated metrics are consistent across individual projects and also with the overall monitoring effort conducted by the CDFW CMP. This involves selecting data-collection sites via a sample frame and rotating-panel design that has been set up by local CDFW biologists. The duration and extent of monitoring will be determined by rescue/reintroduction goals, but monitoring should be conducted for at least 5 years after the release period but could continue over a decade to ascertain the long-term effects of the rescue or reintroduction.

Abundance, spatial structure, and diversity within the donor and release sites and across adjacent reaches and/or the entire watershed should be determined as outlined in the CMP (e.g., using methods such as counting stations, low-flow surveys, snorkel surveys, redd surveys, redd assignments, and genetic sampling). Counting stations combined with low-flow surveys or redd surveys and redd assignment may be used to determine abundance in a system (Boughton et al. 2022). Electrofishing, snorkel surveys, or a combination of both may be used during the low-flow period (i.e., summer or fall) to determine distribution, density, and genetic diversity of *O. mykiss* in a system (Boughton et al. 2022). Seasonal, annual, or extended time series abundance estimates can be used to determine the effectiveness of translocations in meeting recovery goals.

Low-flow surveys during the summer/fall may be used to inform spatial structure in a system and may include electrofishing, snorkel surveys, or a combination of both (Boughton et al. 2022). Detailed electrofishing methods are described in Temple and Pearsons (2007) and Boughton et al. (2022). Advantages of electrofishing include directly handling fish and more accurately identifying fish, while disadvantages include potential impacts on individuals (e.g., stress, injury, or mortality), ineffective use in deep water, and potential constraints due to water quality (Boughton et al. 2022). Detailed snorkel survey methods are described in O'Neal (2007) and Boughton et al. (2022). Advantages of snorkel surveys include lower impacts on aquatic vertebrates and habitat, effective use in deeper pools, simplicity of implementation, and fewer water quality restrictions. Disadvantages include lower fish detection rates, potential constraints due to elevated turbidity, potential for health risks to crews due to polluted surface water, and no direct handling of individuals, which limits the type of data that can be collected.

A combination of counting stations and low-flow surveys (as described above) or redd surveys and redd assignment as described in Boughton et al. (2022) may be used to monitor abundance. A description of potential methods and disadvantages to counting stations is displayed in Table 3 (Table 11 from Boughton et al. 2022). Another disadvantage is the extensive labor required to operate counting stations.

Table 3. Potential methods and disadvantages to different types of counting stations in southern California (Table 11 from Boughton et al. 2022).



Method	Description	Key Issues
Traps	Traditional deployments of fyke nets, resistance-board weirs.	Often incompatible with episodic flow regimes and high bed loads.
Visual-light Video Cameras	Vaki Riverwatcher, overhead video monitors, other systems based on visual light.	Unsuitable for turbid conditions, episodic flow regimes. Interpretation of data as run size.
Sonar Cameras	DIDSON, ARIS sonar-based imaging systems.	Species identification is problematic. Interpretation of data as run size.
Combination Capture/ Sonar	Sonar camera paired with weekly capture session to estimate species proportions.	Low abundance and/or overlapping season may reduce effectiveness of species ID.
Combination Video/Sonar	Overhead video cameras paired with sonar camera.	Partial weir may be necessary. Deposition of bed load may obscure video. Turbidity may still be problematic.
PIT-tagging Approaches	Estimate of smolt production paired with tag-based estimate of marine survival.	Requires a large sample of tagged smolts or juveniles, and effective monitoring stations.

Protocols for redd surveys are described in Gallagher and Gallagher (2005) and Gallagher et al. (2007), and detailed methods for both redd surveys and redd assignments are described in Boughton et al. (2022). Advantages of the redd survey and assignment monitoring route are the high potential for incidental data collection on spawning distribution and spawning habitat and ease of implementation in smaller watersheds. Disadvantages include impacts on habitat, difficulty in collecting information in a large watershed, and difficulty in identifying smaller redds.

Continuous temperature and DO monitoring (e.g., using loggers or sondes in pools) could be used to supplement physio-chemical measurements made during surveys. Data on sand bar breach events may be useful if determining the times and durations of steelhead migration between the lagoon and the ocean.

During fish handling, tissue samples may be gathered via caudal fin-clip for genetic analysis (Boughton et al. 2022). Genetic monitoring may be considered to confirm lineage (hatchery versus coastal steelhead), monitor genetic diversity (to look for evidence of founder effects, inbreeding depression), and look for presence of Omy5 haplotypes. Parentage studies may allow calculations of effective population size and the reproductive contributions of rescued and reintroduced individuals to recipient populations over time.

The survey design would be developed based on considerations such as the action taken (rescue versus reintroduction), physical and environmental conditions in the watershed (e.g., size, water quality, flow, habitat types), available resources (e.g., entity leading survey crew(s), funding, what data collection is already being conducted in the watershed), accessibility (e.g., how accessible it is for crew(s) to access different locations in the watershed, land owner access), and other factors (e.g., considerations resulting from agency discussions, permitting, safety concerns).

Surveys frequency would be developed based on the watershed. Other quantitative methods or survey goals are listed in the abbreviated bulleted list below:

2.6.1.1 Population presence/migration

- PIT antenna arrays
- Outmigrant trapping
- DIDSON (sonar)
- Upstream migrant trapping
- eDNA
- Radio or acoustic telemetry

2.6.1.2 Environmental conditions

- Physio-chemical monitoring of fish habitat
- Continuous temperature and DO monitoring using data loggers

2.6.1.3 Genetics

- Confirm lineage (hatchery versus coastal steelhead)
- Monitor genetic diversity (for evidence of founder effects, inbreeding depression)
- Determine Omy5 haplotypes and ratios of heterozygotes and homozygotes
- Parentage studies
  - Reproductive contributions of rescued and reintroduced individuals to recipient populations over time
  - Estimates of effective population size

2.7 Adaptive Management

The monitoring described above is intended to collect data that will guide decisions under an adaptive management framework. The purpose of the adaptive management framework is to identify specific triggers or criteria for increasing or decreasing the frequency and numbers of fish rescued/reintroduced and to develop strategies dealing with favorable or unfavorable outcomes. We would recommend convening a Technical Advisory Committee (TAC) to evaluate the effects of rescues/reintroductions as part of the adaptive management framework (applications of a TAC is considered within Section 4).

Rescues/reintroductions would cease when the following criteria are met:

- An abundant and viable population is established in recipient waters. This will be location-specific, but could include:
  - Recorded natural densities observed within suitable habitat of all life history stages (based on snorkel or backpack electrofishing surveys).
- There is evidence that rescues/reintroductions are ineffective, including:
  - Low survival, high disease rates, or low growth rates observed within the rescued/reintroduced population.
  - Absence of natural reproduction/one or more life history stages.

  - Population numbers decrease in the recipient habitat.
  - Population numbers remain stable despite increased rescues/reintroductions.
  - For reintroductions, there are negative impacts on the donor population (which in this case, alternative donor populations could be considered).

Recues/reintroductions will continue when:

- There is evidence that rescues/reintroductions are effective, but an abundant and viable population has not yet been established:
  - The density is increasing but has not yet reached densities predicted for the extant environmental conditions (based on snorkel or backpack electrofishing surveys).

3 PART 3 — GENETIC CONSERVATION OPPORTUNITIES

3.1 Introduction

Steelhead in southern California are at the southernmost range limit for the species, and they evolved in streams with highly variable conditions, most notably, high temperatures, and flashy and intermittent surface water. It is thought that steelhead inhabiting streams in their southern range possess genetic adaptations and life history variability that allow them to persist within extreme environments. For example, steelhead in southern California have been shown to have higher temperature tolerances compared to more northern populations with steelhead being capable of tolerating temperatures as high as 29°C (Sloat and Osterback 2013). It is uncertain whether higher temperature tolerances of southern steelhead are due to adaptation or acclimation, but the preservation of southern California steelhead genetics may be key for ensuring more northern populations are capable of developing adaptations to a warming (and more variable) environment. Other adaptations to local conditions in southern California, such as extreme high flow events, drought, intermittent flows, and high suspended sediment loads, could also be valuable for conserving more northern populations.

As described in Part 1 of this Evaluation and Guidance document, steelhead population numbers have been decreasing and many populations have become extirpated due to a myriad of anthropogenic and climate-related factors. Fewer numbers of smaller, fragmented populations make southern California steelhead populations more susceptible to environmental fluctuations, stochastic events, and loss of genetic diversity. In turn, the loss of genetic diversity makes populations more susceptible to other deleterious genetic effects, such as genetic drift. Extreme environmental events that affect populations with low genetic diversity can result in genetic bottlenecks and founder effects. Given life history strategies are controlled, in part, by genetics (Pearse et al. 2014), reduced genetic diversity can also affect the expression of different life history strategies. Life history variability is thought to be critical for steelhead resilience in variable environments of southern California, and recent studies have begun to improve the understanding of the relationships between genetics, life history expression, and metapopulation dynamics of southern California steelhead. Some of the key findings from these studies are summarized below, but the NMFS (2023) 5-year status review presents a more thorough discussion.

Population genetic studies have revealed steelhead in southern California have lower genetic diversity compared to more northern populations (Garza et al. 2014). Studies also revealed steelhead populations in more northern watersheds within the Southern California Steelhead DPS are primarily of native steelhead ancestry (as opposed to hatchery lineage), but more southern watersheds within the DPS showed substantial genetic introgression from hatchery lineages (Abadía-Cardoso et al. 2016). There has also been limited introgression of hatchery genetics into native steelhead populations upstream of barriers despite a history of hatchery stocking (Clemento et al. 2009). Garza et al. (2014) also found genetic distance between populations was associated with geographic distance indicating reduced rates of dispersal with increasing distances.

Anadromous and resident life history forms contribute to overall life history diversity that make southern California steelhead resilient to variable environmental conditions. The decision to migrate to the ocean is related to fish size, sex, environmental conditions, and genetics, and the interactions of these (Martinez et al. 2011, Pearse et al. 2014, Kelson et al. 2020). The life history expression of either anadromy or residency has been linked to different haplotypes at the chromosome Omy5 (Pearse et al. 2019). Juveniles (both females and males) with the homozygous anadromous (AA) or homozygous resident (RR) haplotypes were shown to be more likely to migrate to the ocean and remain in freshwater as residents, respectively (Pearse et al. 2019). Heterozygous (AR) females were also more likely to migrate to the ocean compared to heterozygous males. It should be noted that individuals that are heterozygous (AR) or homozygous resident (RR) haplotypes can still migrate to the ocean, and notably, a resident female can produce offspring that migrate to the ocean and vice versa (Donohoe et al. 2021). Kelson et al. (2020) found that migration may be indirectly controlled by the Omy5 genotypes and is mediated through physiological traits, such as growth rate, which would provide phenotypic plasticity. These new results indicate that a high potential for anadromy can be maintained within resident populations even when migration to the ocean is not possible, such as within populations upstream of barriers. Once migration to the ocean is restored, the frequency of the anadromous genotype could be expected to increase, although would lag behind restoration efforts. However, concern remains that the anadromous phenotype could be lost or modified because of selective pressures imposed by barriers.

Studies have also shown that groups of fish upstream and downstream of barriers are each other's closest relatives compared to fish from neighboring watersheds (Clemento et al. 2009), but the frequency of anadromous and resident haplotypes differs between populations upstream and downstream of barriers with the anadromous haplotype (AA) being more common in populations downstream of barriers with access to the ocean, whereas the resident haplotype is more common in populations upstream of barriers that cannot access the ocean (Clemento et al. 2009, Pearse et al. 2014, Apgar et al. 2017, Pearse et al. 2019). This indicates selection does not favor the anadromous haplotype in populations upstream of barriers, but it is thought that the presence of heterozygous haplotypes maintains the potential for increased frequency of anadromous haplotypes if future conditions provided access to the ocean.

The maintenance of genetic diversity (and life history variability) is among the main objectives of the NMFS (2012) Recovery Plan. Recommendations to maintain genetic and life history diversity include barrier removals, restoring natural flows, and habitat restoration to provide spatial variability that can increase population abundance and allow steelhead to respond more successfully to environmental fluctuations and catastrophic events. As mentioned previously, the recovery actions recommended by the NMFS (2012) Recovery Plan are critical for the long-term viability of steelhead and are focused on addressing the fundamental issues underlying population declines.

Hereafter, we evaluate approaches that focus on conserving genetic diversity with the overall goal being to reduce extinction risk of the Southern California Steelhead DPS. It should be noted that by reviewing a genetic conservation approach, we are not necessarily condoning or promoting its use, rather, we attempt to objectively summarize conservation approaches and consider if or how they could be implemented to support southern California steelhead. The conservation strategies reviewed below were selected because they have largely not been considered (or warrant additional consideration) in other recovery focused documents. These strategies are generally arranged from strategies that we generally recommend (and are potentially viable) to strategies that we do not recommend (or are not viable). In all cases, the genetic conservation strategies align with objectives outlined in the NMFS (2012) Recovery Plan, and some build on specific recommendations from the NMFS (2023) 5-year status review.

3.2 Streamside Incubators

3.2.1 Background

Streamside incubators are a form or captive breeding where gametes are taken from reproductively mature fish, fertilized, and incubated at a stream site where fish will be eventually released. More specifically, gametes are collected from reproductively mature fish, and fertilization occurs streamside. Fertilized embryos are incubated to the eyed stage in a controlled facility and planted in streamside incubators. Incubators can be in-stream or completely contained and separate from the natural stream. Incubators are located at sites with adequate habitat for spawning and/or rearing, and after fry switch to exogenous feeding strategies, swim out of the incubator or are transported to holding pens to acclimate to and imprint on the release location. For the purposes herein, streamside incubators would only be used for embryos from wild, native coastal southern California steelhead lineage parents (Jacobson 2021).

3.2.2 Examples of Use

Streamside or remote-site incubators have been used successfully to reintroduce salmonids (Andrews et al. 2016), as well as to increase survival rates of salmonid embryos in environments with low natural reproduction or poor habitat quality (Donaghy and Verspoor 2000, Kaeding and Boltz 2004, Al-Chokhachy et al. 2009). In British Columbia, streamside incubation boxes have been used for salmonids with the streamside upwelling units placed in side channels fed with groundwater (Miller 1990). Coho salmon young-of-the-year are released from streamside incubators in the Russian River watershed (McClary et al. 2020). In-stream incubators are used for a Chinook salmon supplementation program in the Columbia River watershed and for steelhead in the Salmon River in Idaho (Denny and Tardy 2008). Notably, a system with similar overall goals or reducing domestication selection was developed by the Winemum Wintu. Their system, the Nur Nature Base, was based on indigenous worldview and was used for reintroducing winter-run Chinook salmon to the McCloud River with great success (Earth Island Journal 2023).

3.2.3 Benefits

The benefits of streamside incubators are similar to those described for conservation hatcheries, but with fewer risks. Risk is reduced because the fish are incubated within or directly adjacent to habitat where they will be released. This provides more time for imprinting on natal cues and more closely mimics natural selection pressures during early life stage development. Reduced straying rates would reduce potential negative effects on adjacent populations. Incubators can be simple or complicated, making it easy to place in different environmental conditions.

3.2.4 Challenges, Risks, and Uncertainties

Similar to conservation hatcheries, risks of streamside incubators are primarily related to founder effects, domestication selection, and outbreeding depression, all of which can reduce reproductive success of released individuals and reduce the fitness of wild populations. These risks could be addressed by using eggs collected from reproductively mature, wild fish (as opposed to hatchery fish), but this would require harvesting fish from a population. An alternative would be to harvest fry from a population and raise them in captivity until maturity to reduce impacts on a local population. The major challenges are related to selection of appropriate broodstock and release sites. Broodstock should be from local populations with high genetic diversity and release sites should contain suitable habitat to accommodate all life history stages.

3.2.5 Assessment

Streamside incubators come with fewer risks compared to a conservation hatchery program, but they offer the same benefits. In particular, streamside incubators could be used to reintroduce *O. mykiss* to extirpated habitat in southern California (Jacobson 2021). Experimental implementation of this approach is recommended to evaluate constraints and success.

3.3 Assisted Migration

3.3.1 Background

As defined herein, assisted migration refers to the anthropogenic-assisted movement of salmonids around barriers for conservation. This type of action involves the capture and transport of migratory fish above and/or below a barrier, such as a dam or a waterfall, and is sometimes referred to as "trap and haul" (Kock et al. 2021). The term "assisted migration" can also be used to describe the introduction of fish to habitat outside a species' indigenous range, but in agreement with IUCN and SSC (2013), we refer to this type of translocation as "assisted colonization." Section 3.4 discusses assisted colonization in more detail.

Assisted migrations around barriers entails relocating individuals within the same watershed or waterbody, and this method is often used when dams are too high to support volitional movement of migratory fish or a substantial reservoir is formed behind the dam (Lusardi and Moyle 2017). The goal of assisted migration is to provide opportunities for fish to express different life-history strategies, complete life cycles, provide access to historical habitat, and/or promote gene flow among fragmented populations. The technique can target one life history stage (e.g., moving juveniles downstream) or multiple life history stages (Kock et al. 2021). As previously stated, although the volitional passage of steelhead is the preferred method of conservation, volitional passage in many watersheds is constrained by economic, engineering, permitting, or timeline considerations.

3.3.2 Example of Use

Assisted migration is used extensively along the west coast of the U.S. as a conservation tool for anadromous salmonids. Perhaps the most notable examples are from the Columbia River where adult salmonids are trapped downstream of Bonneville dam and moved upstream of the dam, and juvenile salmonids are trapped and transported downstream by barge (Montgomery 2003). There are fewer examples for salmonids at the southern end of their ranges.

Within California, assisted migration has been recommended for Chinook salmon, but steelhead have been largely overlooked because their life-history plasticity allows for resident and lake-migrant (adfluvial) life-history patterns. One example of a steelhead assisted migration program in California is on the Carmel River where adult steelhead are trapped downstream of Los Padres Dam and relocated upstream of the reservoir. Studies conducted after the release of these steelhead indicate that steelhead are successfully spawning in the Carmel River above Los Padres Reservoir, and pre-spawn mortality associated with the trap-and-haul operation appears to be low (Boughton et al. 2020). In this system, juveniles migrate volitionally downstream using the spillway or a bypass facility, although the proportional use of these downstream passage options by migrating fish appears to be relatively low (20% of fish that entered the reservoir), potentially because fish take up residency or are exposed to predation in the reservoir (Ohms et al. 2022). This example highlights an interesting caveat to providing volitional passage – i.e., that in some situations, volitional passage options may not be effective, and alternatives, such as head of reservoir collectors (i.e., assisted migration), may be more effective at achieving goals. Of course, in all situations, complete barrier removal is the preferred option.

3.3.3 Benefits

As stated above, the goals of assisted migration include providing opportunities for fish to express different life-history strategies, complete life cycles, access to historical habitat, and/or promote gene flow among fragmented populations. Any of these could provide benefits at the individual and population level. However, assisted migration programs are often coupled with other conservation strategies (e.g., hatchery-raised fish released in the same watershed), and individual elements are evaluated rather than a comprehensive review of the process (Kock et al. 2021); hence, the benefits of an assisted migration program are difficult to discern.

Assisted migration programs aim to increase the connectivity of populations upstream and downstream of barriers, which may lead to an improvement in interpopulation genetic diversity. Girman and Garza (2006) and Clemento et al. (2009) found that southern California *O. mykiss* populations upstream of barriers to anadromy were more genetically similar to populations downstream of barriers in the same system than to populations in other watersheds, which indicates the above-barrier populations are descendants of historical contiguous populations (NMFS 2012). Connecting these populations could lead to a reduction in the risk of extinction, improve interpopulation genetic diversity, and increase the population size.

Increased productivity is another goal of assisted migration programs. Genetic parentage studies of Chinook salmon in Oregon found that assisted migration techniques increased the number of returning adults produced by each reintroduced individual that survived, surpassing less fit hatchery pairs (Evans et al. 2016). Within southern California, assisted migration could benefit population productivity, especially for anadromous life history form because most of steelhead historical habitat currently exists upstream of barriers (NMFS 2012). In particular, providing downstream passage opportunities to above barrier populations would increase the numbers of anadromous individuals available to return as adults to natal waters, to other watersheds where they could provide genetic benefits, or to recolonize vacant habitat. In addition, assisted migration for southern California steelhead can serve as an action that can link anadromous populations with climate refuges.

3.3.4 Challenges, Risks, and Uncertainties

Reviews on assisted migration programs show variable results with many uncertainties regarding their efficacy (Anderson et al. 2014, Lusardi and Moyle 2017, Kock et al. 2021). The limited success of assisted migration programs is due to a variety of biological, logistical, financial, and operational challenges. Inherently, handling and transporting any fish comes with sub-lethal stress, which could lead to impaired physiological responses and performance such as reduced swimming performance, disease resistance, growth rates, ability to imprint, and ability to complete smoltification (Maule et al. 1988, Lusardi and Moyle 2017, Kock et al. 2021). It can take weeks for fish to recover from the stress of capture and transport, and stressors can act cumulatively, ultimately reducing reproductive fitness and potentially resulting in mortality (Lusardi and Moyle 2017, Kock et al. 2021). Trap operations can also cause migration delay if the trap is not properly designed and operated to accommodate safe and timely fish passage (Kock et al. 2021).

Stress imposed from handling and transporting fish is unavoidable, but approaches to minimize stress and provide recovery opportunities can be implemented (Kock et al. 2021). For example, in the San Joaquin River restoration pilot study (Sutphin et al. 2018), pre-transport survival of captured juvenile Chinook salmon was 70.6% in 2014, increased to 97.6% in 2015, and 95.1% in 2016 after the installation of a flow diffusing box and second capture box to improve post-capture flow refugia. In-transport survival of Chinook salmon was >99% across all years with fish transported long (>50 mile) distances. Special care was given to ensure transport temperature and DO were maintained with water pumped from the trapping reach. Transport water was slowly adjusted with release site water to ambient release site temperature prior to release, and transfers were made water to water at the release site. These examples suggest that trap-and-haul programs can achieve high survival rates when implementing appropriate design elements and best management practices.

Perhaps the largest factor that reduces the success of assisted migration programs is low collection efficiencies of downstream migrating smolts at trap sites within reservoirs or within rivers during flow conditions that facilitate migration, the later likely being exacerbated in “flashy” southern California streams. Low downstream collection efficiency (or low utilization of collectors) can create ecological traps upstream of dams (Ohms et al. 2022) if the habitat is not suitable. Indeed, assisted migration programs assume that the ecosystems into which steelhead are moved will support introduced steelhead, although several salmonid translocation programs have failed because of inadequate habitat in recipient areas (Harig and Fausch 2002). Thus, the conditions within habitat upstream of dams, as well as conditions downstream such as water quality and connectivity should be considered. Low collection efficiencies can also be advantageous by protecting above barrier populations from removal of individuals and by allowing expression of diverse life history strategies that maintain genetics associated with anadromy. Overall, there is a large amount of uncertainty whether this strategy is an effective action for conserving genetic variability, but the potential to increase the number of anadromous fish is only expected to provide benefits to the DPS.

3.3.5 Implementation in Southern California

Most high-quality spawning and rearing habitat for southern California steelhead is inaccessible to anadromous steelhead because they are upstream of migration barriers. This fragmentation reduces habitat availability and life-history and genetic variability, which increases extinction risk. Thus, barrier removal or modification to provide volitional passage are key recommendations within the NMFS (2012) Recovery Plan. The NMFS (2012) Recovery Plan also suggests that assisted migration could be used as an interim strategy until these recommendations are implemented.

Similar to barrier removals and volitional passage, the goal of assisted migration in southern California would provide population-level benefits by reconnecting fragmented populations and supporting life history variability (e.g., providing opportunities for populations upstream of barriers to express anadromous life history type), which, in turn, would promote genetic diversity and increase population growth. In many cases, implementation of volitional fish passage at dams may not be feasible due to technical, legal, and/or financial constraints, and it is extremely unlikely that some dams would be removed, especially in the near term, due to existing water rights and competing uses from municipal and agricultural sources. In these cases, assisted migration could be appropriate. However, as described in Part 2 of this document, there is concern that implementing assisted migration could provide justification for not implementing volitional passage. On the other hand, there is evidence that volitional passage provided at dams may not be effective (Ohms et al. 2022), and in these cases, dam removal or collectors upstream (or at the head) or reservoirs would be the better options. Overall, assisted migration may be the only feasible option at some locations, at least in the near term.

Below we focus on how downstream and upstream assisted migration programs could impact genetic diversity of southern California steelhead, and major challenges for implementation of such a program.

3.3.5.1 Juvenile and kelt downstream assisted migration

Transporting downstream migrating juveniles from upstream of a barrier to downstream of the barrier has the potential to increase the total number of anadromous smolts produced from the watershed, even if anadromous adults were not transported and released upstream. As described above, current information suggests that resident *O. mykiss* populations upstream of barriers retain genetics associated with anadromy (Pearse et al. 2014, Pearse et al. 2019, Apgar et al. 2017), and some proportion of these populations exhibit this life history strategy. Individuals that attempt to migrate downstream without assisted migration could enter reservoirs, such as Lake Cachuma in the Santa Ynez watershed or Lake Piru in the Piru Creek watershed, where they may be exposed to sub-optimal conditions for growth (e.g., high surface temperatures) and non-native predatory fish species such as the largemouth bass (*Micropterus salmoides*). Hence, fish that enter reservoirs are considered "lost" from the population, which is evidenced by the anadromous phenotype being less common in populations above barriers (Pearse et al. 2014, Pearse et al. 2019, Apgar et al. 2017). Interestingly, in one study, Leitwein et al. (2017) showed that the frequency of the Omy5 A haplotype in above barrier populations was positively associated with reservoir volume, suggesting successful use of larger reservoirs as a surrogate for the ocean.

An assisted migration program that collects fish prior to entering a reservoir (i.e., upstream of a barrier) and passes them downstream of barriers would provide benefits as described above with little risk to the upstream population, although there could be risks to any existing downstream populations if, for example, the fish decides to residualize or disperse into a tributary where steelhead are present. Nevertheless, any potential emigrant released downstream would increase the probability of anadromous adults returning to the watershed or dispersing into other watersheds, which could greatly benefit the genetic diversity of the small anadromous component of below barrier populations. Dispersal into non-natal watersheds or tributaries could increase the odds of recolonizing extirpated habitat or provide genetic mixing among populations.

The major challenge for implementation of assisted migration in southern California is due to low collection efficiency of downstream migrants. As evidenced in (Ohms et al. 2022), floating surface collectors deployed in reservoirs have variable and often low collection efficiencies. Collection sites in streams upstream of reservoirs (or at the head of reservoirs) could increase the numbers of migrants collected compared to a floating surface collector, but due to high flows and debris loads, operation of a collection site within streams is limited during flows that smolts might use for migration. Once again, low collection efficiency of downstream migrant traps could also be considered advantageous because it would ensure only a portion of the migrating population is removed, thereby providing opportunities for continued migratory life history expression (and maintenance of genes associated with anadromy; e.g., Leitwein et al. [2017]) in above barrier populations. Overall, assisted migration of even a small proportion of downstream migrants would provide anadromy opportunities that are extremely limited under existing conditions.

3.3.5.2 Adult upstream assisted migration

As described in Part 1, few adult steelhead are observed migrating across the entire Southern California Steelhead DPS each year, although actual numbers are likely higher than those reported because of monitoring limitations. Assisted migration of anadromous adults would be expected to provide genetic benefits to the population upstream. The potential impacts on genetic diversity are described below.

The evidence indicates adult steelhead will readily breed with resident fish if they were passed upstream. Redd surveys were conducted by NMFS and MPWMD in 2019 above Los Padres Dam on the Carmel River. If adult *O. mykiss* were directly observed building a redd, the life history type (resident versus anadromous) of both the male and female were noted as the origin of the redd. NMFS researchers observed mixed mating pairs (i.e., anadromous female/resident male, anadromous male/resident female), redd superimposition across both life history types, and both anadromous and resident spawning at the same time (Boughton et al. 2020). The production and fitness of the offspring have not been evaluated, though the interactions suggest steelhead and resident *O. mykiss* will readily breed across life history types. Therefore, it is possible transporting small numbers of anadromous adults upstream could result in viable offspring with a higher propensity for anadromy than the progeny of resident spawners, even if they mate with resident *O. mykiss* rather than other anadromous adults.

Increased interactions (breeding) would presumably provide a benefit to both the resident and anadromous populations through increased genetic diversity and overall population resilience. Currently, a resident *O. mykiss* population upstream of a passage barrier is an isolated population. Isolated populations can experience inbreeding depression and genetic drift, resulting in a loss of genetic diversity, which can lead to a reduction in fitness and eventually extirpation. Introduction of anadromous steelhead would help combat these deleterious genetic effects, as well as increase the occurrence of anadromous haplotypes in populations upstream of barriers. For example, in Topanga Creek, spawning contributions from a single anadromous female led to an increase in genetic diversity and an increase in frequency of alleles coding for anadromy that lasted for several years (Dagit et al. 2019).

The major challenges of assisted migration for upstream migrating adult steelhead in southern California are due to low numbers of anadromous adults combined with logistical challenges associated with operating fish traps under highly variable flow conditions. These challenges create uncertainty regarding the numbers of fish that could be successfully trapped. Furthermore, due to the low numbers of adults present in the Southern California Steelhead DPS, even a small chance of mortality associated with capture, handling, and transport is potentially unacceptable. Finally, assisted migration of adults brings up an important regulatory question – what happens to the listing status of an above barrier population once anadromous fish are released into the population? Do these fish not become part of the listed DPS, and how would that impact other regulations such as recreational fishing? Decisions regarding these questions would need to be worked out by NMFS and CDFW.

3.3.6 Assessment

Due to the potential to provide genetic rescue from artificial selection on populations above barriers and the potential for increasing life history expression, strategic implementation of assisted migration programs at locations may be desirable. Consistent with recommendations within the NMFS (2012) Recovery Plan, the most suitable locations to implement would be locations where barrier removal/modification to support volitional passage is infeasible due to technical or other constraints or as an interim strategy while volitional passage is provided. Implementation of assisted migration during construction activities, such as during modifications to existing dams or passage facilities or during dam removal projects, is absolutely recommended. However, it cannot be overstated that this approach should only be considered as an interim strategy until the barrier can be removed or volitional passage provided (or in cases where volitional passage was proven ineffective).

3.4 Assisted Colonization

3.4.1 Background

Assisted colonization is the movement of a species to a location outside of its existing or historical range into a new range where it should survive in future climate scenarios to avoid extinction (IUCN and SSC 2013). Within the context of the Southern California Steelhead DPS, assisted colonization would result in moving individuals from the DPS either north (i.e., into habitat occupied by a more northern steelhead DPS), south (i.e., further south in Baja, Mexico), or inland of the historical range. Moving individuals into non-anadromous waters that were historically accessible to steelhead would be considered a reintroduction translocation, which is covered in Part 2 of this document.

3.4.2 Examples of Use

The species *O. mykiss* (i.e., rainbow trout) has been introduced widely across the globe for recreational fishing or aquaculture purposes, but there are no known examples of *O. mykiss* of native steelhead lineage being introduced into habitats outside of the species historical range for conservation purposes. The large-scale introductions of *O. mykiss* for recreational and aquaculture purposes are not considered to be “assisted colonization” as defined herein because the goal of introductions did not include avoiding extinction. However, it does highlight the species’ high degree of plasticity for colonizing new habitats, even those with environmental conditions (e.g., temperatures) that are considered unsuitable (Chen et al. 2015).

Although not an example of assisted colonization for the purpose of conservation, *O. mykiss* from Sonoma Creek or Russian Creek, California, were introduced into western Australia through hatchery programs (Chen et al. 2015). Due to the source of these fish, they were presumably of steelhead ancestry (although not confirmed). Generations after introductions, the *O. mykiss* populations stemming from these translocated *O. mykiss* have exhibited enhanced thermal tolerance compared to the populations from which they originated in central California (Chen et al. 2015). While these translocations were not for conservation purposes, it highlights the assumption that a translocated *O. mykiss* population will likely adapt to conditions in their new environment.

3.4.3 Benefits

If habitat becomes unsuitable for southern California steelhead due to the impacts of climate change or other issues, assisted colonization could prevent extinction of the DPS by moving populations into habitats that may support the species. This could result in a population of genetic lineage maintained outside of its current or historical range.

As discussed previously, preservation of southern California steelhead genetics may be key for ensuring more northern populations are capable of developing adaptations to a warming (and more variable) environment. Evidence suggests that dispersal from more northern steelhead populations is important for supporting southern California steelhead (NMFS 2012), but relatively less dispersal in the reverse direction (i.e., from southern California into more northern populations) would be expected due to fewer numbers of individuals across more spatially discrete locations in southern California (i.e., there are fewer smolts produced that could stray).

However, few studies have evaluated straying in California steelhead in general, let alone for southern California steelhead (see Section 1.3 for examples). Assisted colonization that moves southern California steelhead into more northern locations that overlap with existing steelhead populations could introduce beneficial genetics into northern populations that would not receive those genetics naturally due to limited dispersal from southern California steelhead populations. Thus, assisted colonization may benefit other populations of protected steelhead, which could be beneficial for the species. However, the potential benefits would come with a host of risks and uncertainties.

3.4.4 Challenges, Risks, and Uncertainties

Introduced fish could be seeded from wild fish from the watersheds within the Southern California Steelhead DPS or from wild broodstock that are held in a facility. The use of wild fish would face similar challenges associated with donor population selection as outlined in Section 2.2.4 of this document (e.g., the effects of population mining on already small populations in anadromous waters). Captive breeding (whether a conservation hatchery or use of streamside incubators) using wild broodstock would likely be the best option for assisted colonization, and the risks associated with these are discussed in further detail in Sections 3.2 and 3.6. There are also issues surrounding feasibility, artificially manipulating dispersal patterns of an already highly mobile species, and there is a chance of the species being introduced into habitat where *O. mykiss* may impact native species. Assisted colonization is an action thought to be potentially applicable for species with small populations, restricted dispersal ability and adaptive potential, and within low-connectivity landscapes (Primack and Mao 1992; Trakhtenbrot et al. 2005; Petit et al. 2008; and Ozinga et al. 2009, as cited in Loss et al. 2010).

If southern California steelhead were moved north of the current range, individuals would be introduced into the habitat already occupied by steelhead from a different DPS, such as the South-Central California Coast (SCCC) DPS. Steelhead in the SCCC DPS are federally threatened, and thus implementation of any actions that could adversely affect their status or viability would be difficult to permit. In addition, while there could be benefits associated with increased genetic diversity, moving fish from the Southern California Steelhead DPS into more northern populations could have unintended consequences to either the introduced individuals or the existing populations due to increased competition at the release sites, introduced diseases or pathogens, or the introduction of deleterious genetic traits.

If the species was introduced to the south of the Southern California DPS range, they would be moved south of the California-Mexican border. Because monitoring is not known to be conducted in this area, there are many uncertainties about the presence of pre-existing populations and habitat within this region. It is assumed the majority, if not all watersheds in this region have habitat that is unsuitable for steelhead due to high temperatures, lack of protections surrounding water and resources stemming from urbanization, water extraction, fishing, pollution, and other issues that would lead to conditions that would not support the species. Finally, increasing temperatures in these locations would be expected under climate change, further reducing suitability.

The species could also be moved inland from the current range into habitat that is outside of the historical range of the species. Assisted colonization to an inland location would likely prevent expression of anadromy and expose fish to different selective pressures (temperatures, flow regimes) compared to historical habitat. Differences in selective pressures could create risks for translocated individuals because they may possess traits that would be maladaptive, but as previously mentioned, the species has shown a high degree of plasticity in new environments.

Moreover, a colonized population of *O. mykiss* would likely accumulate adaptations to the inland environment (e.g., Chen et al. 2015), which would erode the genetics of southern California steelhead. Thus, assisted colonization into inland locations would effectively eliminate any genetic conservation benefits from the action.

Finally, assisted colonization may be a difficult process to permit and current agencies prevent this conservation strategy for endangered animals (Shirey and Lamberti 2010).

3.4.5 Assessment

Based on the information presented above, assisted colonization would not achieve the goals of conserving genetic diversity of the Southern California Steelhead DPS. Assisted colonization into more southern locations would be unsuccessful due to the assumed, unsuitable conditions. Assisted colonization into inland environments would lead to selection and, therefore, would erode the genetics of southern California steelhead. The only potentially feasible assisted colonization action would be moving individuals into more northern locations that are already occupied by *O. mykiss*, which could result in genetic mixing between existing populations and introduced individuals. A benefit would be the introduction of unique traits from southern California steelhead that could make more northern populations more resilient to climate change. However, natural patterns of dispersal could provide these same benefits without the associated risks. The rates of dispersal from south to north is a major gap in understanding metapopulation dynamics.

If there comes a time where habitat is altered within the range of southern California steelhead beyond what the species can handle due to climate change, and it is certain this DPS will be extirpated with or without other forms of intervention, assisted colonization may be a useful tool with the goal of bolstering the population of another DPS through introduction of potentially advantageous traits. However, additional research would be needed to understand whether southern California steelhead possesses unique adaptations.

3.5 Cryopreservation

3.5.1 Background

Cryopreservation of fish gametes can provide a repository of genetic material that can be later used in artificial breeding. Cryopreservation technology is primarily used in hatchery settings but is potentially useful for genetic conservation of wild fish species that are close to extinction or extirpation (Martínez-Páramo et al. 2009, Cabrita et al. 2010). In this conservation context, cryopreservation involves collecting sperm and eggs from wild adult fish and storing them to ensure that they can be viably thawed and used to breed offspring from the wild donors to re-populate areas where populations have been reduced or extirpated.

3.5.2 Examples of Use

Salmonid sperm has been successfully cryopreserved and used to fertilize eggs and create viable offspring since the 1990s, and a few studies have done so using sperm from wild fish. Cloud et al. (1990) trapped wild steelhead and collected sperm from adult males. Sperm was successfully cryopreserved and later thawed and used to fertilize eggs from hatchery females. Martínez-Páramo et al. (2009) cryopreserved sperm from two different wild populations of brown trout. While cryopreserved sperm produced fry with lower survival rates compared to fresh, unpreserved sperm, these survival rates were still determined to be high enough to assist with recovery of brown trout populations. In addition, many studies have optimized the cryopreservation process to ensure maximal survival and fertilization success of salmonid sperm as well as viability of embryos and fry (Lahnsteiner et al. 1996, Cabrita et al. 1998, Cabrita et al. 2001, Fujimoto et al. 2022, Doğan et al. 2023).

It should be noted, however, that salmonid eggs have not yet successfully undergone cryopreservation because of the challenges associated with their large size and yolk content (Chao and Liao 2001). Potential solutions include cryopreservation of primordial germ cells (gametes from larval fish in a developmental stage where they have not yet differentiated into sperm or eggs) and type A spermatogonia (undifferentiated germ cells within mature testes). Kobayashi et al. (2007) successfully preserved primordial germs cells from rainbow trout larvae and were able to eventually thaw and implant them into donor hatchlings. The primordial germ cells then developed along with the donors and differentiated into sperm or eggs depending on the sex of the donor. Lee et al. (2013) cryopreserved whole testes from trout and transplanted type A spermatogonia into sterile male and female surrogates. These cells differentiated into sperm or eggs depending on the sex of the surrogate. Both methods are potential ways to generate eggs from a desired genetic stock from cryopreserved material without the challenges associated with the cryopreservation of salmonid eggs.

3.5.3 Benefits

The preservation of gametes could serve as a stable genetic bank of future restoration efforts or research. Ultimately, this strategy would serve as an ultimate “backstop” for extinction of unique genetics from within the Southern California Steelhead DPS.

3.5.4 Challenges, Risks, and Uncertainties

The main technological challenges with cryopreservation are cell damage due to ice crystal formation and an excess of reactive oxygen species (ROS) during freezing and thawing (Cabrita et al. 2010). Much work has been done to optimize the addition of chemicals that prevent ice crystal and ROS formation. Rainbow trout sperm has been the subject of many of these studies that have successfully used cryopreserved *O. mykiss* sperm to breed viable offspring, so these techniques would presumably be transferrable for preservation of steelhead sperm. However, salmonid eggs are much more difficult to cryopreserve (Chao and Liao 2001), and there does not appear to be a method for achieving this in the literature.

The preservation of primordial germ cells and whole testes containing type A spermatogonia (rather than differentiated gametes) offers a potential solution (Kobayashi et al. 2007, Lee et al. 2013). However, this technique is intensive and would likely require a breeding facility setting. For the primordial germ cell method, sperm and eggs would need to be collected from wild individuals and immediately used to create offspring. When those offspring reached the larval stage, primordial germ cells would need to be excised and preserved. Developing these germ cells into viable sperm and eggs would eventually require larval individuals from the same species to serve as recipients of the cells. For the method involving preservation of whole testes, testes would need to be excised from mature individuals from the wild, which would require killing them. This method would also require a hatchery facility to have sterile individuals available to serve as surrogates.

In order collect gametes from wild individuals, it is necessary to trap reproductively active adults from the wild to collect their gametes or to sacrifice them and collect their whole gonads. Given the low numbers of adults (and particularly migratory adults) in this region, it would likely be difficult to collect gametes from a sufficient number of individuals to preserve genetic diversity. Even if the collection was conducted opportunistically (e.g., during electrofishing surveys), the low adult quantities in the DPS may not contain a high amount of diversity in genetic material that would be collected. Sacrifice is also not optimal given these low numbers. Alternatively, fry could be harvested from a population and reared in captivity to maturity, but this approach increases opportunities for domestication selection. Additionally, cryopreservation requires storage at -80°C or lower, and these systems are susceptible to power outages and failures, risking complete loss of preserved gametes if only stored at a single location. Finally, cryopreservation will eventually require a breeding facility if preserved gametes are used.

3.5.5 Implementation in Southern California

Cryopreservation of genetic material from southern California steelhead populations would preserve any unique adaptations and could later be used for reintroduction purposes or for research (Jacobson 2021). Other than uncertainty related to viability of this approach, a major question for implementation is deciding which populations to target. Abadía-Cardoso et al. (2016) evaluated genetic diversity and ancestry of southern California steelhead populations. This information can be used to identify target populations that have high genetic diversity and low amounts of hatchery introgression. *O. mykiss* populations from more northern watersheds within the DPS, including Santa Maria, Santa Ynez, Ventura, and Santa Clara River watersheds, as well as more southern locations from the East, North and West forks of the San Gabriel River had relatively high genetic diversity and were largely of native coastal steelhead ancestry. Larger-sized populations in these watersheds would be the best candidates to reduce any effects from removing reproductively mature fish from the population. Data on population size is generally lacking, but populations within Sespe Creek and middle Piru Creeks (tributaries to the Santa Clara River), Sisquoc Creek (tributary to the Santa Maria River), and the East and West Fork San Gabriel Rivers are thought to be relatively robust populations that would be resilient to removal of adults for cryopreservation purposes.

3.5.6 Assessment

Based on our review, we do not consider cryopreservation as a viable approach for conserving genetic diversity of southern California steelhead at this time. This is largely because of the anticipated high-costs relative to large amounts of uncertainty regarding its efficacy, as well as the potential for adverse selection. However, if conditions continue to worsen, this method could be considered as a last resort if this approach has been further developed and proven effective.

3.6 Conservation Hatchery

3.6.1 Background

Conservation hatcheries are examined in the NMFS (2012) Recovery Plan and are defined as a program that, "conserves and propagates steelhead taken from the wild for conservation purposes and returns the progeny to their native habitats to mature and reproduce naturally." These can be used for direct supplementation of at-risk natural populations to prevent extirpation and increase natural spawning abundance. To conserve southern California steelhead, the progeny of captively bred wild fish could either supplement existing populations or be used to reintroduce *O. mykiss* into extirpated habitats. This type of program is not the same as hatchery programs that focus on enhancing fishery production or that use broodstock mating pairs that consist of either one or two hatchery origin fish (and are sometimes referred to as "conservation hatcheries" in the literature). The use of hatchery or mixed-spawning (i.e., cross between wild and hatchery origin fish) as part of a conservation hatchery program in the Southern California Steelhead DPS range is not assessed further because there are many well established negative consequences of the practice (i.e., negative genetic and environmental impacts to wild fish) (Naish et al. 2007, Ford et al. 2016).

Rather, conservation hatcheries in the context of this document refers to a program that can be used to breed wild fish (or fertilize their gametes) in captivity and release their offspring into the Southern California Steelhead DPS range. After artificial spawning occurs, fry or juveniles could be released. Alternatively, fertilized eggs or eyed embryos could be placed in a streamside incubator to emerge as fry and rear in the wild, which is a conservation action that is discussed further in Section 3.2. Alternatively, conservation hatcheries could be used for scientific, educational, conservation seed banking, or "insurance population" purposes through maintaining a genetic pool by holding wild progeny in captivity instead of releasing fertilized eggs, fry, or juveniles in the wild.

3.6.2 Examples of Use

Although there are many hatchery programs for steelhead and salmonids, referred to as "conservation hatcheries," across the west coast, most of them rely on some combination of hatchery and wild broodstock, so they do not meet the intent of conservation hatcheries as defined herein—i.e., only using wild broodstock. Although no conservation hatchery programs are currently in place for the Southern California Steelhead DPS, Becker and Reining (2008) stated that a conservation hatchery program was implemented in Sweetwater River for the Southern California Steelhead DPS after a wildfire destroyed habitat and extirpated the population. The program was deemed unsuccessful, and the population is thought to be extirpated. The specifics of the conservation hatchery program are unknown (e.g., where broodstock originated from, how the species was bred and released, and if progeny strayed to adjacent watersheds), and it is unknown whether *O. mykiss* are present in the watershed currently.

Within the Russian River watershed north of the Southern California Steelhead DPS, the Coyote Valley Fish Facility operates a fish ladder on the East Branch of the Russian River to trap upstream migrating steelhead that are then bred as part of a conservation hatchery program. Sexually mature male and female steelhead are placed in holding ponds for the week to spawn until enough fertilized eggs are collected. The fertilized eggs are then transported to a hatchery where they are incubated, hatched, and raised to yearling stage (CDFW 2023b). The yearling steelhead are then transferred back to the Coyote Valley Fish Facility to imprint at the facility and are released into the East Branch of the Russian River. The effects of this action on the overall wild steelhead population in the watershed are not known.

A Supplementation and Research Facility in the Yakima River basin in Washington has a conservation hatchery program that entails releasing wild Chinook salmon (*O. tshawytscha*) progeny after the spawning, incubation, and early rearing occur at a facility (Fast et al. 2015). Since the program started in 1997, the conservation hatchery program has led to increased redd counts, distribution of spawners, maintained wild Chinook returns, and negligible straying (Fast et al. 2015).

In a non-salmonid example, in 2008, USFWS founded a long-term conservation hatchery program for Delta smelt (*Hypomesus transpacificus*) from 2-year-old, wild-origin fish (Lindberg et al. 2013, Lew et al. 2015). Under this ongoing program, the captive population supplies progeny for research purposes and provides a genetic bank in case of further declines or species extinction. Due to careful management to minimize loss of genetic diversity, allele diversity and effective population have increased overall since the program began (Lew et al. 2015).

3.6.3 Benefits

The goals of conservation hatchery programs include the preservation of local populations through increasing the numbers of spawners, preservation of desirable genetic characteristics, reintroduction of populations in restored systems, and research conducted on specific progeny that could be used for future management, conservation, recovery, or protection of the species, pursuant to the CDFW Fish Bulletin for Captive Propagation of Fish, Wildlife and Plants for Conservation Purposes (Naish et al. 2007, NMFS 2012, CDFW 2017a). While there are a number of potential benefits of conservation hatchery programs, there is very little evidence of their benefits within the literature, and most studies point to mixed results and tradeoffs. Studies have shown although the use of local, wild broodstock can increase population abundance (Hess et al. 2012, Janowitz-Kock et al. 2018) and numbers of natural spawners on spawning grounds (Kock et al. 2022), it leads to lower reproductive success of wild spawners (Kock et al. 2022).

3.6.4 Challenges, Risks, and Uncertainties

There are many known risks and uncertainties surrounding the use of conservation hatcheries. The first risk relates to the source of wild broodstock, which could be either anadromous adults, resident *O. mykiss*, or some mixture of the two. The benefit of using anadromous adults only is that because these fish have successfully displayed the anadromous life history type, it could be assumed their genetics are desirable (i.e., genetics with higher likelihood of anadromy). However, it is suspected that a potentially large proportion of anadromous adults in southern California may be strays from northern populations (NMFS 2012, 2016); therefore, these fish may not have desirable genetic traits associated with local adaptations to southern California. These fish may still benefit existing populations through increased genetic diversity, but they may not meet the goals of conserving southern California steelhead genetics. In addition, few anadromous adults have been observed across the entire DPS, so it may not be logistically feasible or appropriate to collect these fish for broodstock purposes, that is, unless we reach a "condor moment"[1] when it becomes a last resort measure.

A second wild broodstock source could reproductively mature resident *O. mykiss*, but the ancestry of these fish should be confirmed to ensure they are of native coastal steelhead ancestry and not influenced by hatchery genetics prior to use. Resident *O. mykiss* can produce anadromous offspring (Donohoe et al. 2021) and would presumably possess potential, desirable genetic traits associated with local adaptation. It may be necessary to confirm the haplotypes of resident *O. mykiss* prior to using as broodstock to ensure they are not homozygous RR fish that are not capable of producing offspring with the allele associated with anadromy. Collection of resident *O. mykiss* downstream of barriers to anadromy would reduce the chances that broodstock have undergone selection upstream of reservoirs, but there are few below barrier populations capable of sustaining removal of individuals. An exception could be the *O. mykiss* population within Sespe Creek in the Santa Clara River. This population is believed to be one of the most robust,

---

[1] California condors nearly went extinct. To rescue the species, the two remaining individuals in the wild were trapped and used in a captive breeding program. When combined with environmental regulations that addressed the source of population declines, the captive breeding program was successful and there are now more than 340 individuals in the wild (USFWS 2022).

but there is limited access and limited, recent monitoring conducted so population size is unknown.

The largest risks of conservation hatcheries are related to effects from breeding and rearing fish within an artificial environment that are released in the wild. Domestication selection has been widely demonstrated in fish reared in captivity because natural selection is relaxed within artificial environments (Frankham 2008). Alarmingly, selection can occur within a single generation (Christie et al. 2012a). Results of domestication selection could include lower reproductive success, reduced genetic diversity, reduced olfactory imprinting, and lower survival in the wild among other impacts (Araki et al. 2007, Araki et al. 2008, Williamson et al. 2010, Christie et al. 2012b). Fish that have undergone domesticated selection can also breed and compete with wild fish, which can affect genetic diversity, fitness, and population dynamics of wild populations (Naish et al. 2007). The progeny of hatchery fish can also introduce novel pathogens into wild populations, further compromising fitness (Naish et al. 2007).

Mixing of conservation hatchery progeny (i.e., fish that have undergone domesticated selection) with wild fish can occur if the progeny of broodstock are directly released into an existing population, if the progeny stray into adjacent populations, or if wild fish stray and recolonize previously extirpated habitat where hatchery progeny were reintroduced. Indeed, hatchery produced fish are more likely to stray due to reduced imprinting to olfactory cues within the wild environment (Schroeder et al. 2001, Williamson and May 2005). Approaches to reduce domestication selection include careful selection of broodstock, releasing fish early in their life stages (e.g., as fry so they can imprint on olfactory cues), and rearing early stages streamside (see Section 3.2 below). Straying of hatchery progeny into wild populations could also increase competition, thereby potentially reducing the fitness of wild fish. Overall, there remains many risks and uncertainties related to the efficacy of conservation hatchery programs for conservation purposes.

3.6.5 Implementation in Southern California

In this section, we discuss the feasibility and additional considerations for implementation of a conservation hatchery program for supporting the Southern California Steelhead DPS. Specifically, we discuss program elements related to broodstock selection, release locations and timing, and availability of facilities to support conservation hatcheries.

Wild fish should only be used as broodstock to support a conservation hatchery program in southern California. As described above, using resident *O. mykiss* populations would be the most feasible option because of the logistical challenges associated with collecting anadromous adults (in addition to the possibility of adults being strays from northern populations). Prior to the use of resident *O. mykiss*, the haplotype at the Omy5 chromosome would need to be confirmed as well as the ancestry to ensure the anadromous allele is present and the fish are not of hatchery origin, respectively. Candidate broodstock source populations could include Sespe Creek in the Santa Clara River watershed, but access could be challenging and little information on the population status in this creek is available. Hilton and Salsipuedes creeks in the Santa Ynez watershed could also be considered. Populations upstream of barriers could be considered for broodstock, but there would be increased risk that the fish have undergone selection upstream of barriers. Depending on the release location, broodstock could be selected from a nearby location with similar conditions.

Release location would depend on multiple considerations. Because of the widely demonstrated impacts of hatchery effects on wild populations (even from entirely wild broodstock sources), it would be risky to release the progeny from captively bred fish into habitat with existing *O. mykiss* or where *O. mykiss* are likely to recolonize in the future. Genetic rescue could be the only reason to consider enhancement of existing populations. Alternatively, release into extirpated habitat could reduce the risks associated with genetic mixing between wild and conservation hatchery offspring and could increase demographic variability. However, progeny from captively bred wild fish may be more likely to stray or wild fish could stray and recolonize the previously extirpated habitat. Therefore, the potential for mixing with wild populations cannot be eliminated. Once again, the concern with mixing between progeny from wild broodstock and wild fish is that the former may have undergone domestication selection, which could result in reduced fitness of wild populations. Release at earlier life stages in the spring would allow fish to begin imprinting on natal cues and would expose released fish to selective pressures over the summer and fall, potentially alleviating some risks of straying while promoting natural selection. Releasing older fish could increase chances of survival to reproductive maturity and increase the changes of anadromy due to ability to achieve larger sizes in captivity, but release of older fish would increase risks related to domestication selection.

Ultimately, a conservation hatchery could be used as part of reintroduction efforts into extirpated habitat. A conservation hatchery program could offer benefits over the reintroduction of wild fish (see Section 2.2) by providing larger numbers of individuals that can be released, which could increase the chance of successful reintroductions by increasing the numbers of spawners and reducing genetic founder effects. A conservation hatchery program could also be used as a conservation seed banking or "insurance population" program, where a population of genetic lineage is maintained and held for future reintroduction or research in the event there is an imminent threat of extinction of the Southern California Steelhead DPS.

Currently, there is no facility in the Southern California Steelhead DPS range that supports captive breeding for wild steelhead. The Fillmore Fish Hatchery is located along the Santa Clara River; however, the facility is designed to rear hatchery strain rainbow trout and nonnative brown trout (*Salmo trutta*) that will ultimately be planted in non-anadromous waters for recreational fishing (CDFW 2023a). It is unknown whether it is feasible for this facility to be used to successfully implement a conservation hatchery program for southern California steelhead. If this facility is not feasible to use, it is likely a separate facility, which could be difficult to permit and would likely be expensive and time intensive to construct, will need to be designed and constructed for this use.

3.6.6 Assessment

Conservation hatchery actions should only be conducted if the wild populations are unlikely to survive without this level of direct intervention, pursuant to the CDFW Fish Bulletin for Captive Propagation of Fish, Wildlife and Plants for Conservation Purposes (CDFW 2017a). The use of conservation hatcheries to promote demographic redundancy across the DPS could be beneficial but is not without many risks. Risks would need to be further assessed through developing potential management, genetic, disease, welfare, release, and contingency strategies/plans for the action. It would be necessary to weigh the potential benefits against the risks prior to developing such a program, and it would be difficult to understand each prior to implementation. If determined to be a necessary recovery action, potential future programs could be modeled based on an ongoing program, such as the long-term Delta smelt conservation hatchery program. A conservation hatchery program with the goals of supplementing existing populations or reintroducing *O. mykiss* to extirpated habitat is unlikely to be necessary at this time but may be needed in the future if other recovery efforts fail to increase the viability of the Southern California Steelhead DPS. However, a conservation hatchery that rears fish for the purposes of genetic banking and research (and not for release into the wild) could be used to conserve genetics and develop a better understanding of physiological, genetic, and genomic adaptations of southern California steelhead.

3.7 Summary of Genetic Conservation Opportunities

Numerous approaches could be used to conserve the genetics of southern California steelhead, but many risks and constraints make their efficacy uncertain at this time. Further, the application of a particular conservation strategy is highly context dependent where the risks and benefits must be carefully weighed. For example, assisted migration could reconnect fragmented populations thereby increasing genetic diversity and providing opportunities for additional life-history expression. However, assisted migration programs are not a long-term solution and may only be warranted as an interim strategy during construction of volitional passage infrastructure or in extreme situations when there are no available alternatives to support volitional passage. As another example, conservation hatcheries could be used to increase demographic redundancy by reintroduction of *O. mykiss* into extirpated habitat, but this strategy risks negative impacts on wild populations if reintroduced fish, even if progeny from wild broodstock, have undergone domestication selection and stray in adjacent populations. In the case of conservation hatcheries, the risks can be minimized through careful selection of reintroduction sites in addition to using rearing techniques that promote natural selection and natal imprinting. The use of streamside incubators shows major promise for reducing risks associated with conservation hatcheries and we recommend experimental implementation to evaluate efficacy using this action. Finally, cryopreservation shows promise as an ultimate last resort to preserve southern California steelhead genetics, and collection of gametes across existing southern California steelhead population should be considered immediately.

Ultimately, as the Southern California Steelhead DPS does not show signs of recovery, a suite of conservation strategies described herein may be warranted. Monitoring, in particular the implementation of the California CMP steelhead monitoring strategy for the Southern Coastal Area (Boughton et al. 2022), of *O. mykiss* populations will be essential for determining when it may be appropriate to implement one or more of these genetic conservation strategies. When exactly more interventionist strategies should be implemented is beyond the scope of this review, and these decisions should be made by a TAC. Because of the uncertainty of southern California steelhead long-term viability, we recommend further development of strategies described above alongside implementation of recovery actions to be prepared for all future scenarios. We further recommend implementing projects such as streamside incubators in the near term under an experimental framework to inform future actions, as well as collecting gametes for cryopreservation for "insurance" purposes.

Research is also needed to increase our understanding of population dynamics. We support the recommendations for research provided in the NMFS (2023) 5-year status review. In particular, research is needed to improve our understanding of the genetic and environmental factors controlling life-history expression, as well as rates of straying among populations. As previously mentioned, it is believed southern California steelhead possess important adaptations to variable and extreme conditions, although there is little direct evidence for these adaptations. Additional research that combines physiological, genetic, and genomic approaches to explore and compare adaptations of southern versus more northern steelhead populations would increase our understanding of local adaptations. Understanding potential limits to adaptations such as thermal tolerance will also aid identification of refuge habitat and predicting future responses to climate change. Due to protections of endangered southern California steelhead, obtaining scientific permits to conduct these studies is challenging. However, the recent NMFS (2023) status review acknowledges there should be strategic support in terms of permitting for research and monitoring activities, such as those described above, to help ensure the best available science is developed to support recovery efforts for the Southern California Steelhead DPS.

4 PART 4 — SUMMARY OF RECOMMENDATIONS AND NEXT STEPS

Based on the evaluation of conservation actions presented within Parts 2 and 3, the following section describes our recommended actions, outlines steps toward their implementation, and provides recommendations for additional information gathering and studies that could address uncertainties.

4.1 TAC Support and Formalization

To aid in development of the Evaluation and Guidance document presented herein, a TAC was convened to provide comments and feedback. Continuing the work of this TAC would guide decision-making and implementation of the conservation actions discussed herein. The TAC could continue to advise on watershed prioritization, implementation timing, donor populations sources, release locations, and overall project success. TAC members include representatives of federal and state agencies, local governmental organizations, academics, Tribal nations, environmental and other non-profit groups, and interested stakeholders from the public. We recommend that going forward the TAC be organized and led by NMFS with the aim that the TAC could also support implementation of recovery actions from the NMFS (2012) Recovery Plan, when practicable. Existing groups such as the Southern California Steelhead Coalition and the Santa Clara River Steelhead Coalition, both facilitated by California Trout, and other local watershed groups could also support this function.

4.2 Programmatic Rescues

Programmatic Rescue programs include rescues in response to environmental, biological, and operational triggers that are predetermined. These types of rescues would be routinely implemented (perhaps annually) due to drying habitat and thus would require consistent leadership, personnel, and funding sources dedicated to these programs. The following steps are recommended to further implementation of Programmatic Rescue programs that would potentially include translocations from impacted watersheds as well as reintroductions to watersheds where extirpation has occurred but where habitat is suitable:

a) Select watersheds for prioritizations using framework provided in appendices (TAC could aid in prioritization, but this could also be driven by local stakeholder groups).
b) Identify leads (leads could include federal and state agencies, local watershed managers such as Resource Conservation Districts, public utilities, nongovernmental organizations (NGOs), or Tribal or local governments).
c) Develop final watershed-specific plans that consider all factors, including project goals/objectives, responsibilities, costs, funding sources, metrics for evaluating success, risks, timing, and an adaptive management plan. These final plans would follow guidelines presented within this document and use watershed-specific information presented within the appendices, which includes information needed to address questions within the CDFW fish rescue policy (i.e., CDFW Bulletin 2013-04).
d) Coincident with items a-c, initiate consultation with regulatory agencies to determine appropriate permitting pathways.
e) Secure approvals, permits, and funding.
f) Begin implementation, monitoring, and adaptive management.

4.3 Isolated Rescues

Isolated Rescues, such as those in progress through CDFW in collaboration with NMFS, will continue to occur in response to relatively unpredictable catastrophic events (e.g., wildfire), to isolated observations of stranded fish, or to operational failures at dams/diversions. Although unpredictable, the information provided in the watershed specific guidelines within the appendices can be used to facilitate quick response times and informed decision-making regarding translocation or reintroduction possibilities. The following steps could also be taken to ensure preparedness in the event an Isolated Rescue is required.

a) Identify/confirm the most appropriate release sites using additional surveys. Release sites both within and external watersheds will be identified within the watershed-specific guideline documents within appendices, but additional surveys may be needed when information was lacking.
b) Coordinate regional response teams that can respond quickly to disturbance if needed. Regional response team could be led by state or federal agencies and could include local stakeholders as a support team to reduce burden on regulatory agencies. Regional response teams could include “local agencies” such as COMB or other groups (RCDs, water districts, etc.) that have an internal fisheries staff that are trained and very capable of performing the needed action. On many occasions, this would reduce the response time and potential mortality. Each regional response team needs equal permit permissions, expertise, and equipment to rapidly respond as needed.
c) Conduct periodic surveys, as required, to confirm habitat suitability and update watershed specific information.

4.4 Reintroductions

An experimental reintroduction is recommended to evaluate its efficacy for establishing a viable population within extirpated habitat. The following steps should be taken in support of an experimental reintroduction:

a) Select a single experimental release site based on information presented in the framework for watershed prioritization (Appendix A) and the reintroduction guidelines (Section 2.2).
b) Identify project leads and funding sources (project leads could include federal and state agencies, local watershed managers such as Resource Conservation Districts, public utilities, NGOs, or Tribal or local governments).
c) Develop final watershed-specific plans that consider all factors, including project goals/objectives, responsibilities, costs, funding sources, metrics for evaluating success, risks, and an adaptive management plan. These final plans would follow guidelines within this document and use watershed-specific information presented within the appendices, which includes information needed to address questions within CDFW translocation policy (i.e., CDFW Bulletin 2017-05).
d) Secure approvals, permits, and funding.
e) Pre-action surveys including rapid DNA testing, habitat surveys, and pathogen screening.
f) Begin implementation, monitoring, and adaptive management.

4.5 Temporary Holding Facility

The availability of a short-term holding facility would benefit both rescue and reintroduction programs. As mentioned, temporary holding facilities are not currently available, although potential sites (e.g., Filmore Fish Hatchery) exist and are in development (CDFW pers. communication 2023). Alternatively, a temporary holding facility could be constructed to meet specific program goals. Multiple stakeholders have expressed interest in pursuing a short-term holding facility to support conservation and research on Southern California steelhead, and building a diverse coalition of stakeholders in support of a temporary holding facility will be essential for implementation. Next steps would include the following:

a) Identify project leads and funding sources (funding would be required for all steps presented below).
b) Coordinate with state and federal agencies to further explore options for a temporary holding facility (existing or new construction) and complete a feasibility study.
c) Identify and select potential temporary holding facility sites.
d) Develop a budget for the construction/modification of the facility and operational costs.
e) Develop a Rescue, Rearing, and Management Plan (RRMP) modeled after the Carmel River Steelhead RRMP.
f) Secure approvals, permits, and funding. This could include an endowment fund to cover long term operational and maintenance costs.
g) Construct a new or modify an existing facility.

4.6 Streamside Incubators

Streamside incubators are promising for reintroducing *O. mykiss* to extirpated habitat while reducing the risks associated with domestication selection in hatcheries. We recommend the use of streamside incubators within an experimental framework to evaluate their efficacy. Recommended steps toward experimental implementation follow:

a) Identify project leads and funding sources (funding would be required for all steps presented below).
b) Identify candidate reintroduction sites using guidelines described in Section 2.2. The most suitable candidate watersheds would be those identified for reintroductions (see Section 4.4).
c) Coordinate with state and federal agencies.
d) Develop a reintroduction plan using streamside incubators that builds on the work of the Southern California Native Trout Subpopulation Expansion Plan (Jacobson 2021). The plan should include additional information required within CDFW policies for propagation (CDFW Bulletin 2017-04) and translocation (CDFW Bulletin 2017-05) and information on donor populations, breeding plan, risk assessment, methodologies, monitoring, and adaptive management.
e) Secure approvals, permits, and funding.
f) Begin implementation, monitoring, and adaptive management.

4.7 Widespread Genetic Testing

Additional genetic data on ancestry, genetic diversity, and presence of Omy5 haplotypes would improve the baseline understanding of *O. mykiss* populations across the DPS, which would inform conservation needs and planning. Much of these data have already been collected, but data are either lacking or missing for certain additional populations. These efforts should be led by research scientists from NMFS and CDFW. It could take years for a comprehensive effort, and thus, in the short-term, rescue and reintroduction actions would need to rely on individual population genetic testing or available data.

4.8 Additional Recommendations

The recommendations below include additional data collection, monitoring, and studies needed to improve our understanding of southern California steelhead biology to inform decisions regarding implementation of conservation actions described herein.

- *Increased monitoring* – Consistent with the NMFS (2012) Recovery Plan, 5-year status reviews (NMFS 2016, 2023) and the CMP (Boughton et al. 2022), additional DPS-wide monitoring is needed to monitor existing populations and to increase our understanding of population dynamics. In particular, implementation of the California CMP steelhead monitoring strategy for the Southern Coastal Area (Boughton et al. 2022) will provide information for decision-making regarding when and where to implement additional conservation actions. Continuous updates should be added to specific watershed conditions documents over time to reflect on the ground changes. Access to these watershed condition documents will be limited to protect sensitive information.
- *Identify drought refugia* – Locations, both upstream and downstream of barriers, that are currently suitable and are projected to remain suitable under future climate change scenarios should be prioritized for protection and could serve as reintroduction locations. While the watershed-specific guidance documents within appendices attempt to identify these, additional data collection and modeling using Geographic Information System (GIS) data would aid identification of these habitats. Furthermore, the future suitability of refugia should be evaluated under climate change scenarios.
- *Develop quantitative habitat suitability relationships for southern California O. mykiss* – Quantitative relationships between habitat variables and *O. mykiss* presence and density would inform population dynamics and could be used to inform appropriate stocking densities. Habitat suitability curves are available for *O. mykiss*, but these are based on populations from more northern locations.
- *Research on population dynamics* – In agreement with the NMFS 5-year status review (NMFS 2023), research focused on improving the understanding of population dynamics of southern California steelhead is needed. Studies on the genetic and environmental factors controlling life-history expression, straying rates, and contributions of resident and anadromous life-history types to overall population dynamics would improve our ability to predict population responses to restoration and climate change.
- *Research on adaptation* – Studies are needed to evaluate adaptations of southern California steelhead to extreme conditions (e.g., high water temperatures, low DO). Genetic, genomic, and physiological approaches (and combinations of these) could be used to increase the understanding of the mechanisms that allow steelhead to tolerate extremes.

5 CONCLUSIONS

Southern California steelhead populations have experienced continued declines and extirpation because of numerous anthropogenic factors. Of upmost concern is climate change, which is predicted to increase the frequency and severity of disturbances, further compromising the effectiveness of recovery actions and the persistence of the Southern California Steelhead DPS. This Evaluation and Guidance document assessed conservation actions (rescues and reintroductions) in an effort to identify feasible, short-term actions that could support the persistence and recovery of the Southern California Steelhead DPS and contribute to recovery policies and plans that are currently in place. Additional genetic conservation opportunities were also evaluated in an attempt to objectively summarize each approach and consider their feasibility for implementation within the Southern California Steelhead DPS. Potential risks and uncertainties were weighed against anticipated benefits to determine an overall assessment of each opportunity. Within appendices to the Evaluation and Guidance document, watershed-specific guidance is presented for rescues, reintroductions, or in some cases, no immediate action.

Rescues and reintroductions were considered as feasible and effective short-term conservation actions that could be used to maintain population abundance, prevent extirpation, and introduce demographic redundancy, all of which will make the DPS more resilient to climate change and reduce the risk of extinction. The major challenges associated with rescues and reintroductions are selecting suitable release sites and donor populations, and, ultimately, these actions should be implemented under an adaptive management framework. Candidate watersheds for implementation are identified within appendices to this Evaluation and Guidance document, and watersheds in the southern portion of the DPS, where there are few extant populations and natural recolonization is unlikely, should be prioritized for reintroductions.

Other genetic conservation approaches that were assessed include streamside incubators, cryopreservation, assisted migration, assisted colonization, and only as a potential last resort, conservation hatcheries. Each of these genetic conservation approaches could be used as an interim strategy under specific circumstances if the Southern California Steelhead DPS does not show signs of recovery, but they all present risks to the population. Given the current population trends, implementation of some of these strategies within an adaptive management framework should be considered, and the continued convening of a TAC is recommended to aid in decision-making. Increased monitoring via implementation of the CDFW CMP and additional research on population dynamics, genetics, and local adaptation are also needed to facilitate decision-making regarding implementation and to address risks and uncertainties, but these are not required before implementation of the strategies recommended herein.

Ultimately, implementation of rescue and reintroductions across locations within the Southern California Steelhead DPS would help prevent extirpation and increase demographic variability in the short-term, which in turn would increase the effectiveness of long-term recovery actions. Although these actions are not without risk, the status quo also presents risks. Rescues and reintroductions, while human interventions, could be strategically implemented to ensure more intensive interventions, such as conservation hatcheries, are not necessary in the future.

6 REFERENCES

Abadía-Cardoso A., D. E. Pearse, S. Jacobson, J. Marshall, D. Dalrymple, G. Kawasaki F, Ruiz-Campos, and J. C. Garza. 2016. Population genetic structure and ancestry of steelhead/rainbow trout (*Oncorhynchus mykiss*) at the extreme southern edge of their range in North America. *Conservation Genetics*, 17: 675–689.

Adams, P. B., L. B. Boydstun, S. P. Gallagher, M. K. Lacy, T. McDonald, and K. E. Shaffer. 2011. California coastal salmonid population monitoring: strategy, design, and methods. Fish Bulletin. State of California, Department of Fish and Game 180: 4–82.

Aguilar, A., and J. C. Garza. 2006. A comparison of variability and population structure for major histocompatibility complex and microsatellite loci in California coastal steelhead (*Oncorhynchus mykiss* Walbaum). Molecular Ecology 15: 923–937.

Al-Chokhachy, R., M. Peacock, L. G. Heki, and G. Theide. 2009. Evaluating the reintroduction potential of Lahontan Cutthroat Trout in Fallen Leaf Lake, California. North American Journal of Fisheries Management 29: 1,296–1,313.

Anderson, J. H., G. R. Pess, R. W. Carmichael, M. J. Ford, T. D. Cooney, C. M. Baldwin, and M. M. Mclure. 2014. Planning Pacific Salmon and Steelhead reintroductions aimed at long-term viability and recovery. North American Journal of Fisheries Management 34:1 72–93.

Andrews, T. C., B. B. Shepard, A. R. Litt, C. G. Kruse, M. L. Nelson, P. Clancey, A. V. Zale, M. L. Taper, and S. T. Kalinowski. 2016. Performance of Juvenile Cutthroat Trout Translocated as Embryos from Five Populations into a Common Habitat. North American Journal of Fisheries Management 36: 926–941.

Apgar, T. M., D. E. Pearse, and E. P. Palkovacs. 2017. Evolutionary restoration potential evaluated through the use of trait-linked genetic marker. Evolutionary Applications 10: 485–497.

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

Araki, H., B. A. Berejikian, M. J. Ford, and M. S. Blouin. 2008. Fitness of hatchery-reared salmonids in the wild. Evolutionary Applications 1: 342–355.

Becker, G.S. and I.J. Reining. 2008. Steelhead/Rainbow Trout (*Oncorhynchus mykiss*) resources south of the Golden Gate, California. Center for Ecosystem Management and Restoration.

Becker, G. S., K. M. Smetak, and D. A. Asbury. 2010. Southern Steelhead Resources Evaluation: Identifying Promising Locations for Steelhead Restoration in Watersheds South of the Golden Gate. Cartography by D.A. Asbury. Center for Ecosystem Management and Restoration, Oakland, California.

Bell, E., R. Dagit, F. Ligon. 2011. Colonization and Persistence of a Southern California Steelhead (*Oncorhynchus mykiss*) Population. Bulletin Southern California Academy of Sciences 110: 1–16.

Bett, N. N., and S. G. Hinch. 2016. Olfactory navigation during spawning migrations: a review and introduction of the Hierarchical Navigation Hypothesis. Biological Reviews 91: 728–759.

Boughton, D. A., and H. A. Ohms. 2022. Response of a threatened steelhead trout population to water provisioning scenarios for the Carmel River, California.

Boughton, D. A., A. D. Chargualaf, K. Liddy, T. Kahles, and H. A. Ohms. 2020. Carmel River Steelhead Fisheries Report 2020. California American Water Company. Pacific Grove, California.

Boughton, D., J. Nelson, and M. K. Lacy. 2022. Integration of Steelhead Viability Monitoring, Recovery Plans and Fisheries Management in the Southern Coastal Area. Fish Bulletin 182.

Boughton, D., and H. Ohms. 2022. Response of a threatened steelhead trout population to water provisioning scenarios for the Carmel River, California

Cabrita, E., R. Alvarez, L. Anel, K. J. Rana, and M. P. Herraez. 1998. Sublethal damage during cryopreservation of rainbow trout sperm. Cryobiology 37: 245–253.

Cabrita, E., F. Martinez, M. Alvarez, and M. P. Herraez. 2001. The use of flow cytometry to assess membrane stability in fresh and cryopreserved trout spermatozoa. Cryo Letters 22: 263–272.

Cabrita, E., C. Sarasquete, S. Martínez-Páramo, V. Robles, J. Beirão, S. Pérez-Cerezales, and M. P. Herráez. 2010. Cryopreservation of fish sperm: applications and perspectives. Journal of Applied Ichthyology 26: 623–635.

CDFW (California Department of Fish and Wildlife). 2013. Policy and Procedural Guidance for Fish Rescue (Departmental Bulletin No. 2013–04).

CDFW. 2017a. Captive Propagation of Fish, Wildlife and Plants for Conservation Purposes (Departmental Bulletin No. 2017–04).

CDFW. 2017b. Policy and Procedures for Conservation Translocations of Animals and Plants (Departmental Bulletin No. 2017–05).

CDFW. 2023a. Fish Species Raised at Fillmore Hatchery. Available at: https://wildlife.ca.gov/Fishing/Hatcheries/Fillmore/Species-Raised.

CDFW. 2023b. Coyote Valley Satellite Facility. Available at: https://wildlife.ca.gov/Fishing/Hatcheries/Warm-Springs/Coyote-Valley.

CEC (California Energy Commission). 2020. Exploring California's Climate Change Research. Available at: https://cal-adapt.org/.

Chao, N. H., and I. C. Liao. 2001. Cryopreservation of finfish and shellfish gametes and embryos. Reproductive Biotechnology in Finfish Aquaculture 197: 161–189.

Chen, Z., M. Snow, C. S. Lawrence, A. R. Church, S. R. Narum, R. H. Devlin, and A. P. Farrell. 2015. Selection for upper thermal tolerance in rainbow trout (*Oncorhynchus mykiss* Walbaum). The Journal of Experimental Biology 218: 803–812.

Cheng, L. J. 2021. Upper ocean temperatures hit record high in 2020. Adv. Atmos. Sci. 38: 523−530.

Christie, M. R., M. L. Marine, R. A. French, and M. S. Blouin. 2012a. Genetic adaptation to captivity can occur in a single generation. Proceedings of the National Academy of Sciences 109: 238–242.

Christie, M. R., M. L. Marine, R. A. French, R. S. Waples, and M. Blouin. 2012b. Effective size of a wild salmonid population is greatly reduced by hatchery supplementation. Heredity 109: 254-260.

Clemento, A. J., E. C. Anderson, D. Boughton, D. Girman, and J. C. Garza. 2009. Population genetic structure and ancestry of *Oncorhynchus mykiss* populations above and below dams in south-central California. Conservation Genetics 10: 1,321–1,336.

Cloud, J. G., W. H. Miller, and M. J. Levanduski. 1990. Cryopreservation of sperm as a means to store salmonid germ plasm and to transfer genes from wild fish to hatchery populations. *The Progressive Fish-Culturist*, *52*(1), 51-53.

COMB (Cachuma Operation and Maintenance Board Fisheries Division). 2013. 2011 Annual Monitoring Summary and Trend Analyses.

COMB. 2021. WY2020 Annual Monitoring Summary.

COMB. 2022. WY 2021 Annual Monitoring Summary for The Biological Opinion for the Operation and Maintenance of the Cachuma Project on the Santa Ynez River in Santa Barbara County, California.

Cooper, S. D., K. Klose, D. B. Herbst, J. White, S. M. Drenner, and E. J. Eliason. 2021. Wildfire and drying legacies and stream invertebrate assemblages. Freshwater Science 40: 659–680.

Dagit, R., D. Alvarez, A. D. Bella, S. Contreras, B. Demirci, P. House, A. Kahler, R. Kieffer, E. Montgomery, H. Nuetzel. 2019. Steelhead abundance monitoring in the Santa Monica Bay, January 2017–November 2019. Prepared for California Department of Fish and Wildlife Contract No. 1650904. Prepared by the Resource Conservation District of the Santa Monica Mountains, Topanga, California.

Dagit, R., M. T. Booth, M. Gomez, T. Hovey, S. Howard, S. D. Lewis, S. Jacobson, M. Larson, D. McCanne, and T. H. Robinson. 2020. Occurrences of steelhead trout (*Oncorhynchus mykiss*) in southern California, 1994–2018. California Fish and Wildlife 106: 39–58.

Denny, L. P., and K. A. Tardy. 2008. Supplementation, Monitoring, and Evaluation Program Annual Report October 2006-September 2007. Prepared for: United States Fish and Wildlife Service. Boise, Idaho.

Dittman, A. and T. Quinn. 1996. Homing in Pacific salmon: mechanisms and ecological basis. Journal of Experimental Biology 199: 83–91.

Doğan, M., E. Can, and F. K. Kocabaş. 2023. Spermatozoa cryopreservation of sex-reversed rainbow trout (*Oncorhynchus mykiss*): the effect of dilution rate and supplementation of a N-(2-

Mercaptopropionyl)-glycine-based extender on sperm motility and fertilizing capacity. Turkish Journal of Agriculture-Food Science and Technology 11: 303–306.

Donaghy, M. J., and E. Verspoor. 2000. A new design of instream incubator for planning out and monitoring Atlantic Salmon eggs. North American Journal of Fisheries Management 20: 521–527.

Donohoe, C. J., D. E. Rundio, D. E. Pearse, and T. H. Williams. 2021. Straying and life history of adult steelhead in a small California coastal stream revealed by otolith natural tags and genetic stock identification. North American Journal of Fisheries Management 41: 711–723.

Earth Island Journal. 2023. Rewilding Baby Salmon Using Indigenous Knowledge. November 7, 2023. https://www.earthisland.org/journal/index.php/articles/entry/re-wilding-baby-salmon-according-to-indigenous-knowledge/

Evans, M. L., M. A. Johnson, D. Jacobson, J. Wang, M. Hogansen, and K. G. O’Malley. 2016. Evaluating a multi-generational reintroduction program for threatened salmon using genetic parentage analysis. Canadian Journal of Fisheries and Aquatic Science 73: 1–9.

Fast, D. E., W. J. Bosch, M. V. Johnston, C. R. Strom, C. M. Knudsen, A. L. Fritts, G. M. Temple, T. N. Pearsons, D. A. Larsen, A. H. Dittman, and D. May. 2015. A synthesis of findings from an integrated hatchery program after three generations of spawning in the natural environment. North American Journal of Aquaculture 77: 377–395.

Fischer, J., and D. B. Lindenmayer. 2000. An assessment of the published results of animal relocations. Biol Conserv 96: 1–11.

Flagg, T. A., and C. F. Nash. 1999. A conceptual framework for conservation hatchery strategies for Pacific Salmonids. NOAA Tech. Memo. NMFS-NWFSC-38.

Flosi, G., S. Downie, J. Hopelain, M. Bird, R. Coey, and B. Collins. 2010. California salmonid stream habitat restoration manual. Fourth Edition. California Department of Fish and Game.

Ford, M. J., A. R. Murdoch, M. S. Hughes, T. R. Seamons, E. S. LaHood. 2016. Broodstock History Strongly Influences Natural Spawning Success in Hatchery Steelhead (*Oncorhynchus mykiss*). PLoS ONE 11: e0164801.

Frankham, R. 2008. Genetic adaptation to captivity in species conservation programs. Mol Ecol 17:3 25–333.

Fujimoto, T., T. Kaneyasu, M. Endoh, Y. Kogame, J. Nynca, A. Ciereszko, and K. Arai. 2022. Cryopreservation of masu salmon sperm using glucose-methanol extender and seminal plasma biomarkers related to post-thaw sperm motility. Aquaculture 557: 738305.

Gallagher, S. P., and C. M. Gallagher. 2005. Discrimination of chinook salmon, coho salmon, and steelhead redds and evaluation of the use of redd data for estimating escapement in several unregulated streams in northern California. North American Journal of Fisheries Management 25: 284–300.

Gallagher, S. P., P. K. Hahn, and D. H. Johnson. 2007. Redd counts. Pages 197–234 *in* D. H. Johnson, B. M. Shrier, J. S. O’Neil, J. A. Knutzen, X. Augerot, T. A. O’Neil, and T. N. Pearsons, editors. Salmonid field protocols handbook: techniques for assessing status and trends in salmon and trout populations. American Fisheries Society, Bethesda, Maryland.

Garza, J. C., and A. Clemento. 2007. Population genetic structure of *Oncorhynchus mykiss* in the Santa Ynez River, California. Final Report. Project partially funded by the Cachuma Conservation Release Board.

Garza, J. C., E. A. Gilbert-Horvath, B. C. Spence, T. H. Williams, H. Fish, S. A. Gough, J. H. Anderson, D. Hamm, and E. C. Anderson. 2014. Population structure of steelhead in coastal California. Transactions of the American Fisheries Society 143: 134–152.

Gavery, M. R., K. M. Nichols, B. A. Berejikian, C. P. Tatara, G. W. Goetz, J. T. Dickey, D. M. Van Doornik, and P. Swanson. 2019. Temporal Dynamics of DNA Methylation Patterns in Response to Rearing Juvenile Steelhead (*Oncorhynchus mykiss*) in a Hatchery versus Simulated Stream Environment. Genes 10: 356.

George, A. L., B. R. Kuhajda, J. D. Williams, M. A. Cantrell, P. L. Rakes, and J. R. Shute. 2009. Guidelines for propagations and translocation for freshwater fish conservation. Fisheries 34: 529–545.

Girman, D. and J. C. Garza. 2006. Population Structure and Ancestry of O. mykiss populations in South-Central California Based on Genetic Analysis of Microsatellite Data. Final Report for California Department of Fish and Game Project No. P0350021 and Pacific State Marine Fisheries Contract No. AWIP-S-1.

Gustafson, R. G., R. S. Waples, J. M. Myers, L. A. Weitkamp, G. J. Bryant, O. W. Johnson, and J. J. Hard. 2007. Pacific salmon extinctions: quantifying lost and remaining diversity. Conservation Biology 21: 1,009–1,020.

Harig, A. L., and K. D. Fausch. 2002. Minimum habitat requirements for establishing translocated Cutthroat Trout populations. Ecological Applications 12: 535–551.

Hasler, A. D., and A. T. Scholz. 1983. Olfactory Imprinting and homing in salmon. Springer-Verlag, New York

Hayes, S. A., M. H. Bond, C. V. Hanson, E. V. Freund, J. J. Smith, E. C. Anderson, A. J. Ammann, and R. B. MacFarlane. 2008. Steelhead growth in a small central California watershed: upstream and estuarine rearing patterns. Transactions of the American Fisheries Society 137: 114–128

Hayes, F. H., and N. P. Banish. 2017. Translocation and reintroduction of native fishes: a review of bull trout *Salvelinus confluentus* with applications for future reintroductions. Endangered Species Research 34: 191–209.

Hendry, A. P., J. K. Wenburg, P. Bentzen, E. C. Volk, and T. P. Quinn. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science 290: 516–518.

Hess, M. A., C. D. Rabe, J. L. Vogel, J. J. Stephenson, D. D. Nelson, and S. R. Narum. 2012. Supportive breeding boosts natural population abundance with minimal negative impacts on fitness of a wild population of Chinook salmon. Molecular Ecology 21: 5,236–5,250.

Hovey, T. E. 2004. Current status of southern steelhead/rainbow trout in San Mateo Creek, California. California Fish and Game 90: 140–154

IUCN and SSC (International Union for Conservation of Nature and Species Survival Commission). 2013. Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. IUCN Species Survival Commission, Gland, Switzerland.

Jacobson, Sandra. 2021. Southern California Native Trout Sub-population Expansion Plan. Prepared by California Trout.

Janowitz-Koch, I., C. Rabe, R. Kinzer, D. Nelson, M. A. Hess, and S. R. Narum. 2018. Long-term evaluation of fitness and demographic effects of a Chinook Salmon supplementation program. Evolutionary Applications 12: 456–469.

Jenkins Jr, T. M., S. Diehl, K. W. Kratz, and S. D. Cooper. 1999. Effects of population density on individual growth of brown trout in streams. Ecology 80: 941-956.

Kaeding, L. R., and G. D. Boltz. 2004. Use of remote-site incubators to produce Arctic Grayling fry of wild parentage. North American Journal of Fisheries Management 24: 1,031–1,037.

Keefer, M. L., and C. C. Caudill. 2014. Homing and straying by anadromous salmonids: a review of mechanisms and rates. Reviews of Fish Biology and Fisheries 24: 333–368.

Kelson, S. J., S. M. Carlson, and M. R. Miller. 2020. Indirect genetic control of migration in a salmonid fish. Biology Letters 16: 20200299.

Kendall, N. W., J. R. McMillan, M. R. Sloat, T. W. Buehrens, T. P. Quinn, G. R. Pess, K. V. Kuzishchin, M. M. McClure, and R. W. Zabel. 2014. Anadromy and residency in steelhead and rainbow trout (*Oncorhynchus mykiss*): a review of the processes and patterns. Canadian Journal of Fisheries and Aquatic Sciences 72: 319–342.

Kobayashi, T., Y. Takeuchi, T. Takeuchi, and G. Yoshizaki. 2007. Generation of viable fish from cryopreserved primordial germ cells. Molecular Reproduction and Development: Incorporating Gamete Research 74: 207–213.

Kocik, J. F., S. A. Hayes, S. M. Carlson, and B. Cluer. 2022. A Resist-Accept-Direct (RAD) future for Salmon in Maine and California: Salmon at the southern edge. Fisheries Management and Ecology 29: 456–474.

Kock, T. J., J. W. Ferguson, M. L. Keefer, C. B. Schreck. 2021. Review of trap-and-haul for managing Pacific salmonids (*Oncorhynchus* spp.) in impounded river systems. Reviews in Fish Biology and Fisheries 31: 53–94.

Kovach, R. P., R. F. Leary, D. A. Bell, S. Painter, A. Lodmell, and A. R. Whiteley. 2022. Genetic variation in westslope cutthroat trout reveals that widespread genetic rescue is warranted. Canadian Journal of Fisheries and Aquatic Science 79: 936–946.

Lahnsteiner, F., B. Berger, T. Weismann, and R. Patzner. 1996. Changes in morphology, physiology, metabolism, and fertilization capacity of rainbow trout semen following cryopreservation. The Progressive Fish-Culturist 58: 149–159.

Larsen, D. A., D. L. Harstad, A. E. Fuhrman, C. M. Knudsen, S. L. Schroder, W. J. Bosch, P. F. Galbreath, D. E. Fast, B. R. Beckman. 2019. Maintaining a wild phenotype in a conservation hatchery program for Chinook salmon: The effect of managed breeding on early male maturation. PLoS ONE 14: e0216168

Lee, S., Y. Iwasaki, S. Shikina, and G. Yoshizaki. 2013. Generation of functional eggs and sperm from cryopreserved whole testes. Proceedings of the National Academy of Sciences 110: 1,640–1,645.

Leitwein, M., J. C. Garza, and D. E. Pearse. 2017. Ancestry and adaptive evolution of anadromous, resident, and adfluvial rainbow trout (*Oncorhynchus mykiss*) in the San Francisco Bay area: application of adaptive genomic variation to conservation in a highly impacted landscape. Evolutionary Applications 10: 56-67.

Lew, R. M., A. J. Finger, M. R. Baerwald, A. Goodbla, B. May, and M. H. Meek. 2015. Using next-generation sequencing to assist a conservation hatchery: a single-nucleotide polymorphism panel for the genetic management of endangered Delta Smelt. Transactions of the American Fisheries Society 144: 767–779.

Lindberg, J. C., G. Tigan, L. Ellison, T. Rettinghouse, M. M. Nagel, K. M. Fisch. 2013. Aquaculture Methods for a Genetically Managed Population of Endangered Delta Smelt. North American Journal of Aquaculture 75: 186–196.

Loss, S. R., L. A. Terwilliger, and A. C. Peterson. 2011. Assisted colonization: Integrating conservation strategies in the face of climate change. Biological Conservation 144: 92–100.

Luers, A. L., and S. C. Moser. 2006. Preparing for the impacts of climate change in California: opportunities and constraints for adaptation. Prepared by Climate Change Center, Sacramento, California.

Lusardi, R. A., and P. B. Moyle. 2017. Two-way trap and haul as a conservation strategy for anadromous salmonids. Fisheries 42: 478–487.

Martinez, A., J. C. Garza, and D. E. Pearse. 2011. A microsatellite genome screen identifies chromosomal regions under differential selection in steelhead and rainbow trout. Transactions of the American Fisheries Society 140: 829–842.

Martínez-Páramo, S., S. Pérez-Cerezales, F. Gómez-Romano, G. Blanco, J. A. Sánchez, and M. P. Herráez. 2009. Cryobanking as tool for conservation of biodiversity: effect of brown trout sperm cryopreservation on the male genetic potential. Theriogenology 71: 594–604.

Maule, A. G., C. B. Schreck, C. S. Bradford, and B. A. Barton. 1988. Physiological effects of collecting and transporting emigrating juvenile Chinook past dams on the Columbia River. Transactions of the American Fisheries Society 117: 245–261.

McClary, A., Z. Reinstein, N. Bauer, A. Bartshire, and M. Obedzinski. 2020. UC Coho Salmon and Steelhead Monitoring Report: Summer 2019. Santa Rosa, California.

McClure, M., J. Anderson, G. Pess, T. Cooney, R. Carmichael, C. Baldwin, J. Hesse, L. Weitkamp, D. Holzer, M. Sheer, and S. Lindley. 2018. Anadromous Salmonid Reintroductions:

General Planning Principles for Long-Term Viability and Recovery. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-NWFSC-141.

McClure, M. M., F. M. Utter, C. Baldwin, R. W. Carmichael, P. F. Hassemer, P. J. Howell, P. Spruell, T. D. Cooney, H. A. Schaller, and C. E. Petrosky. 2008. Evolutionary effects of alternative artificial propagation programs: implications for viability of endangered anadromous salmonids. Evolutionary Applications 1: 356–375.

McElhany, P., M. H. Ruckelshaus, M. J. Ford, T. C. Wainwright, and E. P. Bjorkstedt. 2000. Viable salmonid populations and the recovery of evolutionarily significant units. NOAA Technical Memo. NMFS-NWFSC-42.

McEwan, D., and T. A. Jackson. 1996. Steelhead Restoration and Management Plan for California. Department of Fish and Game.

McKenzie, D. J., D. J. Y. Zhang, E. J. Eliason, P. M. Schulte, G. Claireaux, F. R. Blasco, J. J. H. Nati, and A. P. Farrell. 2020. Intraspecific variation in tolerance of warming in fishes. Journal of Fish Biology 98: 1,536–1,555.

Meek, M. H., M. R. Stephens, K. M. Tomalty, B. May, and M. R. Baerwald. 2014. Genetic considerations for sourcing steelhead reintroductions: investigating possibilities for the San Joaquin River. San Francisco Estuary and Watershed Science: 12.

Miller, E. M. 2020. Ocean ecology of steelhead as revealed by pop-up satellite archival tags. Monterey Bay Aquarium. https://mlml.sjsu.edu/2020/01/20/emily-miller-seminar/

Miller, W. H. 1990. Analysis of Salmon and Steelhead Supplementation. U.S. Fish and Wildlife Service. Prepared for Thomas Vogel, Project Leader.

Modrick, T.M. and K.P. Georgakakos, 2015: The character and causes of flash flood occurrence changes in mountainous small basins of Southern California under projected climatic change. Journal of Hydrology Regional Studies 3: 312–336.

Montgomery. D. 2003. King of fish: the thousand-year run of salmon. Westview Press, Denver Colorado.

MPWMD (Monterey Peninsula Water Management District). 2018. Carmel River steelhead rescue and rearing management plan.

Naish, K. A., J. E. Taylor III, P. S. Lavin, T. P. Quinn, J. R. Winton, D. Huppert, R. Hilborn. 2007. An Evaluation of the Effects of Conservation and Fishery Enhancement Hatcheries on Wild Populations of Salmon. Advances in Marine Biology 53: 62–194.

National Research Council. 1996. Upstream: salmon and society in the Pacific northwest. National Academies Press, Washington, D.C.

Nielsen, J.L. 1999. The evolutionary history of steelhead (*Oncorhynchus mykiss*) along the US Pacific Coast: developing a conservation strategy using genetic diversity. ICES Journal of Marine Science 56: 449-458.

Nielsen, J. L., T. E. Lisle, and V. Ozaki. 1994. Thermally stratified pools and their use by steelhead in northern California streams. Transactions of the American Fisheries Society 123: 613–626.

NMFS (National Marine Fisheries Service). 2000. Guidelines for Electrofishing Waters Containing Salmonids Listed Under the Endangered Species Act.

NMFS. 2012. Southern California Steelhead Recovery Plan. Southwest Region, Protected Resources Division, Long Beach, California.

NMFS. 2015. Endangered Species Act (ESA) Section 7(a)(2) Biological Opinion and Magnuson-Stevens Fishery Conservation and Management Act Essential Fish Habitat Consultation: Programmatic Consultation on funding and permitting restoration projects within watersheds of San Luis Obispo, Santa Barbara, Ventura, Los Angeles, Orange and San Diego counties, California. NMFS Consultation Number: 2014-00285.

NMFS. 2016. 5-Year Review: Summary and Evaluation of Southern California Coast Steelhead Distinct Population Segment. National Marine Fisheries Service. West Coast Region. California Coastal Office. Long Beach, California.

NMFS. 2023. 5-Year Review: Summary and Evaluation of Southern California Coast Steelhead Distinct Population Segment. National Marine Fisheries Service. West Coast Region. California Coastal Office. Long Beach, California.

NMFS and CDFW. 2021. California Interagency Anadromous Fish Rescue Strategy 2021 to 2026.

NOAA (National Oceanic and Atmospheric Administration). 2018. Anadromous Salmonid Reintroductions: General Planning Principles for Long-Term Viability and Recovery. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-NWFSC-141.

Ohms H. A., D. N. Chargualaf, G. Brooks, C. Hamilton, E. P. Palkovacs, and D. A. Boughton. 2022. Poor downstream passage at a dam creates an ecological trap for migratory fish. Canadian Journal of Fisheries and Aquatic Sciences 00: 1–12.

O'Neal, J. S. 2007. Snorkel Surveys. Pages 325–340 *in* D. H. Johnson, B. M. Shrier, J. S. O'Neil, J. A. Knutzen, X. Augerot, T. A. O'Neil, and T. N. Pearsons, editors. Salmonid field protocols handbook: techniques for assessing status and trends in salmon and trout populations. American Fisheries Socieity, Bethesda, Maryland.

Ozinga, W.A., Romermann, C., Bekker, R.M., Prinzing, A., Tamis, W.L.M., Schaminee, J.H.J., Hennekens, S.M., Thompson, K., Poschlod, P., Kleyer, M., Bakker, J.P., van Groenendael, J.M., 2009. Dispersal failure contributes to plant losses in NW Europe. Ecol. Lett. 12, 66–74.

Peacock, M. M., M. L. Robinson, T. Walters, H. A. Mathewson, and R. Perkins. 2010. The evolutionarily significant unit concept and the role of translocated populations in preserving the genetic legacy of Lahontan Cutthroat Trout. Transactions of the American Fisheries Society 139: 382–395.

Pearse, D. E., and J. C. Garza. 2015. Unscrambling an egg: population genetic structure of *Oncorhynchus mykiss* in the California Central Valley inferred from combined microsatellite and SNP data. San Francisco Estuary Watershed Science 13: 3.

Pearse, D. E., C. J. Donohoe, and J. C. Garza. 2007. Population genetics of steelhead (*Oncorhynchus mykiss*) in the Klamath River. Environmental Biology of Fishes 80: 377–387.

Pearse, D. E., S. A. Hayes, M. H. Bond, C. V. Hanson, E. C. Anderson, R. B. Macfarlane, and J. C. Garza. 2009. Over the falls? Rapid evolution of ecotypic differentiation in steelhead/rainbow trout (*Oncorhynchus mykiss*). Journal of Heredity 100: 515–525.

Pearse, D. E., N. J. Barson, T. Nome, G. T. Gao, M. A. Campbell, A. Abadía-Cardoso, E. C. Anderson, D. E. Rundio, T. H. Williams, K. A. Naish, T. Moen, S. X. Liu, M. Kent, M. Moser, D. R. Minkley, E. B. Rondeau, M. S. O. Brieuc, S. R. Sandve, M. R. Miller, L. Cedillo, K. Baruch, A. G. Hernandez, G. Ben-Zvi, D. Shem-Tov, O. Barad, K. Kuzishchin, J. C. Garza, S. T. Lindley, B. Koop, G. H. Thorgaard, Y. Palti, and S. Lien. 2019. Sex-dependent dominance maintains migration supergene in rainbow trout. Nature Ecology and Evolution 3: 1,731–1,742.

Pearse, D. E., M. R. Miller, A. Abadı´a-Cardoso, and J. C. Garza. 2014. Rapid parallel evolution of standing variation in a single, complex, genomic region is associated with life history in steelhead/rainbow trout. Proceedings of the Royal Society Biological Sciences 281: 20140012

Petit, R.J., Hu, F.S., Dick, C.W., 2008. Forests of the past: a window of future changes. Science 320, 1450–1452

Pierce, D. W., J. F. Kalansky, and D. R. Cayan. 2018. Climate, drought, and sea level rise scenarios for the fourth California climate assessment. California's Fourth Climate Change Assessment. www.climateassessment.ca.gov.

Primack, R.B., Mao, S.I., 1992. Dispersal can limit local plant distribution. Conserv. Biol. 6, 513–519.

Robert, A., D. Couvet, and F. Sarrazin. 2007. Integration of demography and genetics in population restorations. Ecoscience 14: 463–471.

Schroeder, R. K., R. B. Lindsay, and K. R. Kenaston. 2001. Origin and straying of hatchery winter steelhead in Oregon coastal rivers. Transactions of the American Fisheries Society 130: 431–441.

Shapovalov, L., and A. C. Taft. 1954. The Life Histories of the Steelhead Rainbow Trout (*Salmo gairderneri gairderneri*) and Silver Salmon (*Oncorhynchus kisutch*) with Special Reference to Waddell Creek, California, and Recommendations Regarding Their Management. Fish Bulletin No. 98.

Shirey, P. D., and G. A. Lamberti. 2010. Assisted colonization under the U.S. Endangered Species Act. Conservation Letters 3: 45–52.

Sloat, M. R., and A. M. K. Osterback. 2013. Maximum stream temperature and the occurrence, abundance, and behavior of steelhead trout (*Oncorhynchus mykiss*) in a southern California stream. Canadian Journal of Fisheries and Aquatic Sciences 70: 64–73.

Sutphin Z., J. Hutcherson, C. Hueth, R. Root, and D. Portz. 2018. 2016 Juvenile Trap and Haul Program. Bureau of Reclamation, Denver Technical Service Center, Colorado.

Taylor, E. B. 1991. A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. Aquaculture 98: 185–207.

Temple, G. M., and T. N. Pearsons. 2007. Electrofishing: backpack and drift boat. Pages 95–132 *in* D. H. Johnson, B. M. Shrier, J. S. O'Neil, J. A. Knutzen, X. Augerot, T. A. O'Neil, and T. N. Pearsons, editors. Salmonid field protocols handbook: techniques for assessing status and trends in salmon and trout populations. American Fisheries Socieity, Bethesda, Maryland.

Thompson, L. M., A. J. Lynch, E. A. Beever, A. C. Engman, J. A. Falke, S. T. Jackson, T. J. Krabbenhoft, D. J. Lawrence, D. Limpinsel, R. T. Magill, T. A. Melvin, J. M. Morton, R. A. Newman, J. O. Peterson, M. T. Porath, F. J. Rahel, S. A. Sethi, J. L. Wilkening. 2021. Responding to Ecosystem Transformation: Resist, Accept, or Direct? Fisheries Magazine. 46: 8–21.

Trakhtenbrot, A., R. Nathan, G. Perry, and D. M. Richardson. 2005. The importance of long-distance dispersal in biodiversity conservation. Diversity and Distributions 11: 173–181.

USFS (United States Forest Service). 2018. Final Los Padres National Forest 2018 Steelhead Monitoring Report.

USFWS (United States Fish and Wildlife Service). 2022. California Condor Population Graph, 1980–2022. https://fws.gov/media/california-condor-population-graph-1980-2022.

Verkaik, I., M. Rieradevall, S. D. Cooper, J. M. Melack, T. L. Dudley, and N. Prat. 2013. Fire as a disturbance in Mediterranean climate streams. Hydrobiologia 719: 353–382.

Waples, R. S., G. R. Pess, and T. Beechie. 2008. Evolutionary history of Pacific salmon in dynamic environments. Special Issue: Evolutionary Perspectives on Salmonid Conservation and Management 1: 189–206.

Weeks, A. R., C. M. Sgro, A. G. Young, R. Frankham, N. J. Mitchell, K. A. Miller, M. Byrne, Coates DJ,
M. D. B. Eldridge, P. Sunnucks, M. F. Breed, E. A. James, and A. A. Hoffmann. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evol App 4: 709–725.

Williams, J. E., D. W. Sada, and C. D. Williams. 1988. American Fisheries Society guidelines for introductions of threatened and endangered fishes. Fisheries 13: 5–11.

Williamson, K. S., and B. May. 2005. Homogenization of fall-run Chinook salmon gene pools in the central valley of California, USA. North American Journal of Fisheries Management 25: 993–1,009.

Williamson, K. S., A. R. Murdoch, T. N. Pearsons, E. J. Ward, and M. J. Ford. 2010. Factors influencing the relative fitness of hatchery and wild spring Chinook salmon (*Oncorhynchus tshawytscha*) in the Wenatchee River, Washington, USA. Canadian Journal of Fisheries and Aquatic Science 67: 1,840–1,851.

Yackulic, C. B., D. R. Van Haverbeke, M. Dzul, L. Bair, K. L. Young. 2021. Assessing the population impacts and cost-effectiveness of a conservation translocation. Journal of Applied Ecology 58: 1,602–1,612.

Appendices

Appendix A

Framework for Watershed Prioritization and Assignment of Potential Actions

This section summarizes the information used to prioritize watersheds and match watersheds with potential conservation actions (e.g., rescue as described in Section 2.1 or reintroduction as described further in Section 2.2) while considering any potential risk to rescued populations, donor populations, or other native species in the surrounding habitat. In addition to rescue and reintroductions, we also considered "No Action" to be a viable option under certain circumstances that are described below. The proposed decision framework is summarized in Figure A-1 and discussed below.

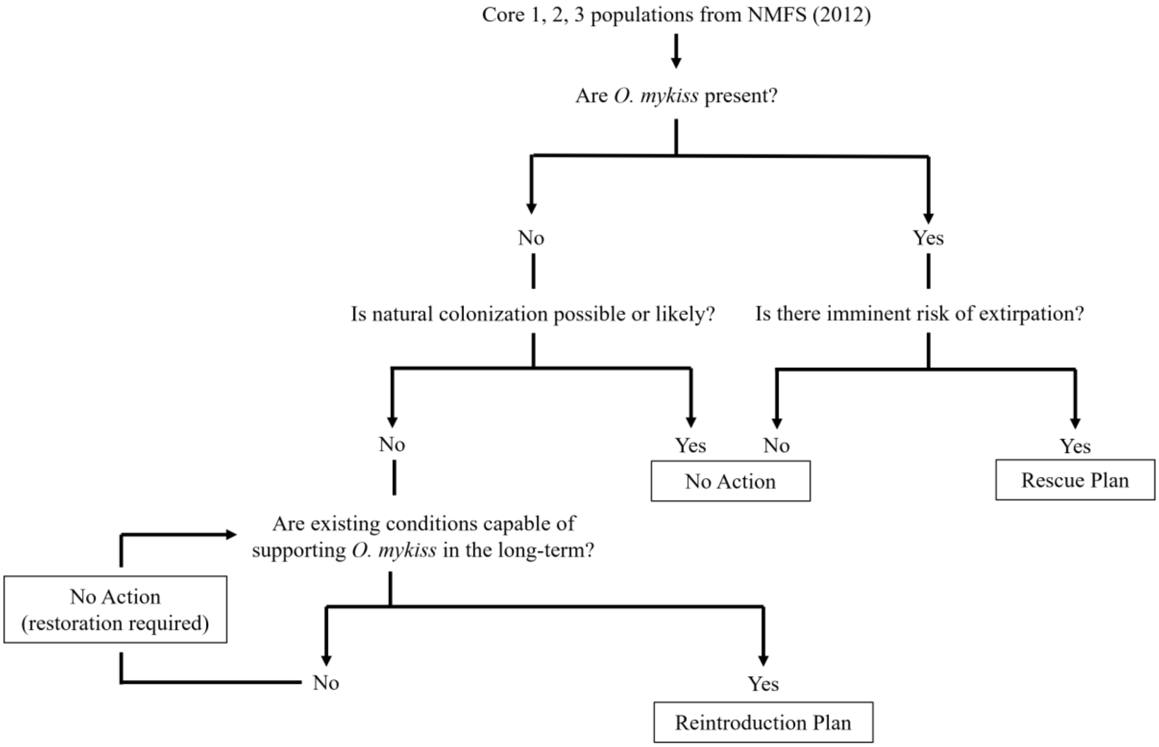


Figure A-1. Decision framework for determining appropriate conservation actions for individual watersheds in southern California. Boxes indicate decision endpoints. Figure adapted from decision framework presented in Anderson et al. (2014). Additional details and considerations presented in text.

All watersheds identified as Core 1, 2, and 3 within the NMFS (2012) recovery plans were considered candidates for either rescue, reintroduction, or no action. Core 1 through 3 watershed-specific guidance documents are included within appendices. For each Core watershed, it was first determined whether *O. mykiss* were present or extirpated (or population status was unknown), which informs whether they become candidates for potential rescue (when fish are present) or a reintroduction (when fish are absent) actions. Next, the spatial distribution and abundance of *O. mykiss* were considered within the watershed. For example, if *O. mykiss* were abundant and widely distributed within a watershed, it could be assumed *O. mykiss* within the watershed would be resilient to extreme events (i.e., unlikely to be extirpated from a single disturbance event) and reaches could recolonize naturally from other populations within the watershed following a disturbance event as long as major barriers were not present. In these cases, neither rescue nor reintroductions were deemed appropriate (i.e., "No Action" was required within the watershed). Other factors that could also contribute to a "No Action" decision include poor habitat quality, information gaps about fish and habitat, and accessibility.

After a conservation action was assigned to a watershed, conditions within a watershed, including habitat quality, barriers, and genetics, among other factors, were used to prioritize watersheds and guide specific actions. For example, watersheds with “high” quality habitat and an extirpated population would be prioritized for reintroductions over watersheds with “moderate” quality habitat to increase the likelihood of success. Habitat conditions within a watershed were also used to develop watershed specific plans, such as where to potentially collect and release rescued or reintroduced fish. The presence and number of barriers were also considered. In some situations, the presence of barriers to anadromy could be advantageous for a given action due to reduced permitting constraints. For example, collection of *O. mykiss* above a barrier to anadromy (for rescue or reintroduction actions) requires different permits (e.g., state scientific collection permit) than collection of *O. mykiss* within anadromous waters because the later are not protected under federal or state ESAs. For reintroductions, watersheds without barriers (or locations downstream of barriers) to anadromy would also be prioritized for reintroductions to promote the anadromous life-history type, although reintroductions to locations above barriers to anadromy are still considered to promote demographic redundancy.

Genetics were also considered for each population. Several published studies have evaluated the genetic structure of steelhead populations across California (Aguilar and Garza 2006; Pearse et al. 2007, 2014; Clemento et al. 2009; Garza et al. 2014; Pearse and Garza 2015; Abadía-Cardoso 2016). Notably, Abadía-Cardoso (2016) analyzed both microsatellite and single nucleotide polymorphism (SNP) data to evaluate the population genetic structure, steelhead ancestry (native coastal versus hatchery), and Omy5 chromosomal inversion across many populations of *O. mykiss* in southern California. In addition to aiding prioritization of native coastal steelhead lineages over those with hatchery lineage, genetic information was also used to inform donor populations for translocations and release locations.

Additional considerations included existing monitoring, access, funding, and social/cultural conditions. Generally, watersheds that have existing monitoring, are accessible, have funding for restoration, and have governmental, public, and/or tribal support would be prioritized. The degree of restoration (both completed and planned) was also considered. Sites that have undergone successful restoration would be prioritized over sites in need of restoration, and in some cases, planned restoration efforts would influence the timing of implementation. For example, in the Malibu Creek watershed, if a reintroduction was recommended, it should occur only after the planned deconstruction of Rindge Dam, to avoid a reintroduction prior to substantial disturbance.

The information outlined above was used to summarize Core 1 through 3 watersheds. Table A-1 lists all Core watersheds and identifies the status of the *O. mykiss* (present/absent) population in each.

The results of the decision framework (i.e., what type of action may be appropriate for each Core watershed, if any, as summarized in the appendices) is a suggested approach to combat the decreases in steelhead populations observed throughout the DPS. It should be noted that specific details of any conservation action would be highly watershed-specific due to differences in exiting conditions, steelhead population status, threats, restoration actions, and risks among other considerations.

Table A1. Core 1–3 watersheds organized by Biogeographic Population Groups (BPG) as defined in NMFS (2012) and associated presence/absence of *O. mykiss*. Core 1–2 watersheds are indicated by bold text. Presence/absence has not been systematically assigned to each watershed at this time and thus presence/absence designations are made to the best of our knowledge.

BPG/Population	Focus for recovery	Present/Absent/Unknown
Monte Arido Highlands		
Santa Clara River	**Core 1**	Present
Santa Maria River	**Core 1**	Present
Santa Ynez River	**Core 1**	Present
Ventura River	**Core 1**	Present
Conception Coast		
Carpinteria Creek	**Core 1**	Absent
Mission Creek	**Core 1**	Present
Rincon Creek	**Core 1**	Absent
Canada de la Gaviota	**Core 2**	Present
Goleta Slough Complex	**Core 2**	Present
Agua Caliente	Core 3	Absent
Arroyo Burro	Core 3	Absent
Arroyo Hondo	Core 3	Absent
Arroyo Paredon	Core 3	Unknown
Arroyo Quemado	Core 3	Absent
Bell Canyon	Core 3	Absent
Canada de Santa Anita	Core 3	Unknown
Canada del Capitan	Core 3	Absent
Canada del Corral	Core 3	Absent
Canada del Refugio	Core 3	Absent
Canada del Venadito	Core 3	Absent
Canada San Onofre	Core 3	Unknown
Carpinteria Salt Marsh Complex	Core 3	Absent
Dos Pueblos Canyon	Core 3	Unknown
Eagle Canyon	Core 3	Absent
Gato Canyon	Core 3	Absent
Jalama Creek	Core 3	Absent
Montecito Creek	Core 3	Present
Oak Creek	Core 3	Unknown
Romero Creek	Core 3	Absent
San Ysidro Creek	Core 3	Unknown
Tajiguas Creek	Core 3	Absent
Tecolote Canyon	Core 3	Absent
Santa Monica Mountains		
Malibu Creek	**Core 1**	Absent
Topanga Canyon	**Core 1**	Present
Arroyo Sequit	**Core 2**	Absent
Big Sycamore Canyon	Core 3	Absent

BPG/Population	Focus for recovery	Present/Absent/Unknown
Solstice Creek	Core 3	Absent
Mojave Rim		
San Gabriel River	**Core 1**	Present (above barriers)
Santa Ana River	**Core 2**	Present (above barriers)
Los Angeles River	Core 3	Present (above barriers)
Santa Catalina Gulf Coast		
San Juan Creek	**Core 1**	Present (above barriers)
San Luis Rey River	**Core 1**	Present (above barriers)
San Mateo Creek	**Core 1**	Unknown
Santa Margarita River	**Core 1**	Present
San Dieguito River	**Core 2**	Unknown
San Onofre Creek	**Core 2**	Absent
Otay River	Core 3	Unknown
San Diego River	Core 3	Unknown
Sweetwater River	Core 3	Unknown
Tijuana River	Core 3	Unknown