Gabilan Creek Watershed Monitoring Report

January 2020 – May 2021

Submitted To:
Monterey County Water Resources Agency

Prepared By:
Jack Eschenroeder
Dana Lee

FISHBIO
1617 S. Yosemite Ave.
Oakdale, CA 95361
209.847.6300
www.fishbio.com

July, 2021

Table of Contents

Executive Summary

In late 2019, FISHBIO developed a survey plan to assess the Gabilan Creek/Tembladero Slough Watershed to determine the suitability of this system to support the South-Central California Coast (SCCC) steelhead, the migratory form of *Oncorhynchus mykiss* (*O. mykiss*) present in many Central Coast watersheds. The Gabilan Creek/Tembladero Slough Watershed Habitat Assessment consisted of three components, which were intended to update and expand available information on (1) the current presence or distribution of steelhead (adults or juveniles) using environmental DNA (eDNA) surveys during peak migration season, (2) the overall connectivity of the watershed by assessing potential and previously known barriers to migration, and (3) the quantity and quality of habitat in the watershed capable of supporting populations of steelhead.

The Gabilan Creek/Tembladero Slough Watershed was selected for assessment after discussions with NOAA Fisheries staff, ICF, and Monterey County Water Resources Agency (MCWRA). This watershed has been heavily impacted by agricultural development, water infrastructure, and urbanization. The Gabilan/Tembladero Slough watershed makes up approximately 1.5% of the total available habitat within the Salinas River watershed (Becker et al. 2010). While rainbow trout (i.e., non-migratory *O. mykiss*) have been documented in the upper portions of the watershed in past surveys and in the current study, there has been only one confirmed observation of a steelhead (i.e., migratory *O. mykiss*) in the watershed, a gravid female that was found dead below a barrier in March of 2004. FISHBIO intended to complete the aforementioned components of the watershed assessment during the late winter and early spring of 2020, but field efforts were first reduced due to lack of rain and consequent lack of connectivity in the system, and were later suspended due to COVID-19 and shelter-in-place orders. Due to the suspension of field activities, only the eDNA assessment and flow monitoring were completed in the spring of 2020. Additional eDNA sampling and surveys of habitat in the upper watershed were completed in the winter of 2020 and spring of 2021. This report provides a summary of the findings from all surveys conducted during the 2020 and 2021 monitoring seasons, including a summary and discussion of habitat surveys conducted in the upper watershed.

During the 2020 monitoring season, from January 9 through April 10, 2020, FISHBIO personnel collected eDNA and water quality samples from strategic locations throughout the Gabilan Creek/Tembladero Slough Watershed in Monterey County (Figure 1). In addition, water level was monitored at two key locations in mainstem Gabilan Creek from January 24 through April 16, 2020. From its mouth at Tembladero Slough 14 km upstream to at least the San Jon Road crossing of Gabilan Creek, the system appeared to have had sufficient flow to facilitate the passage of adult anadromous (migratory) *O. mykiss* for the duration of the study. However, based on data recorded by water level loggers and conservative estimates of flow requirements for adult steelhead, the 6.32 km reach of mainstem Gabilan Creek between Little River Drive and the Hebert Road crossing may have been passable for only approximately seven of the total 1,837 monitored hours (0.38% of the monitoring period) during 2020. Further, the barrier at the uppermost assessed site on Gabilan Creek (Old Stage Road crossing) appeared impassable for the duration of the study. This barrier is formed by an undercut retaining wall and an old pipeline. Concrete riprap below this barrier acts as a further obstacle to migration and prevents the formation of a scour pool. Similarly, both Natividad and Alisal creeks, two main tributaries to Gabilan Creek, were impassable due to lack of flow for the entire duration of the study. However, it is important to note that 2020 saw the driest February on record for the state of California, and the hydrological regime observed in the Gabilan Creek/Tembladero Slough Watershed in spring of 2020 was representative of below-average flows.

Of the 100 eDNA samples submitted for processing and analysis in 2020, 13 indicated the presence of *O. mykiss* DNA. The majority of these (n = 8) were collected at the two most upstream sites at Hebert Road and Old Stage Road. Due to pervasive lack of watershed connectivity and numerous suspected migration barriers, these positive detections likely resulted from the resident rainbow trout population in the perennial reaches of the upper watershed, rather than from the presence of migratory steelhead. Positive samples were also collected as far downstream as San Jon Road (once), but the interpretation of this eDNA detection is inconclusive. It remains unclear whether the detection resulted from adult steelhead migrating upstream, from resident *O. mykiss* DNA being transported downstream from the upper watershed, and/or from juveniles from the resident population that smolted and outmigrated. As no positive samples were collected from the two most downstream sites at Tembladero Slough and Old Salinas River in 2020, there was no direct indication of adult steelhead entering the watershed.

During the 2021 monitoring season, from December 17, 2020 through February 8, 2021, FISHBIO staff collected eDNA and water quality samples from a subset of the same key locations that were sampled during 2020. Samples were collected once a week from only San Jon Road, Tembladero Slough, and the Old Salinas River (Figure 1). The decision to sample only the lower watershed was based on the reasoning that the occurrence of positive eDNA detections at the sites further upstream (Hebert Road and Old Stage Road) were likely due to the presence of rainbow trout, and because very low precipitation and a consequent lack of flow led to short and limited periods of connectivity between Littler River Drive and the Hebert Road crossing. Weekly sampling was discontinued after February 8 due, as it was one of the driest springs on record for the region and this led to lack of connectivity throughout the system, which would have precluded the upstream migration of steelhead. Although water level loggers were strategically placed in the streambed at Boronda Road and near the Hebert Road crossing, both were stolen during the monitoring season and therefore the assessment of flow and connectivity in the system is limited to comparisons of precipitation in 2021 with precipitation and water level data from 2020.

Of the 29 eDNA samples submitted for processing and analysis in 2021, three indicated the presence of *O. mykiss* DNA. Two of these were collected at the Old Salinas River site, and one was collected at the Tembladero Slough site. These detections in the most downstream sites in the watershed may indicate the presence of one or more steelhead in the lower river, but it remains unclear whether any steelhead moved upstream as no *O. mykiss* eDNA was detected at the more upstream site (San Jon Road).

FISHBIO was granted access from two landowners to conduct habitat surveys in the upper watershed, which were completed in May 2021. These surveys documented several kilometers of suitable spawning and rearing habitat for *O. mykiss*, as well as the presence of an additional potential barrier to steelhead migration. Significant portions of the assessed streambed above Old Stage Road were dry, but juvenile *O. mykiss* were detected in all but one of the wetted reaches that were assessed. The uppermost reaches of the watershed on Gabilan Ranch remain unassessed, therefore the absolute upstream limit to migration in the upper watershed remains unclear.

Although FISHBIO's surveys of the upper watershed documented habitat that appears suitable for steelhead spawning and rearing, our monitoring of connectivity throughout the lower watershed and the presence of multiple potential barriers to migration suggest that significant restoration may be necessary in the lower watershed to allow steelhead access to the upper watershed. Even if such restoration were completed, functional connectivity throughout the watershed would only occur periodically (in wet or very wet years). Further, obtaining definitive proof of historic or contemporary anadromy in the system would require additional studies. In particular, microchemistry analysis of otoliths collected from resident *O. mykiss* in the upper watershed would allow for assessment of the proportion of anadromous ancestry. Alternatively (or additionally), collection of tissue or scale samples and subsequent analysis of *Omy5* genotypes from upper watershed fish may provide some insight into the potential for expression of anadromous behavior in the population. PIT tagging of juvenile *O. mykiss* in the upper watershed and operation of a PIT tag antenna at a strategic location downstream to detect outmigration may also be implemented, either by itself or in conjunction with genetic and otolith studies, to evaluate the occurrence of the migratory life history expression in the Gabilan *O. mykiss* population.

Project Background and Objectives

The Monterey County Water Resources Agency (MCWRA) is in the process of developing a Habitat Conservation Plan (HCP) for a portion of the Salinas River Watershed and the Gabilan Creek/Tembladero Slough Watershed, which drains to the Old Salinas River channel (collectively called the HCP study area). A primary goal of the HCP is the protection of steelhead (the migratory life history form of *O. mykiss*) belonging to the South-Central California Coast (SCCC) Distinct Population Segment (DPS) within the HCP study area. The population of steelhead in the HCP study area has consistently declined due to a number of factors, including severely altered habitat, land-use changes, and anthropogenic water uses. This decline resulted in the listing of this DPS as threatened under the U.S. Endangered Species Act (ESA) in 1997 (National Marine Fisheries Service 1997). The bulk of the extant population of steelhead in the HCP study area appears to be primarily confined to the Arroyo Seco River, which contains the majority of spawning habitat and half of the rearing habitat in the Salinas River Watershed (National Marine Fisheries Service 2013). However, other tributaries to the Salinas River are still considered critical habitat for steelhead (National Marine Fisheries Service 2005) and are known to support small populations of resident rainbow trout (the non-migratory life history form of *O. mykiss*), which persist above manmade and natural barriers. Some of these tributaries may also have the potential to occasionally support small numbers of steelhead in years when hydrologic conditions are exceptionally favorable (Boughton and Goslin 2006).

The Gabilan Creek/Tembladero Slough Watershed is one such tributary to the Salinas River that supports a resident population of *O. mykiss* in the upper watershed, but it is relatively small in size and contains significant obstacles to steelhead migration. In addition to highly impaired water quality, any steelhead attempting to migrate to the upper reaches of the Gabilan Creek/Tembladero Slough Watershed must migrate through a series of agricultural drainage ditches and the highly fragmented urban portion of the system before reaching potential spawning habitat in the upper watershed. These challenges likely prevent steelhead from migrating upstream in most years. This hypothesis is supported by the fact that there has only been one documented observation of a steelhead in the watershed – a gravid female that was found dead in Gabilan Creek in 2004. The cause of death was not explicitly determined but was posited to be a combination of low flows and a possible migration barrier (Casagrande and Watson 2006). However, several studies have documented *O. mykiss* in the headwaters of Gabilan Creek and described habitat hypothesized to support steelhead throughout the upper drainages of the watershed (Gabilan, Natividad, Alisal, Towne, and Mud creeks; Hager 2001, Casagrande et al. 2003, Boughton et al. 2006, Boughton and Goslin 2006, Casagrande and Watson 2006, Becker et al. 2010).

Due to the continuously low abundance of steelhead in the HCP study area and a lack of information pertaining to actual or potential steelhead use of the Gabilan Creek/Tembladero Slough Watershed, FISHBIO was contracted to survey the aquatic habitat of the watershed to determine the suitability of this system to support steelhead. A similar assessment was conducted in this watershed in the early 2000s but was unable to confirm the presence of steelhead in the watershed and did not survey substantial portions of the upper watershed (Casagrande and Watson 2006). Nonetheless, the 2006 report provides the current best science on the watershed and served as a baseline for this assessment. The primary objectives of this assessment were to 1) evaluate the temporal extent of watershed connectivity and passability at known barriers (i.e., those identified by Casagrande and Watson [2006], Casagrande [2010], and Hager [2015]) throughout peak steelhead migration season, 2) detect any migrating *O. mykiss* throughout the watershed during periods of connectivity via analysis of environmental DNA (eDNA) in filtered water samples, and 3) assess the habitat quality in the watershed and its potential to support populations of resident and migratory *O. mykiss*.

South-Central California Coastal Steelhead

All steelhead in the study area belong to the South-Central California Coast (SCCC) Distinct Population Segment (DPS), which is federally listed as Threatened (NMFS 2006). The SCCC DPS includes all naturally spawned anadromous *O. mykiss* populations below natural and manmade impassable barriers in streams from the Pajaro River (inclusive) to, but not including, the Santa Maria River in Santa Barbara County, California. The Gabilan Creek/Tembladero Slough Watershed is included in the Interior Coast Range Biogeographical Population Group (BPG), which is comprised of populations in the Pajaro River, the Lower Salinas Basin, and the Upper Salinas Basin. The Gabilan Creek/Tembladero Slough Watershed provides an estimated 0.9 stream miles of available rearing and spawning habitat for steelhead (Becker et al. 2010) that is located in the upper watershed (i.e., upstream of Old Stage Road) where cool water temperatures, complex habitat, and riparian cover provide favorable habitat conditions.

To complete the migratory phase of their life cycle, steelhead require connectivity with the ocean during several time periods throughout their lives. Land use practices, in-stream development, and water management practices have gradually reduced connectivity, available habitat, and instream flows in the Gabilan Creek/Tembladero Slough Watershed. Changes in channel configuration from channelization, loss of riparian habitat, and agricultural encroachment into the floodplain have all affected surface flows, leading to a flashy flow regime including long periods without connectivity. Although Gabilan Creek historically went dry during parts of the year, water use in the watershed and several partial (or possibly complete) barriers to migration have severely exacerbated these issues, leading to impairment of upstream migration of adult steelhead and downstream migration of juveniles.

Based on watershed size, location, ecological context, and overall status of SCCC steelhead, a viable steelhead population in the Gabilan Creek/Tembladero Slough Watershed would be important to the conservation of ecological diversity of the SCCC DPS and the Lower Salinas Basin BPG. As such, the Gabilan Creek/Tembladero Slough Watershed has been listed as critical habitat for SCCC steelhead (National Marine Fisheries Service 2005). However, only one confirmed observation of a steelhead has ever occurred in the watershed. Moreover, given the lack of evidence that the watershed historically supported a steelhead population, even during periods with more favorable conditions (i.e., prior to significant anthropogenic changes to the watershed), it seems unlikely that a viable, self-sustaining population could be established in the future. Furthermore, SCCC steelhead are adapted to the variable flow and precipitation regime of Central Coast rivers, which are characterized by long dry periods interspersed with intense storms and flashy stream flow. As such, steelhead are opportunistic in their migrations, as they must be completed during very short, specific time periods. If flows are optimal, migration by a few individuals can occur, even in seemingly poor habitat. While these migrations can provide evidence of stream connectivity and availability of migratory habitat under optimal conditions, they do not necessarily indicate the quality of the aquatic ecosystem as a whole (i.e., spawning and rearing habitat) or reflect the size or health of the *O. mykiss* population.

O. mykiss in the Gabilan Creek/Tembladero Slough Watershed

Due to the persistent population of resident *O. mykiss* in the upper watershed, it is possible that opportunistic steelhead migration has occurred in the Gabilan Creek/Tembladero Slough Watershed. However, there are no historic accounts or archaeological evidence of adult steelhead in the watershed, and most observations of *O. mykiss* have been small individuals, which are likely resident rainbow trout (Day 1959; Hager 2001; Casagrande 2010). Like other streams in the Salinas Basin, rainbow trout have also been stocked in upper Gabilan Creek on multiple occasions (Day

1959). Previous studies have confirmed the presence of *O. mykiss* in the upper watershed (Hager 2001; Casagrande 2010), with observations ranging from as far downstream as the Hebert Road crossing up to the headwaters of Gabilan Creek.

Although there have been several reported observations of adult steelhead in Gabilan Creek/Tembladero Slough Watershed, only one of these was confirmed by state or federal agency biologists. In March 2004, a gravid female (~30 in or ~762 mm in total length) was found dead at the base of the Little River Drive grade control structure in the city of Salinas. The biologists determined that this individual died as a result of the migration barrier and lack of suitable flow. Several observations of Chinook salmon (*Oncorhynchus tshawytscha*) have also been confirmed in the Gabilan Creek/Tembladero Slough Watershed, with the furthest upstream observation at Little River Drive, where the salmon eventually died.

Environmental Setting

Gabilan Creek, which originates in the Gabilan Mountain Range and drains an area of 157 square miles (~407 square kilometers) in the northeast corner of the Salinas Valley, is one of the streams in the HCP study area that is thought to have historically provided habitat for steelhead in the upper watershed. However, it is estimated that this area makes up only approximately 1.5% of the total available steelhead habitat within the Salinas River watershed (Becker et al. 2010), and downstream reaches are characterized by poor habitat and water quality conditions.

In the upper reaches of the Gabilan Creek/Tembladero Slough Watershed, perennial sections of the stream are cool and shaded, and provide habitat for resident rainbow trout (Becker et al. 2010; Casagrande and Watson 2006; Hager 2001). As Gabilan Creek enters the valley, it eventually merges with Natividad and Alisal creeks at Carr Lake, an ephemeral lake, in the City of Salinas. Downstream of this confluence, the stream is known as the Reclamation Ditch (or "Rec Ditch"), which was created in the early 20th century for flood protection and agricultural drainage. The Rec Ditch flows northwest through a series of natural low points (often forming lakes during wet weather) which are linked by a system of drainage ditches. Near the town of Castroville, the Rec Ditch flows into Tembladero Slough and eventually reaches the Old Salinas River before it discharges through the tide gates at Potrero Road into Moss Landing Harbor.

Gabilan Creek exhibits variable stream conditions throughout the watershed. At higher elevations, the stream is steep, narrow, and rocky, and includes spatially intermittent perennially wetted habitat (i.e., pools). Dense riparian vegetation surrounds the stream, resulting in cooler water temperatures and complex habitat with large woody debris. The lower sections of the Gabilan Creek/Tembladero Slough Watershed primarily discharge agricultural runoff throughout most of the year and only become fully connected to the upper watershed briefly during and immediately after large precipitation events in the winter and spring. During periods of precipitation, urban runoff from Salinas and increased levels of agricultural drainage from surrounding farms also flow into the Rec Ditch and lead to poor water quality (e.g., fertilizer and pesticide runoff, low dissolved oxygen, high sediment loads; Casagrande and Watson 2006). Section 303(d) of the Federal Clean

Water Act (CWA) has identified the Gabilan Creek/Tembladero Slough Watershed as having significantly impaired water quality (California State Water Resources Control Board 2016). It is noted that Gabilan Creek itself suffers from contamination by ammonia, nitrates, and fecal coliforms, exhibits agricultural toxicity, and has high turbidity and low pH. Pesticide contamination is also noted throughout certain portions of the watershed, including Natividad Creek and Tembladero Slough. This contamination has the potential to reduce *O. mykiss* survival in the system.

Migration Barriers

Steelhead attempting to migrate to the upstream reaches of the Gabilan Creek/Tembladero Slough Watershed must overcome several barriers within the lower watershed. The furthest downstream impediments are the Potrero Road tide gates, located just upstream of Moss Landing Harbor. The tide gates consist of a box culvert with flap gates that are passable only during outgoing tides and periods of high flow when the differential water pressure is sufficient to open the gates. In some cases, strong tides in the spring can counter high outflows and impede access to Tembladero Slough. Tembladero Slough and the Rec Ditch are relatively free of barriers with the exception of the San Jon Road crossing, where a concrete chute and rip rap form a small barrier.

Upstream of Carr Lake, an elevated culvert under East Laurel Drive likely creates a partial barrier during low flow conditions, where any migrating steelhead must navigate several rock boulders and a small jump to enter the culvert. However, this barrier appears passable at moderate to high flows as evidenced by sightings of salmonids at upstream locations. Further upstream, a significant barrier is formed by the concrete grade control structure at Little River Drive. At this location the stream is funneled through a steep, narrow concrete chute which forms a long cascade at high flows. The pool below the barrier appears to be the upstream extent of connectivity during low-flow conditions, as urban runoff into the pool provides semi-consistent flows downstream. This barrier has been identified as a significant impediment to migration under most if not all flow conditions (Casagrande 2010), and the pool below the barrier was the location where a single gravid female steelhead was found dead in 2004 (Casagrande and Watson 2006). Another concrete grade control structure is located just upstream of Boronda Road. The control structure is approximately 1.2 meters in height and consists of a concrete lip on the right side of the river and a gently sloped concrete ramp on the left, which causes shallow, high-velocity laminar flows during periods of high discharge.

The next upstream barrier is a concrete weir located underneath Hebert Road. The weir is greater than one meter in height (as measured from the downstream streambed), but this height fluctuates depending on discharge and the level of scour in the sandy pool below the weir. This barrier appears to be passable during high flows but presents a significant obstacle during low to moderate flows due to the height of the jump and the shallow depth of the downstream pool. Further upstream, an undercut retaining wall and pipeline form what appears to be a complete migration barrier for adult steelhead underneath the Old Stage Road crossing. Downstream of the barrier, concrete riprap extends for approximately three meters, creating obstacles to migration and preventing the formation of a scour pool below the wall and pipeline.

An additional barrier of concrete riprap is present approximately 2.55 km (1.58 miles) upstream of the Old Stage Road crossing. This barrier has an approximate height of 1.68 meters and has accumulated a significant amount of large woody debris and fallen willow trees. No observations of this barrier were made during periods of flow, but it may be passable during periods of high precipitation and associated high discharge.

Elsewhere in the basin, it appears that poor habitat conditions (e.g., low annual runoff, narrow streambed, porous substrate) and numerous migration barriers prevent migration of adult steelhead into Natividad and Alisal creeks. It is unclear if resident *O. mykiss* persist in the upper reaches of those tributaries, or in Towne and Mud creeks.

2020 Sampling – Methods

eDNA Sampling Site Description

On January 9th, 2020, FISHBIO staff surveyed the Gabilan Creek/Tembladero Slough Watershed from the confluence of the Old Salinas River and Tembladero Slough to the most upstream point of Gabilan Creek not located on private land (Old Stage Road crossing). In addition, exploratory visits were made to key tributaries and sites, including Mud, Towne, Alisal, and Natividad creeks, as well as the confluence of Natividad, Alisal, and Gabilan creeks (at this point also referred to as Reclamation Ditch or Rec Ditch). The intention of this initial survey was to locate and evaluate key barriers identified by Casagrande and Watson (2006), and to identify suitable locations for weekly collection of eDNA and water quality samples. While conducting the survey, FISHBIO staff collected eDNA and water quality samples from all sites where flow was present (see *eDNA Sampling Protocol* and *eDNA Monitoring* sections below). Ultimately, nine sites ranging from the Old Salinas River to the Old Stage Road crossing of Gabilan Creek were selected for routine sampling (Table 1; Figure 1).

The two locations furthest downstream were at the Monterey Dunes Way crossing on the Old Salinas River (OSR; Figure 2) approximately 180 meters upstream of its confluence with Tembladero Slough, and at the Molera Road crossing of Tembladero Slough (TEM; Figure 3), approximately 100 meters upstream of its confluence with the Old Salinas River. The intention of monitoring TEM for *O. mykiss* eDNA was to detect any individuals moving upstream towards the Gabilan Creek watershed, whereas monitoring at OSR was to determine whether steelhead might be using the Old River as a migratory pathway in and out of the Salinas River, particularly during periods of sandbar closure at Salinas Lagoon.

The next upstream site was at the San Jon Road crossing of Gabilan Creek (SJR; Figure 4). This site is located between the cities of Castroville and Salinas and is surrounded by agricultural fields. Frequent irrigation on these fields likely contributes to runoff in this reach. The next two sites were located on mainstem Gabilan Creek just below (LRD) and above (LRU) the barrier at Little River Drive in the City of Salinas (Figure 5). Upstream of the Little River Drive barrier, there is a large concrete grade control structure just to the north of the Boronda Road crossing. Unfortunately, difficulties with access precluded sampling at this location, but it was presumed that the analysis of samples collected at Little River Drive and Hebert Road crossing, described below, would provide insight into whether fish were passing this structure.

Water quality and eDNA samples were also collected just above the concrete barrier at the Hebert Road site (HEB; Figure 6). The uppermost sampling site on Gabilan Creek was located at the Old Stage Road crossing (SRC; Figure 7), where samples were collected just above this possibly complete migration barrier (below the Old Stage Road bridge).

In addition to these seven sites (OSR, TEM, SJR, LRD, LRU, HEB, SRC), the Old Stage Road crossings of Natividad (NAT) and Alisal (ALI) creeks were visited routinely. However, as these sites were dry during the initial survey on January 9, and only NAT was observed to have experienced a very small amount of flow on a single day (see *Flow Monitoring* section below), no eDNA or water quality samples were collected there. The Mud Creek, Towne Creek, and Reclamation Ditch confluence sites were sampled during the initial survey on January 9 (Table A1) but were not included in the routine sampling for the remainder of the study.

Table 1. Site codes, locations, and sampling frequencies.

Code	Site	Coordinates	Sample Frequency
TEM	Tembladero Slough	36.772187, -121.787768	Bi-weekly
OSR	Old Salinas River	36.771886, -121.789785	Bi-weekly
SJR	San Jon Road	36.704949, -121.705084	Bi-weekly
LRD	Little River Road – Downstream of Barrier	36.705410, -121.621810	Bi-weekly
LRU	Little River Road – Upstream of Barrier	36.705410, -121.621810	Bi-weekly
HEB	Hebert Road	36.755801, -121.610458	Weekly
SRC	Old Stage Road	36.780490, -121.585460	Weekly
ALI	Alisal Creek	36.692490, -121.569012	Weekly
NAT	Natividad Creek	36.730224, -121.596080	Weekly

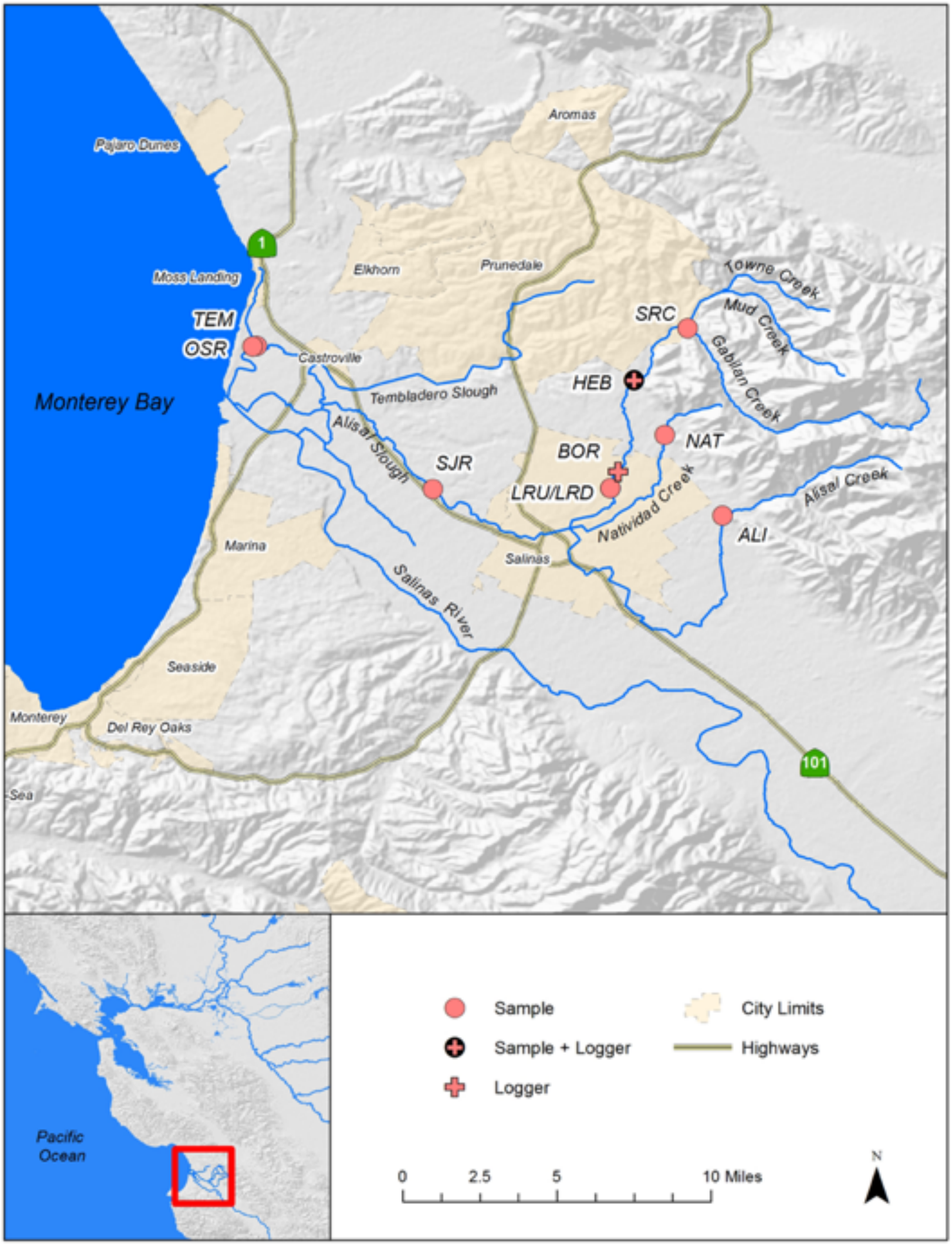

Figure 1. A map depicting the Gabilan Creek/Tembladero Slough Watershed. Labels indicate eDNA sampling locations and water level logger placement. See Table 1 for explanation of site codes.

Figure 2. Sampling site at the Monterey Dunes Way crossing of Old Salinas River (OSR). Photo taken April 7, 2020.

Figure 3. Sampling site at the Molera Road crossing of Tembladero Slough (TEM). Photo taken March 31, 2020.

Figure 4. The sampling site located at the San Jon Road crossing of Gabilan Creek (SJR). This photo was taken during a period of high flow on January 17, 2020.

Figure 5. The barrier and pool located at Little River Drive in the City of Salinas. Although the sample site at the downstream end of the pool (LRD) maintained flow for the duration of the study, the streambed upstream of the barrier (LRU) was frequently dry. Photo taken April 7, 2020.

Figure 6. The sampling site located at the Hebert Road crossing (HEB). The height from the top of the barrier to the substrate below was 1.22 meters (four feet), as measured during a period of no flow. Photo taken April 7, 2020.

Figure 7. Sampling underneath the bridge at the Old Stage Road crossing of Gabilan Creek (SRC), just above the barrier formed by a retaining wall and pipeline. Photo taken January 31, 2020.

Flow Monitoring Protocol

During the initial site survey, which occurred immediately following a precipitation event on January 9th, 2020, it was noted that the middle portion of the Gabilan Creek/Tembladero Slough Watershed between LRD and SRC rapidly lost flow. In an effort to evaluate the duration of connectivity across this reach following precipitation events, two HOBO U20-L water level loggers were deployed on January 24, 2020 (Table 2). The first was placed on the edge of the streambed at Boronda Road (BOR), approximately one kilometer upstream of LRD/LRU. The second was placed at the base of the concrete barrier at HEB. The intention was to use water level data from this site to determine the height difference between the water's surface to the top of the barrier during periods of flow, but this was complicated by shifting sediment (see *Flow Monitoring* section below). The loggers were programmed to record hourly pressure and temperature.

Loggers were retrieved following the final precipitation event of the monitoring period, on April 6, 2020. On April 10, 2020, the logger at BOR was recovered from the dry streambed. The HEB logger was not recovered until April 16, 2020, as flow persisted longer at that site and the logger had become buried under a significant amount of sediment. The data from both loggers was downloaded and processed using HOBOware Pro software. Ambient barometric pressure data recorded at the nearby Salinas Airport were downloaded from the Automated Surface Observing System (ASOS; available at https://mesonet.agron.iastate.edu/request/download.phtml), and input into HOBOware Pro to generate estimated water depths over the period of deployment based on the difference between the logger's pressure readings and ambient atmospheric pressure. Due to the flashy nature of the Gabilan system, and the rapidity with which this particular reach lost flow following precipitation events, the loggers were only submerged for brief periods.

Table 2. Water level logger sites and duration of deployment.

Site	Coordinates	Dates Deployed
BOR	36.713108, -121.618065	1/24/2020 - 4/10/2020
HEB*	36.7558010, -121.6104580	1/24/2020 - 4/16/2020

*Logger deployed at the same site where eDNA samples were collected.

eDNA Sampling Protocol

On January 9th, 2020, FISHBIO personnel surveyed the Gabilan Creek/Tembladero Slough Watershed to collect initial eDNA samples and to identify locations suitable for weekly eDNA sampling. Samples were collected from nine locations, of which five (TEM, OSR, SJR, LRU, LRD; Table 1, Figure 1) were selected for bi-weekly sampling. An additional four sites (HEB, SRC, ALI, NAT; Table 1, Figure 1) were selected for weekly sampling, and two of these four (ALI and NAT) were dry at the time of the initial site visit. Routine sampling began on January 17, 2020, at which time all sites were visited and sampled if flow was present. At the beginning of the following week only the lower sites (TEM, OSR, SJR, LRD) were sampled. This pattern – lower sites at the beginning of the week and all sites at the end of the week – continued through February 14, 2020. Thereafter, sampling was reduced to only the lower sites once a week due to a lack of rain and associated loss of flow throughout much of the watershed. No notable changes in flow occurred until a large precipitation event in mid-March (see *Flow Monitoring* section below), after which all sites continued to be checked once a week and sampled when flow was present. This continued until flows from the last large rain event in early April tapered off, and the last samples were collected on April 10, 2020.

Water samples were filtered using a Smith Root eDNA sampler backpack equipped with 5µm filters. Technicians filtered either a minimum of one liter of stream water or as much as possible using two filters per site. During periods of high turbidity (i.e., during and immediately following heavy rain events) filters became clogged more quickly, sometimes before it was possible to filter a total of one liter. In addition to filtered eDNA samples, technicians used a YSI water quality meter to record temperature (˚C), dissolved oxygen (mg/L), total dissolved solids (mg/L), salinity (ppt), conductivity (µS/cm), and specific conductivity (µS/cm). Staff also recorded river stage at sites where a gauge was present (OSR, SJR). Any abnormal conditions (e.g., oil sheen on surface, signs of eutrophication) as well as observations on apparent passability of barriers were noted.

In total, 121 eDNA samples were collected between January 9, 2020, and April 10, 2020. At the end of the season, redundant or non-informative samples were identified (i.e., repeated samples from LRD during extended periods of no connectivity, low-volume samples from a site where a second sample of larger volume was collected, and samples of volumes less than 0.4 L). Ultimately, 100 of these samples were submitted to Precision Biomonitoring (Guelph, Ontario) for analysis (Table 3). The bulk of these samples (n = 94) came from TEM, OSR, SJR, LRU, LRD, HEB, and SRC, but an additional six were collected from various other sites throughout the watershed during the initial survey on January 9, 2020 (Table 3).

Table 3. Total number of eDNA samples by site and respective date ranges covered.

Site	Samples Analyzed	Sample Date Range
TEM	24	1/9/2020 - 4/10/2020
OSR	26	1/9/2020 - 4/10/2020
SJR	26	1/9/2020 - 4/10/2020
LRU	2	3/16/2020 - 4/7/2020
LRD	5	1/9/2020 - 4/7/2020
HEB	6	1/9/2020 - 4/10/2020
SRC	5	1/9/2020 - 4/7/2020
TWN*	1	1/9/2020
MUD*	2	1/9/2020
GAB*	2	1/9/2020
REC*	1	1/9/2020
Total	**100**	

* Sites were visited and sampled only once during the initial watershed survey, see Table A1 in Appendix for locations.

2020 Sampling – Results

Flow Monitoring

Very little precipitation occurred in the Gabilan/Tembladero Slough Watershed in the winter of 2019 and spring of 2020. February 2020 was the driest February on record for the state of California, and no precipitation was measured at the Salinas Airport during that month. As a result, much of the Gabilan/Tembladero Slough Watershed was without flow for extended periods of time. Even following the few large precipitation events that occurred later in the spring, flow was ephemeral between HEB and LRD. This portion of the creek bed, and particularly the location where the BOR water level logger was placed, was often completely dry within one or two days following a rain event. As such, the spring of 2020 clearly did not represent the "exceptionally favorable" hydrologic conditions that were posited by Boughton and Goslin (2006) to be necessary in order for the upper watershed to support steelhead. Nonetheless, the watershed experienced several very brief periods of connectivity when passage by anadromous adults may have been possible.

The BOR water level logger was deployed for 46 days (January 24 through April 10, 2020) near the Boronda Road crossing (Figure 1; Figure 8), and the HEB water level logger was deployed for 52 days (January 24 through April 16, 2020) at the base of the concrete barrier at the Hebert Road crossing (Figure 1; Figure 9). During this period, precipitation was measured at Salinas Airport on 20 days (Table 4). Three of these rain events occurred between January 16 and 26, and the remaining 17 occurred throughout March and early April 2020. Logged water levels at both sites appeared to peak approximately one day after rain events. Resulting flows were not sustained for more than 24 hours at BOR. At HEB flows following large rain events or multiple days of rain generally persisted for at least 48 hours, but it is unlikely that the water level remained high enough for the concrete barrier (Figure 10) to be passable for the entirety of that duration.

Table 4. Days when precipitation occurred during and shortly before the water level logger deployment period (January 24 through April 16, 2020) and total amount of precipitation as measured at the Salinas Airport (source: NOAA National Centers for Environmental Information, available at https://www.ncdc.noaa.gov/).

Date	Precipitation (cm)	Date	Precipitation (cm)
1/16/20	1.83	3/19/20	0.08
1/17/20	0.05	3/21/20	0.18
1/26/20	0.28	3/22/20	0.99
3/1/20	0.10	3/23/20	0.03
3/7/20	0.03	3/24/20	0.23
3/10/20	0.20	3/25/20	0.28
3/14/20	0.28	3/26/20	0.13
3/15/20	0.94	3/28/20	0.05
3/16/20	1.24	4/5/20	2.29
3/17/20	0.08	4/6/20	0.46
		Total	**9.75**

Figure 8. BOR logger site at the time of deployment (January 24, 2020). Arrow indicates the position of the logger.

Figure 9. HEB logger site at the time of deployment (January 24, 2020). Arrow indicates the position of the logger, black line indicates height of the barrier, which was 1.22 m above the substrate.

Figure 10. The large concrete barrier located just north of the Boronda Road crossing on Gabilan Creek. Photo was taken on April 7, 2020, 24 hours after a large precipitation event. Laminar flow down the concrete ramp suggests this barrier may have remained impassable at all but the highest flows experienced in 2020.

The standard operating procedure for critical riffle analysis for fish passage in California (Haas 2017) states the minimum depth of instream flow required to facilitate adult steelhead passage is 0.7 feet (~21 cm). Review of the water level logger data indicates that BOR was only above this threshold for one hour on March 16 (Figure 11) and for six hours on April 5. Although the BOR logger was placed near the eastern bank of the creek, the lack of a clear thalweg in this stretch along with the presence of a storm sewer inflow pipe immediately adjacent to the logger suggest that it likely experienced as much or greater depth than any other location across the width of the stream bed. Moreover, even if this threshold is conservative, it is unlikely the barrier located approximately 0.25 km upstream near the Boronda Road crossing (Figure 10) would have been passable at lower flows.

Calculated water levels based on data from the HEB logger were significantly higher and more persistent than those for BOR. This was expected, as the HEB logger was placed at the lowest point in a pool immediately underneath the small waterfall caused by the concrete barrier under Hebert Road. According to NOAA's Guidelines for Salmonid Passage at Stream Crossings (National Marine Fisheries Service 2001), salmonids require a pool depth of at least 1.5 times the jump height or a minimum of 0.6 meters (whichever is deeper) in order to successfully clear a barrier. Filtering the HEB logger data indicates that these standards were met from April 5 through April 13, however, this time period coincides with the largest precipitation event during the monitoring period (2.29 cm on April 5; Table 4), at which time the logger became buried under a significant amount of sediment (~71 cm; Figure 12). As such, the pressure readings during this period were not an accurate reflection of water depth, and it is highly unlikely that a jump height to depth ratio greater than 1.5 was maintained for this entire period, although the initial readings during the rain event and those shortly thereafter may be more accurate. If the minimum ratio is reduced from 1.5 to 1.25 times, as suggested by other sources (e.g., Robison et al. 2000), then the level logger data indicate that the standards were met for one hour on March 26 as well as during the likely erroneous early April period.

Plotting the calculated water levels and precipitation shows a pattern of ephemeral peaks following rain events (Figure 13; Figure 14). Data from April 1st onward were excluded from the HEB plot, as excessive positive and negative water level estimates during this period suggest erroneous pressure readings. However, site visits confirmed that flow was maintained at HEB at least through April 10. The apparent peak in flow detected at HEB between the evenings of February 3 and February 5 is unexplained. Although the site was not visited during this period, it was found to be devoid of flow on January 31 and February 7. Although it is possible that flow from an unknown source (e.g., irrigation runoff from surrounding crop fields) occurred during this period, the atypical shape of the curve and the lack of precipitation during this period seems to suggest that these readings were either the result of a malfunction or tampering with the logger. Regardless, estimated water depth during this period peaked at 29 cm (approximately 11.4 inches), suggesting the barrier would likely have remained impassable.

Based on the standards discussed above, it appears that mainstem Gabilan Creek had sufficient flow to facilitate the passage of an adult steelhead for only seven of 1,837 monitored hours, or 0.38% of the monitoring period. Moreover, the barriers at BOR (Figure 10; no photos from peak flow available), LRD/LRU (Figure 15), and HEB (Figure 16) may have only been passable for an even shorter period. Following periods of high flow, the pool at LRD quickly lost upstream flow, and its outflow was also occasionally blocked by debris (Figure 17). Although flow at HEB was often maintained for a longer period of time following precipitation events (generally ~48 hours), the rapid loss of flow between Boronda Road and Little River Drive (generally ~24 hours) may have prevented any steelhead from reaching the pool at HEB. It is also important to note that water quality conditions at the Little River Drive pool quickly became unsuitable for steelhead during periods of zero flow input from upstream. Measured DO in the pool was frequently below 4 mg/L, and at one point dropped below 1 mg/L. Water quality may have been further impaired by storm drain input, as an oil sheen was evident on the surface of the water on at least one occasion.

Figure 11. The BOR water level logger location during the high flow event on March 16, 2020. Arrow indicates the position of the logger.

ALI and NAT were visited frequently throughout the early portion of the monitoring period (i.e., through February 14), but site visits were halted during the extended rain-free period. Later in the spring (i.e., mid-March to early April), these sites were revisited following rain events. No flow was ever detected at ALI, likely due in large part to a retaining pond located approximately 120 meters to the east of the Old Stage Road crossing of Alisal Creek. Low flow was observed at NAT on March 16 (Figure 18), but downstream flow was minimal. No eDNA sample was collected. A disconnected puddle was present below the culvert at NAT on April 7, but there was no outflow. Notably, a fence along the property just west of the Old Stage Road crossing of Natividad Creek runs across the entirety of the creek bed, and would likely prevent adult steelhead passage even if flows were sufficient. At the points along Old Stage Road, neither Natividad nor Alisal creeks appear to permit fish passage, and they were certainly never passable during the spring of 2020.

The barrier present at the highest site in the watershed that was surveyed in the 2020 monitoring season (SRC; Figure 1) did not appear passable even after the large flow events in March and April (Figure 19). Although water quality at this site was consistently suitable for *O. mykiss*, it appears unlikely that any anadromous individuals would have been able to reach it. Construction activity was noted under the Old Stage Road bridge on March 16 (Figure 20), but without apparent consequence for fish passage.

Flow at the two sites (TEM and OSR) closest to the ocean was continuous, although they experienced substantial tidal variation. For example, stage readings at OSR varied from 0 (i.e., sea level) to 2 feet (~61 cm). Flows were also maintained through the entire study period further upstream at SJR, where stage varied from 0.7 to 1.3 feet (21 to 40 cm). At all three of these sites, water quality appeared to remain suitable for *O. mykiss* throughout the study period.

Figure 12. The Hebert Road (HEB) water level logger was buried under approximately 71 cm (~28 inches) of sand sometime during or following the large rain event on April 5. Photo taken April 16, 2020.

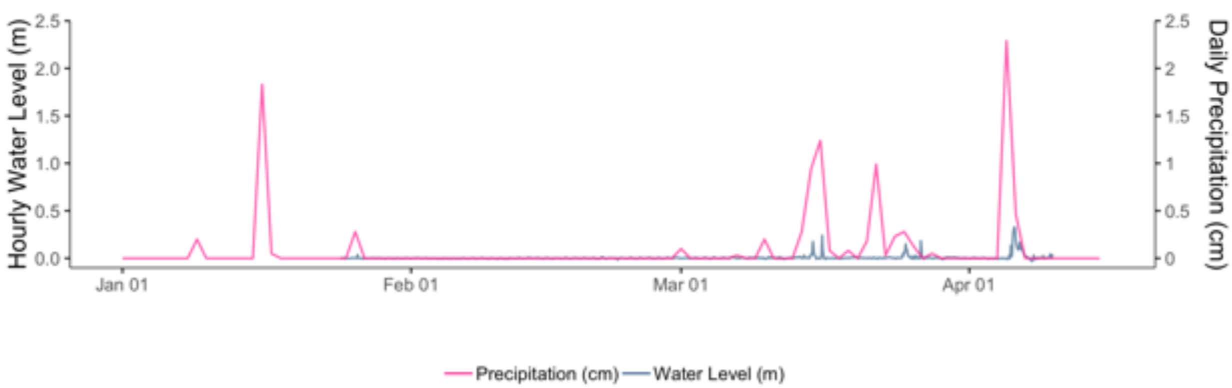

Figure 13. Logged water level at BOR and daily precipitation, as measured at Salinas Airport. Water level data begins on January 24, 2020 when the BOR logger was deployed.

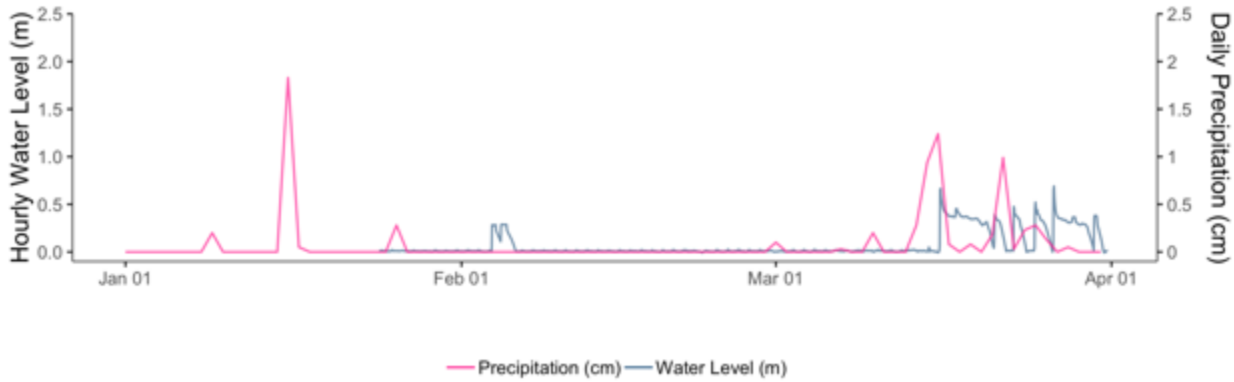

Figure 14. Logged water level at HEB and daily precipitation, as measured at Salinas Airport. Water level data begins on January 24, 2020, when the HEB logger was deployed. Water level data collected beyond March 31 are excluded, as both excessively high and excessively low values during this period suggest that shifting sediment interfered with accurate pressure readings. The apparent peak in water level between the evening of February 3 and the evening of February 5 is unexplained. See text for details.

Figure 15. High flow at Little River Drive (LRD) on March 16, 2020, following substantial rain.


Figure 16. High flow at Hebert Road (HEB) on March 16, 2020, following substantial rain.


Figure 17. The pool at Little River Drive (LRD) on March 24, 2020. High flows during the preceding week washed a large amount of woody debris into the pool, which blocked nearly all outflow at the pool's downstream end.

Figure 18. Natividad Creek (NAT) during the high flow period on March 16, 2020. Outflow was minimal, and the presence of a fence suggests passage by adult steelhead may be impossible.

Figure 19. Old Stage Road crossing (SRC) on March 16, 2020, after a large precipitation event. The barrier consisting of a retaining wall and an old pipeline did not appear passable at any time during the 2020 monitoring season.

Figure 20. Bridge construction at Old Stage Road crossing (SRC). Photo taken March 31, 2020.

eDNA Monitoring

A total of 100 eDNA samples (Table A2) were shipped to Precision Biomonitoring (Guelph, Ontario) on May 5, 2020. Although all 100 samples yielded workable levels of DNA, PCR was partially or entirely inhibited due to significant amounts of sediment adhered to the filters, which necessitated an additional step of optimization and dilution. The laboratory indicated that the use of soil-based DNA extraction kits may be warranted in any future sampling of this system.

Overall, *O. mykiss* eDNA was detected in 13 of the 100 samples (Table 5). Three technical replicates were run for each sample, and of the positive samples, only three exhibited 100% detection across all three replicates: sample 002 from GAB and samples 027 and 117 from SRC (Table 5). Further, sample 117 from SRC exhibited the highest DNA concentration of the positive samples, which likely reflects proximity and/or greater abundance of *O. mykiss* near the sample site at the time of collection. Spatially, positive detections ranged across all sites between SRC and SJR, although detections at the two most upstream sites (HEB and SRC) were more frequent. Field protocols to reduce contamination risk make it exceedingly unlikely that detections resulted from cross contamination. The only potential indication of a discrepancy is seen in sample numbers 006 and 005. Sample 006 was collected in the pool immediately downstream of the barrier at HEB (Figure 9; Figure 16) and tested negative for *O. mykiss* DNA, while sample 005 was taken at the same time in the stream immediately above the plunge pool and tested positive for *O. mykiss* DNA. If eDNA was present in the upstream sample, it is reasonable to expect that it would also be present in a sample collected less than a meter downstream. However, it is possible that higher turbidity (resulting in sediment trapped on the filter) in the plunge pool may have prevented the detection of DNA. Only one of the three PCR technical replicates for sample 005 successfully amplified *O. mykiss* DNA, lending further support for this explanation, as it suggests that either the turbidity of the water was resulting in interference even in the above barrier sample, and/or that the amount of DNA in stream was already minimal before being further diluted in the plunge pool. Either of these factors may have overwhelmed the assay's ability to detect any DNA that was present in the pool.

Most of the positive samples (8 of 13) were collected at HEB and SRC and are indicative of the presence of resident *O. mykiss* in the upper watershed. Fine particulate organic matter (FPOM) – including partial or intact cells or clusters of cells that may contain DNA – settles out of the water column more quickly and therefore experiences less downstream drift during periods of low flow (Wipfli et al. 2007). Conversely, periods of high flow are likely to result in FPOM being transported greater distances downstream. Understanding of the dynamics of eDNA transport in lotic systems is limited (Wilcox et al. 2017), but studies in small river systems have indicated that eDNA may be detected anywhere from 50 meters to nine kilometers downstream of a target organism (Deiner and Altermatt 2014; Pilliod et al. 2014). As such, it follows that the positive detections at HEB and SRC may have resulted from downstream transport of eDNA from *O. mykiss* in the reaches of the upper watershed. On one occasion (January 9, 2020), the sample collected at HEB was positive while the sample collected at SRC was negative, which would suggest that *O. mykiss* were present somewhere between the two sites, or that there was a false negative detection at SRC. The other single occasion when HEB was the only positive was March 16, 2020, but no sample was collected from SRC on this date. Because the shelter-in-place order precluded surveys of the system above SRC during the 2020 monitoring season, there was no opportunity to visually confirm the presence of fish in the upper watershed. However, past surveys and FISHBIO's surveys conducted in 2021 indicate that there is a population of resident *O. mykiss* occurring in the portions of the system above SRC (see *2021 Sampling – Results* section below). The only site within nine kilometers of SRC and the upper watershed was HEB, whereas all others exceeded the maximum expected distance for downstream transport of DNA from upper watershed resident trout (Table 6).

The positive samples further downstream at LRU and LRD may also have resulted from transport of eDNA from HEB and reaches slightly further upstream. For example, the numerous detections among samples collected on April 7, 2020 (Table 5) may have resulted from increased drift of FPOM and associated eDNA resulting from the 2.75 cm of precipitation that occurred over the preceding two days. In addition to transporting FPOM, individual *O. mykiss* may have migrated or been displaced downstream over apparent unidirectional barriers such as those at SRC (Figure 19) and Boronda Road (Figure 10) during these high flow events, which may have resulted in their detection at more downstream sites.

Table 5. All eDNA samples that tested positive for *O. mykiss*. A total of three technical replicates were run on each sample, and the number of positive detections indicates how many of these replicates resulted in successful amplification of *O. mykiss* DNA.

Sample ID	Site	Date	Time	Volume Filtered (L)	Number of Positive Detections
002	GAB	1/9/2020	8:40	0.52	3
003	GAB	1/9/2020	8:50	0.56	1
005	HEB	1/9/2020	11:15	0.51	1
027	SRC	1/17/2020	11:47	1.08	3
051	SRC	1/31/2020	9:30	2.22	1
074	SRC	2/14/2020	10:10	3.04	1
100	HEB	3/16/2020	10:15	1.00	1
109	SJR	3/31/2020	9:01	1.00	1
113	LRD	4/7/2020	8:17	1.01	2
114	LRU	4/7/2020	8:33	1.50	1
115	HEB	4/7/2020	9:07	0.40	2
117	SRC	4/7/2020	9:45	1.36	3
121	HEB	4/10/2020	8:56	1.01	2

Table 6. Distance (in river kilometers) between each pair of sample sites above the diagonal. Sites where positive *O. mykiss* detections occurred on the same day are indicated by * below the diagonal.

	TEM	OSR	SJR	GAB	LRD/LRU	HEB	SRC
TEM	-	0.27	14.01	21.77	24.99	31.31	35.59
OSR		-	13.74	21.50	24.72	31.04	35.32
SJR			-	7.76	10.98	17.30	21.58
GAB				-	3.22	9.54	13.82
LRD/LRU					-	6.32	10.60
HEB				*	*	-	4.28
SRC					*	*	-

As noted above, the window of time during which flows were sufficient to allow for upstream migration beyond the barrier at Little River Drive was quite limited, but it is conceivable that fish were able to move at least as far as LRD for most of the monitoring period. On the day of a positive detection at GAB (January 9, 2020) the only other positive was at HEB and samples from all other sites (REC, TWN, MUD, SRC, LRD, SJR, TEM, and OSR) were negative. The extensive sample coverage on that day and the fact that flow was disconnected between HEB and GAB (at the LRD/LRU barrier; Figure 17) suggest that it is unlikely downstream transport of eDNA was responsible for the positive, although it is still possible one or more fish were physically transported downstream during the apparent period of connectivity that occurred immediately prior to sampling (as indicated by persistent shallow pools above LRD). On the day when a positive detection occurred at SJR (March 31, 2020), the only other sites sampled were OSR and TEM (both negative), so it is not possible to ascertain whether transport from a more upstream site was responsible.

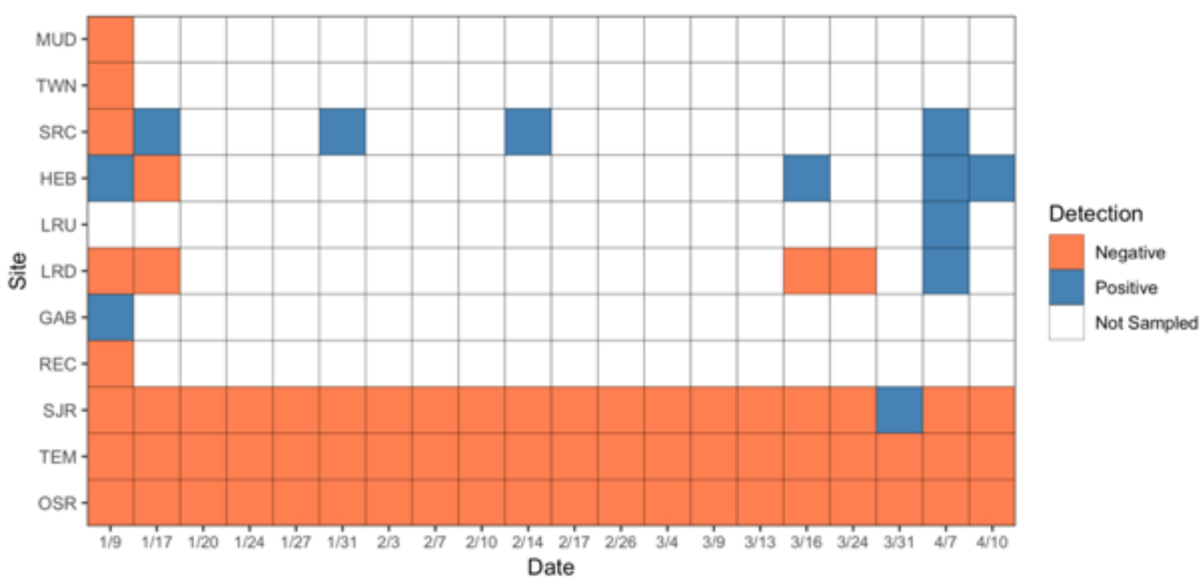

Figure 21. Positive and negative detections across all 100 assessed samples from the 2020 monitoring season, with sites in descending order from upstream to downstream. Note that MUD, TWN, REC, and GAB were only sampled once on January 9, 2020, and that HEB and LRU were dry for most of the monitoring period. Samples from lower sites (TEM, OSR, SJR) were prioritized for analysis, as the lack of connectivity dry above LRD precluded the possibility of steelhead accessing more upstream locations for much of the monitoring period. Further, some of the tiles represent multiple samples collected from a single site on a single day, and some of the "not sampled" dates were sampled but samples were not analyzed (e.g., LRD during extended periods with no flow input from upstream). See Table A2 for the full list of analyzed eDNA samples.

If upstream migrating adult steelhead were responsible for the detections at these lower sites, it is somewhat surprising that no detections occurred at one or both of the two most downstream sites (OSR and TEM) over the entire monitoring period. It is possible that higher levels of total dissolved solids at these lower sites led to greater interference with the assay, but samples collected from the sites further upstream were also laden with significant amounts of sediment.

There is also the possibility that tidal mixing could have influenced the detection at these sites, but studies indicate that eDNA in tidally influenced habitats tends to reflect organisms occurring within said habitat (Kelly et al. 2018). As such, if a steelhead was passing through the slough on its way upstream, its DNA signal should have been detectable. Further, it is likely that tidal flux would keep eDNA suspended in the water column at a given location for a longer period of time than in a reach that is not tidally influenced. The frequency with which TEM and OSR were sampled reduces the possibility that upstream passage of an adult steelhead remained undetected. However, samples were only collected twice a week, leading to periods of up to four consecutive days when fish could have passed through without a sample being collected. Also, the relative rarity of steelhead in the watershed, in conjunction with the larger volume of water at OSR and TEM may have resulted in eDNA concentrations below the detection limit. Lastly, a possible explanation for downstream detections may be that juveniles derived from the resident population smolted and outmigrated during periods of higher flow. Offspring produced by individuals with a resident life history may include individuals that express an anadromous life history (Kendall et al. 2015), which may occur occasionally among the upper watershed population in the Gabilan system. However, the apparent lack of connectivity with the upper watershed on the date of the positive detection at GAB suggests this explanation is unlikely. While the current resolution of sampling data does not permit definitive identification of the source of DNA detected at SJR and GAB, it can be concluded that 1) based on the low frequency of detections, steelhead (juvenile or adult) occurrence in the lower watershed is exceedingly rare; 2) opportunity for adult steelhead to access the upper watershed for spawning is extremely limited; 3) a resident population of *O. mykiss* in the upper watershed results in frequent detection of *O. mykiss* eDNA at SRC and HEB; and 4) that ephemeral stream sections are an impediment to migration opportunity, in both directions.

2021 Sampling – Methods

Overview

In the late winter of 2020 and spring of 2021, FISHBIO personnel conducted additional monitoring and assessments in the Gabilan watershed. In addition to gathering more data on flow and connectivity throughout the middle and lower watershed and collecting additional eDNA samples in the lower watershed to detect the presence of any returning adult steelhead, the main objective of the 2021 monitoring effort was to conduct an assessment the available habitat and presence of *O. mykiss* in the upper watershed.

Replicating the protocol of the 2020 monitoring, two HOBO U20-L water level loggers were once again deployed at HEB and BOR on December 21, 2020 to gather more data about connectivity and flow in the watershed following precipitation events. However, both loggers were stolen prior to attempted recovery on April 6, 2021, and therefore no new water level data were obtained. The theft of the equipment may have been partially due to riparian brush clearing at both BOR and HEB, which made difficult to effectively conceal the loggers. Because of the lack of water level data, assessments of flow for the 2021 monitoring season are limited to visual observations by FISHBIO personnel during eDNA and water quality sample collection, and comparisons between 2020 and 2021 precipitation and water level data.

The main activities in 2021 monitoring were continuation of eDNA and water quality sampling at sites in the lower watershed (SJR, TEM, and OSR; Figure 1), and fish sampling and habitat surveys in previously unsurveyed portions of the upper watershed that flow through two privately owned ranches upstream of the Old Stage Road crossing. The methods and results of these activities are described below.

eDNA Sampling Protocol

Collection of eDNA samples during the 2021 monitoring season was limited to the three most downstream sites (SJR, TEM, OSR; Figure 1), as detection of *O. mykiss* DNA at sites in the upper watershed would provide no new information (see discussion on downstream transport of eDNA from resident trout in the upper watershed).

The eDNA sampling protocols used during the 2020 monitoring season were replicated in 2021. Samples were collected at SJR, TEM, and OSR once per week from December 17, 2020, through January 25, 2021, and then twice per week from January 25 through February 8, 2021. Monitoring after February 8 was to be limited to periods following significant precipitation (i.e., greater than 1 cm within a 24-hour period) as water level monitoring during 2020 indicated that at least that much would be necessary to achieve sufficient flow over a reasonable timeframe to allow for passage between LRD and HEB (see the *2020 Sampling Results – Flow Monitoring* section above). However, no substantial rain events occurred beyond February 8, 2021, and no additional samples were collected. No additional eDNA samples were collected during the upper watershed surveys that occurred in May, as *O. mykiss* were observed and captured in all but one of the wetted stream reaches that were assessed.

In total, 38 eDNA filters were collected between December 17, 2020 and February 8, 2021. At the end of the season, redundant or non-informative samples were identified (i.e., lower volume samples from a site where a second sample of larger volume was collected). Ultimately, 29 of these samples were submitted to Jonah Ventures (Boulder, Colorado) for analysis (Table 7). Samples were collected at all three sites during each sampled date, with the exception of TEM on January 29, 2021, as extremely high turbidity precluded effective sample collection.

Table 7. Analyzed eDNA samples from the 2021 monitoring season by site and date

Site	Samples Analyzed	Sample Date Range
TEM	9	12/17/2020 - 2/8/2021
OSR	10	12/17/2020 - 2/8/2021
SJR	10	12/17/2020 - 2/8/2021
Total	**29**	

Upper Watershed Fish and Habitat Surveys

FISHBIO personnel were granted access to two privately owned ranches – Vierra Ranch and Bardin Ranch – which collectively include the stretch of Gabilan Creek from the Old Stage Road crossing to approximately 9.8 km upstream where the stream descends from the steep, uppermost reaches in the Gabilan Mountains and enters the upper valley. Fish and habitat assessments conducted on each of these properties included electrofishing, water quality, barrier measurements and simple visual surveys, based on habitat conditions and the preferences of the respective landowners.

On May 5, 2021, FISHBIO personnel surveyed the reach within Vierra Ranch (the property immediately adjacent to Old Stage Road). Personnel descended into the streambed where access was possible in order to conduct multiple pass backpack electrofishing, and to record water quality using a YSI water quality meter (i.e., temperature [˚C], dissolved oxygen [mg/L], total dissolved solids [mg/L], salinity [ppt], conductivity [µS/cm], and specific conductivity [µS/cm]). In addition, depth and width measurements were taken for accessible pools, and dimensions of a potential migration barrier were recorded. Because of the very incised nature of the stream, extremely dense vegetation, and abundant poison oak, examination of the entire 3.3 km of streambed located on this ranch was not possible. However, visual observations were made along its entire length from the vantage point of an access road that parallels the stream, and the extent of wetted channel was recorded.

On May 6, 2021, FISHBIO personnel surveyed the reach within the Bardin Ranch, the further upstream of the two properties that includes the portion of the creek from where it descends out of the Gabilan Mountains downstream to the border of the Vierra Ranch (approximately 6.5 km of stream). This survey involved an escorted tour provided by the landowner, during which time photographs, GPS coordinates, and visual observations of wetted reaches and fish were recorded. Again, examination of the entire ~6.5 km of streambed was not possible, but the extent of wetted channel was estimated based on visual observation of multiple points throughout the stream's length on the property.

During both surveys, anecdotal information was provided by the ranch manager and landowner, respectively. This included typical occurrence and extent of flows across time and space, estimated sizes of fish that have been captured and observed in the stream over the years, and seasonal and interannual variation in stream conditions.

2021 Sampling – Results

Flow Monitoring

As noted above, no additional water level data were obtained due to the deployed water level loggers being stolen prior to recovery. However, during several eDNA and water quality sampling events, FISHBIO staff traveled to additional sites further upstream to visually assess flow. These included evaluations of LRD and BOR on January 5, 2021 after 0.06 cm (~0.02 inches) of rain over the preceding two days, and on January 29, 2021 after 7.7 cm (~3 inches) of rain over the preceding three days (the largest precipitation event that occurred during the 2021 monitoring season, and larger than any precipitation event recorded in 2020; Figure 22, Table 8). During both of these assessments, there was no flow at BOR (Figure 23), no flow above LRD (Figure 24), and very little flow downstream of LRD (Figure 25). Despite the lack of flow at BOR and LRD on January 29, very high flows were observed at SJR (Figure 26), and the stage levels at TEM (Figure 27) and OSR (Figure 28) were extremely high. Notably, January 29, 2021 was also the date when the Salinas Lagoon was breached.

Table 8. Days when precipitation occurred from December 1, 2020, through May 31, 2021, as measured at as measured at the Salinas Airport (source: NOAA National Centers for Environmental Information, available at https://www.ncdc.noaa.gov/).

Date	Precipitation (cm)	Date	Precipitation (cm)
12/11/20	0.41	2/2/21	0.18
12/13/20	0.33	2/11/21	0.38
12/17/20	0.43	2/12/21	0.05
12/26/20	0.05	2/13/21	0.08
12/27/20	0.03	2/15/21	0.03
12/28/20	0.03	2/20/21	0.13
12/30/20	0.03	3/6/21	0.13
12/31/20	0.15	3/9/21	0.69
1/3/21	0.03	3/10/21	0.84
1/4/21	0.03	3/14/21	0.30
1/22/21	0.58	3/18/21	0.43
1/24/21	0.36	3/19/21	0.08
1/26/21	0.03	4/20/21	0.05
1/27/21	6.48	4/25/21	0.05
1/28/21	1.19	5/14/21	0.03
1/29/21	0.10	**Total**	**13.71**

The lack of flow upstream of LRD on the morning of January 29 despite 7.7 cm (~3 inches) of precipitation over the preceding three days is in line with findings from 2020, which indicated that the Gabilan Creek between HEB and LRD dries rapidly even after large precipitation events. The combination of highly porous, sandy substrate and a highly channelized streambed likely contribute to the flashy flows typical of the system. Although water level data are unavailable, the fact that 2.29 cm (~0.9 inches) of rain on April 5, 2020 resulted in an estimated six hours of flow conditions potentially suitable for adult steelhead passage (see the *2020 Sampling Results – Flow Monitoring* section above) and only two days in the 2021 monitoring season surpassed this level of precipitation (January 26 and 27, 2021; Figure 22, Table 8) would suggest a maximum of two days of passable conditions. In reality, the actual period of connectivity was likely shorter. Flow was also visually assessed during the upper watershed surveys, and more information is provided in the *Upper Watershed Fish and Habitat Surveys* section below.

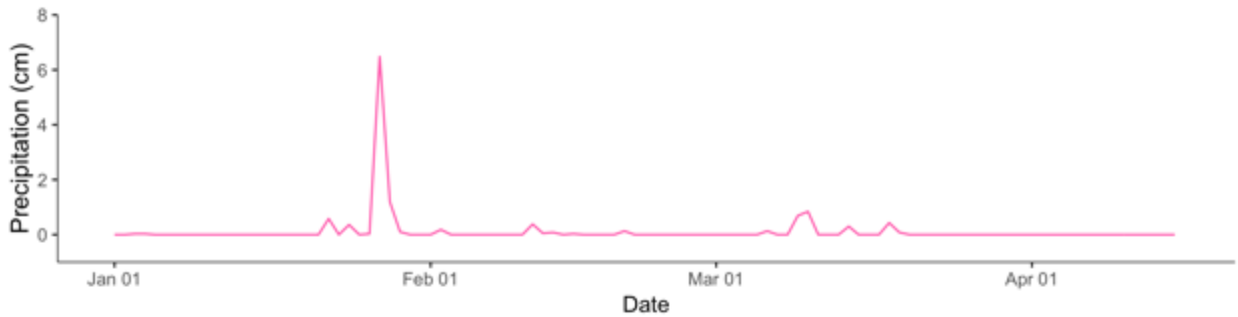

Figure 22. Daily precipitation, as measured at Salinas Airport, from January 1 through April 15, 2021.

Figure 23. BOR on January 29, 2021, after 7.7 cm of rain fell in Salinas over the preceding three days. Although some small puddles suggest flow may have been recently present, no surface flow was observed between LRD and the Boronda Road crossing.

Figure 24. The barrier at LRD on January 29, 2021, after 7.7 cm of rain fell in Salinas over the preceding three days. Despite the heavy precipitation, there was no flow observed above the barrier at LRD.

Figure 25 Facing downstream from the barrier at LRD on January 29, 2021, after 7.7 cm of rain fell in Salinas over the preceding three days. Although water remained in the pool at LRD, there was no downstream flow.

Figure 26. SJR on January 29, 2021, after 7.7 cm of rain fell in Salinas over the preceding three days. The river stage was 3.7 feet as measured by the gauge at the San Jon Road crossing, which was 1.64 feet higher than the next highest stage observed across the entirety of 2020-2021 monitoring.

Figure 27. TEM on January 29, 2021, after 7.7 cm of rain fell in Salinas over the preceding three days. The river stage was 18 feet as measured by the gauge at the Molera Road crossing. No eDNA sample was collected from the site on this date due to excessive sediment that repeatedly clogged the collection filters.

Figure 28. OSR on January 29, 2021, after 7.7 cm of rain fell in Salinas over the preceding three days. The river stage was 3.5 feet as measured by the gauge at Monterey Dunes Way, which was 0.5 feet higher than the next highest stage observed across the entirety of 2020-2021 monitoring.

eDNA Monitoring

A total of 29 eDNA samples (Table A3) were shipped to Jonah Ventures (Boulder, Colorado) on May 12, 2021. *O. mykiss* eDNA was detected in three of the 29 samples (Table 9; Figure 29). Three technical replicates were run for each sample, and none of the positive samples exhibited 100% detection across all three replicates. Sample 217 exhibited replication in two of the three replicates, and samples 218 and 220 exhibited replication in only one of the three technical replicates. As such, all three of these samples were considered weak positives.

Positive detections were recorded only at the two sampling locations located furthest downstream (OSR and TEM). It is unlikely that any of these positive detections resulted from cross contamination of negative samples with a positive sample, as field protocols were specifically followed to mitigate the risk of this occurring. It is unlikely that the detection of *O. mykiss* eDNA in the Old Salinas River and Tembladero Slough was the result of a juvenile steelhead smolt outmigrating, as Gabilan Creek remained dry from at least LRD up to HEB, and outmigration of *O. mykiss* or downstream transport of eDNA from the upper watershed was not possible.

As such, a likely explanation for these detections is the presence of one or more adult steelhead in the lower watershed shortly before or when these samples were collected. It is unlikely these steelhead would have moved upstream in Gabilan during this time, as the low flows mentioned above would have prevented them from passing upstream. This hypothesis is supported by the lack of detections at the next most upstream site (SJR) at the time of detection in the lower sites and in the subsequent weeks. Notably, the Salinas River Lagoon remained closed during these positive detections, and would not breach until January 29, 2021, 11 days after the last detection at OSR. The potential presence of adult steelhead in the Old Salinas River even during periods when a sandbar prevents direct entry to the Salinas River raises the question of whether individuals are using or attempting to use the Old River channel to gain access to the Salinas via the lagoon tidegate. However, the effects of tidal mixing and the collection of eDNA samples from only two sites in the lower watershed prohibit a more precise analysis of where and when adult steelhead may have been moving.

Similar to sampling in 2020, high turbidity following large precipitation events made collection of eDNA samples difficult, as collection filters quickly clogged with sediment. On January 29, 2021, when water levels at OSR, TEM, and SJR were at their highest (Figure 28, Figure 27, and Figure 26, respectively), it was not possible to collect a sample at TEM due to the excessive amount of sediment in the water (see "Not Sampled" tile in Figure 29). Sediment trapped in the filter may also inhibit the amplification of *O. mykiss* DNA during the analysis process. These factors, combined with the dilution of any DNA present in the watershed during periods of high river stage, suggest that the times when flows are high (i.e., the times when steelhead are most likely to be migrating) are also the times when it is most difficult to detect their presence using eDNA.

Table 9. All eDNA samples from the 2021 monitoring season that tested positive for *O. mykiss*. A total of three technical replicates were run on each sample, and the number of positive detections indicates how many of these replicates resulted in successful amplification of *O. mykiss* DNA.

Sample ID	Site	Date	Time	Volume Filtered (L)	Number of Positive Detections
217	OSR	1/12/2021	10:15	1.05	2
218	TEM	1/12/2021	10:48	1.07	1
220	OSR	1/18/2021	9:44	1.01	1

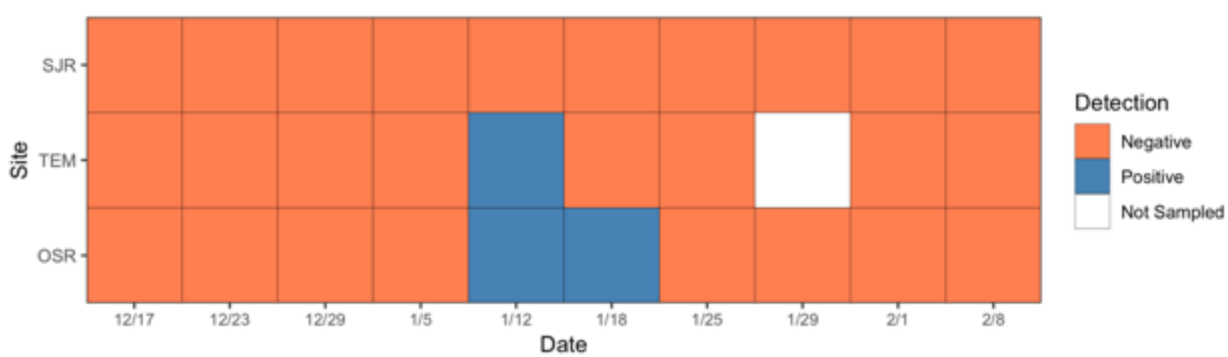

Figure 29. Positive and negative detections across all 29 assessed samples, with sites in descending order from upstream to downstream. See Table A3 for the full list of analyzed eDNA samples collected in 2021.

Upper Watershed Fish and Habitat Surveys

The findings of the upper watershed fish and habitat surveys are presented in spatial order from the furthest upstream site (BDR1) to the most downstream site (VRR2; Table 10, Figure 30). The Bardin Ranch property encompasses Gabilan Creek from its origin in the Gabilan Mountains downstream to the point where it flows onto Vierra Ranch property. FISHBIO personnel were provided a tour of the stream by ranch owner Bob Swanson on May 6, 2021. During this survey no physical sampling was conducted, but data were recorded on locations where flow was present, presence of substrates suitable for spawning, and observations of *O. mykiss*.

The furthest upstream site (BDR1; Table 10, Figure 30) was near the emergence of Gabilan Creek from a steep, boulder filled canyon in the Gabilan Mountains and enters the uppermost portion of the valley floor. Although FISHBIO personnel were unable to access the canyon itself, the landowner indicated that the streambed is very steep and flows through numerous boulders that are greater than 10 feet in diameter. BDR1 was a short run containing a minimal amount of water, and the streambed was dry immediately upstream and downstream, making it functionally a long, shallow puddle at the observed water level (Figure 31, Figure 32). It appeared the streambed was completely dry upstream from BDR1 to at least the entry to the canyon. Notably, this was the only wetted reach in the upper watershed where no fish were observed. The landowner indicated that this upper reach is typically dry by June, and that there had been no flow in this portion of the creek during the spring of 2021. The streambed at this site, and for essentially the entirety of its length on the Bardin Ranch property, consisted primarily of cobble and gravel substrates, which appeared to be suitable for *O. mykiss* spawning.

Downstream of BDR1, the creek remained dry for approximately 5.7 km, and the presence of large amounts of leaves and other detritus in the streambed suggested that little or no flow had occurred recently (Figures 33, 34). This long, dry section of riverbed was characterized by gentle gradient, gravel and cobble substrate, and nearly complete riparian cover, indicating high potential as quality spawning and rearing habitat when wetted. The next wetted channel reach was not observed until reaching BDR2 (Table 10, Figures 35, 36), which was a short run section that flowed downstream into BDP1 (Table 10, Figure 37), a pool on an outside bend. Gravel was the predominant substrate at both sites, and multiple *O. mykiss* were observed (estimated average fork length 100-150 mm). The landowner indicated that small *O. mykiss* can reliably be caught with hook and line at BDP1.

Flow appeared to be continuous over the approximately 0.5 km between BDP1 and BDR3 (Table 10, Figure 30). However, observation of the entire streambed was not possible due to dense riparian vegetation and the deviation of the access road away from the stream, therefore it is possible there were intermittent dry reaches. BDR3, located approximately 0.25 km upstream of the Vierra Ranch property line, was the furthest downstream site with observed flow on the Bardin Ranch property. FISHBIO personnel observed multiple *O. mykiss* in BDR3, but very dense riparian vegetation precluded direct access to the stream. Immediately downstream of this site, near the Bardin Ranch-Vierra Ranch border (BDVRB; Table 10, Figure 30), the streambed was dry again. It appears there was no flow between this point and the furthest upstream pool observed on the Vierra Ranch (approximately 0.75 km; Figure 30). From this point onward, dominant substrates shifted from gravel and cobble to sand and silt, which appeared to remain the dominant substrates for the downstream remainder of the creek's length. Based on substrate alone, it appears that *O. mykiss* spawning in Gabilan creek is limited to the 7.25 km reach between the potential barrier on the Vierra Ranch (rKm 16.35) and the valley portion of the Bardin Ranch (ending where the creek flows out of the canyon at approximately rKm 23.6).

On May 5, 2021, FISHBIO staff assessed the portion of Gabilan Creek that flows through the Vierra Ranch property, which begins at the Old Stage Road crossing and includes approximately 3.3 km of the stream up to the border with the adjacent ranch (Bardin Ranch). FISHBIO was granted full access to the stream by the landowner and ranch manager. Walking the entire length of the stream on the property was not feasible due to extremely dense riparian vegetation, accumulated woody debris, abundant poison oak, and the very incised nature of the stream channel. Instead, FISHBIO personnel traveled along an access road to the upstream end of the stream on the property and conducted surveys at multiple points along the stream where access was possible (Table 10, Figure 30).

The uppermost point that was assessed on the property was a riprap barrier in the streambed (VRB; Table 10, Figure 30). Approximately 1.68 meters (~5.5 feet) tall, this structure consisted of concrete with rebar and large boulders, and a significant amount of woody debris and fallen willow trees had accumulated on its upstream edge (Figure 38). It was unclear why or how this barrier was formed, but due to the height of the structure, this appeared to present a potential barrier to migration for any upstream migrating steelhead, especially at lower flows. At the time of the surveys, there did not appear to be any flow on the Vierra Ranch property from this barrier upstream to the border of the Bardin Ranch, and a very small puddle at the bottom of the barrier was the furthest upstream surface water observed during the Vierra Ranch survey. Approximately 15 meters downstream of the puddle below the barrier was a small pool that was 18 cm deep at its deepest point, and measured approximately 2.3 m (7.5 feet) by 2.6 m (8.5 feet) in size (VRP1; Table 10, Figures 30,39). Water quality measurements indicated that dissolved oxygen was very low (1.31 mg/L; Table A4). Despite the poor water quality conditions, three passes of electrofishing resulted in the capture of eight *O. mykiss* ranging in size from 93-157 mm fork length (Table 11, Figure 40). A second pool (VRP2; Table 10, Figure 30) approximately five meters downstream of VRP1 was also sampled. This pool was 15 cm deep and approximately 1.8 m (6 feet) by 2.7 m (9 feet) in size. The dissolved oxygen in this pool was slightly higher, at 3.72 mg/L (Table A4), and three passes of electrofishing resulted in the capture of a single *O. mykiss* (fork length = 90 mm; Table 11). A third pool (VRP3; Table 10, Figures 30,40) approximately 50 meters downstream of SRP2 was sampled as well. This pool was 18 cm deep and approximately 2.7 m (9 feet) by 2.4 m (8 feet) in size. The dissolved oxygen level was nearly as low as VRP1 (1.37 mg/L; Table A4). Three pass electrofishing resulted in the capture of a single *O. mykiss* (fork length = 100; Table 11). This series of three pools (VRP1, VRP2, and VRP3) were connected by minimal flow, which was too shallow to allow for passage of *O. mykiss* between pools. The surprisingly high abundance of *O. mykiss* in VRP1 (eight fish in a pool of approximately one cubic meter in volume) suggests that they may have moved downstream from habitats further upstream on the Bardin Ranch property during a period of higher flow and became trapped in a drying pool as flows rapidly receded.

Downstream of VRP3, vegetation in the channel was too dense to penetrate. Notably, visual surveys from the access road indicated there was at least minimal surface flow from SRP3 downstream to the next site, but there were likely reaches with insufficient flow to allow for fish movement (such as the surface flow between VRP1, VRP2, and VRP3) and there may have been small, intermittent dry sections that were not visible from the road. The next assessed site (VRR1; Table 10, Figure 30) was a short run section approximately 20 m in length, which ended in a small pool that was 25 cm deep (Figure 42). The water at this location had a substantially higher dissolved oxygen content (6.59 mg/L; Table A4), and three pass electrofishing from the upstream end to the downstream end resulted in the capture of three *O. mykiss* ranging in fork length from 90-141 mm (Table 11). Flow was minimal downstream of the pool at VRR1, and dense vegetation once again required return to the access road for travel further downstream (Figures 42,43). Visual surveys from the road indicated there was at least minimal surface flow between VRR1 and the next site (Figures 42,43).

The final assessed site (VRR2; Table 10, Figure 30) was another run that was approximately 35 m in length, with a 25 cm-deep pool at the upstream end. This run curved from upstream to downstream. Riparian erosion appeared to be due in large part to cattle wallowing. There was also a significant amount of cattle manure in the streambed, but dissolved oxygen was higher than at other assessed sites on the Vierra Ranch (7.81 mg/L; Table A4). Three pass electrofishing resulted in detection of two *O. mykiss* at this site, but only one of these was successfully captured (fork length = 91; Table 11).

Visual inspection of the stream at the Old Stage Road crossing prior to conducting the surveys on the Vierra Ranch revealed that a minimal amount of flow was present. However, upon leaving the ranch at 5pm the streambed no longer had flow at the crossing, suggesting that it had dissipated at some point over the course of the day. Visual assessment of the stream along the access road between VRR2 and the Old Stage Road crossing suggested that minimal flow was present nearly all the way to the crossing (Figure 45). Visits to HEB and BOR on the subsequent day (in an attempt to recover the flow loggers) revealed that there was no flow in the system between LRD and SRC. The lack of flow at SRC allowed for precise measurement of the retaining wall barrier downstream of the crossing, which was 0.98 m (~3.2 feet) in height (Figure 47).

In addition to the surveys conducted by FISHBIO personnel, the Bardin Ranch landowner and Vierra Ranch manager provided anecdotal information on historical patterns in stream conditions on their respective properties. Neither had ever encountered an *O. mykiss* large enough to potentially be a steelhead (i.e., ≥ 14 inches), nor had they ever heard of anyone observing large fish in the stream. However, both indicated that small *O. mykiss* (i.e., ≤ 6 inches) were common. As noted above, Bob Swanson (Bardin Ranch) indicated that the upper portions of the creek on his property typically dry by June, and that this year had been particularly dry with no flow observed in the upper reaches during the spring. Further, he indicated that a number of springs or seeps are located throughout the surrounding mountains (Figure 48), which feed into Gabilan Creek in the valley below. However, a number of these springs had gone dry this year, which to his knowledge was the first time they had done so. Tony Temen, the ranch manager for Vierra Ranch, indicated that the upper portion of the stream on their property typically ran dry in the spring, but he noted that there was always flow in the lower portion.

Table 10. Site codes, locations, and observations of flow and *O. mykiss* from the upper watershed surveys on May 5 and 6, 2021. Refer to Figure 30 for a map of sampling locations.

Date	Code	Site	rKm	Coordinates	Flow Present?	*O. mykiss* Observed?
4/6/21	BDR1	Bardin Ranch Run 1	23.6	36.73722 -121.51722	Yes	No
4/6/21	BDR2	Bardin Ranch Run 2	17.87	36.75057, -121.56695	Yes	Yes
4/6/21	BDP1	Bardin Ranch Pool 1	17.82	36.75111, -121.56705	Yes	Yes
4/6/21	BDR3	Bardin Ranch Run 3	17.33	36.75473, -121.56982	Yes	Yes
4/6/21	BDVRB	Bardin/Vierra Ranch Border	17.09	36.75721, -121.56884	No	No
4/5/21	VRB	Vierra Ranch Barrier	16.35	36.76291, -121.57123	No	No
4/5/21	VRP1	Vierra Ranch Pool 1	16.34	36.76299, -121.57127	Yes	Yes
4/5/21	VRP2	Vierra Ranch Pool 2	16.34	36.76299, -121.57114	Yes	Yes
4/5/21	VRP3	Vierra Ranch Pool 3	16.31	36.76319, -121.57103	Yes	Yes
4/5/21	VRR1	Vierra Ranch Run 1	16.23	36.76395, -121.57109	Yes	Yes
4/5/21	VRR2	Vierra Ranch Run 2	15.47	36.76948, -121.57472	Yes	Yes

Table 11. Capture location, length, and weight of all *O. mykiss* captured during electrofishing activities on Gabilan Creek within Vierra Ranch on May 5, 2021.

Date	Time	Site Code	Species	Fork Length (mm)	Total Length (mm)	Weight (g)
4/5/21	13:51	VRP1	RBT	133	141	24.9
4/5/21	13:51	VRP1	RBT	157	168	44.5
4/5/21	13:51	VRP1	RBT	100	106	11.5
4/5/21	13:51	VRP1	RBT	106	113	12.2
4/5/21	13:51	VRP1	RBT	104	110	12.3
4/5/21	13:51	VRP1	RBT	95	101	9.3
4/5/21	13:51	VRP1	RBT	117	124	17.6
4/5/21	13:51	VRP1	RBT	93	98	8.2
4/5/21	14:12	VRP2	RBT	90	97	8.9
4/5/21	14:28	VRP3	RBT	100	107	Not measured
4/5/21	15:15	VRR1	RBT	141	147	32.5
4/5/21	15:15	VRR1	RBT	90	95	9
4/5/21	15:15	VRR1	RBT	112	119	19.8
4/5/21	16:22	VRR2	RBT	91	96	10.2
4/5/21	16:22	VRR2	RBT	Not measured	Not measured	Not measured
Total = 15 fish			**Average**	**109.21**	**115.86**	**16.99**

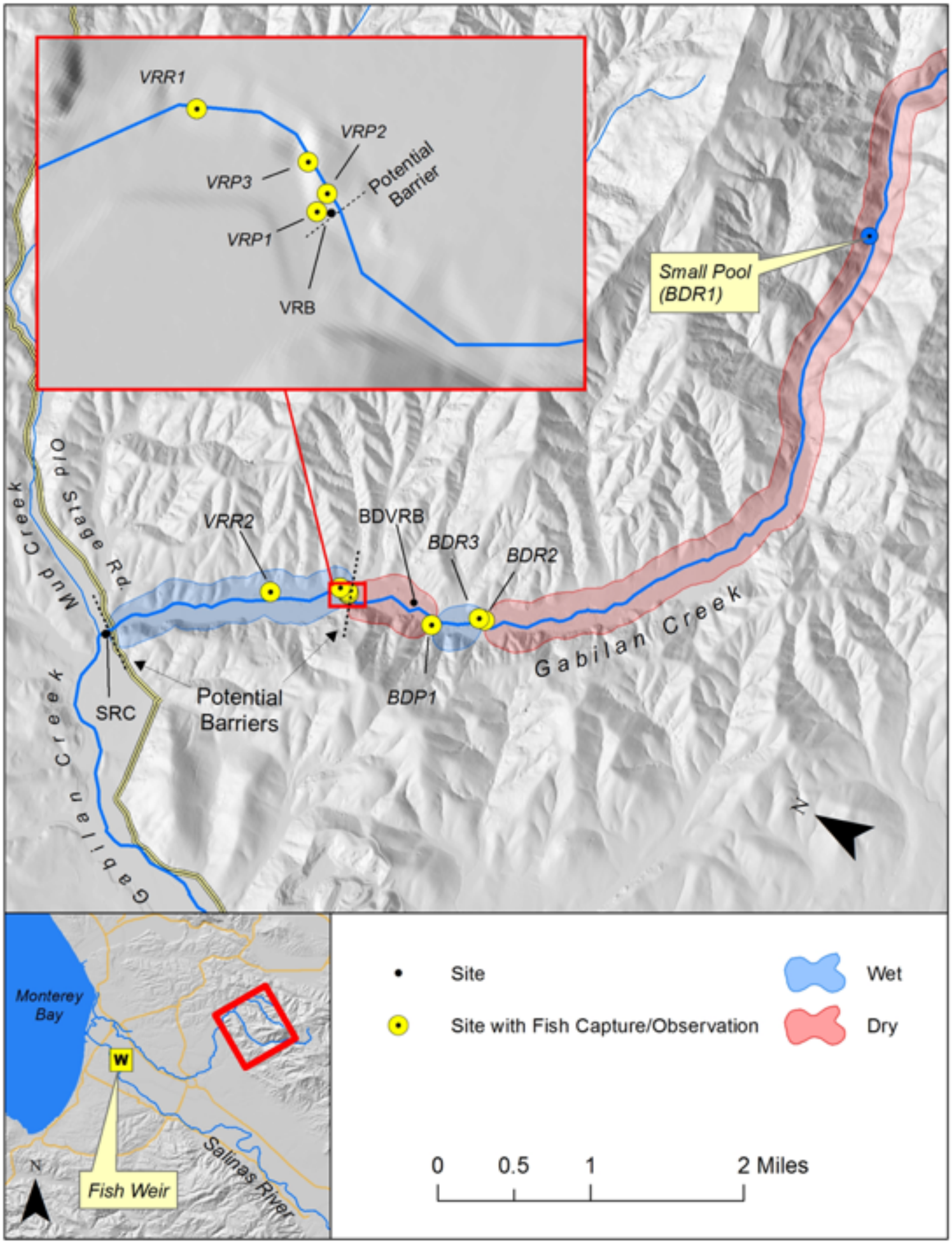

Figure 30. Map of sampling locations and potential migration barriers in the upper Gabilan Watershed. Site codes refer to the sampling locations listed in Table 10 and Table 11.

Figure 31. BDR1 facing upstream. This was the furthest upstream observation of wetted channel in the system.

Figure 32. BDR1 facing downstream. The streambed was dry for approximately 5.73 km below this point.

Figure 33. A dry portion of the streambed between BDR1 and BDR2, facing downstream. The predominant substrate was cobble, and the accumulation of leaf litter suggests no flow had occurred recently.

Figure 34. A dry portion of the streambed between BDR1 and BDR2, facing upstream. The predominant substrate was cobble, and the accumulation of leaf litter suggests no flow had occurred recently.

Figure 35. BDR2 facing upstream. Multiple *O. mykiss* were observed in this reach.

Figure 36. BDR2 facing upstream.

Figure 37. BDP1 facing downstream.

Figure 38. VRB facing upstream. This barrier of concrete, rebar, and woody debris stood approximately 1.68 m tall (5.5. feet). The very shallow puddle visible at the bottom was the furthest upstream observation of water on the Vierra Ranch property.

Figure 39. VRP1 facing upstream, approximately 15 m downstream of VRB. Despite very poor water quality and restricted habitat, eight *O. mykiss* were captured in this pool.

Figure 40. *O. mykiss* captured during electrofishing surveys on Vierra Ranch.

Figure 41. VRP3 facing downstream. There appeared to be continuous flow downstream of this location, but dense vegetation prevented FISHBIO personnel from travelling further along the streambed.

Figure 42. VRR1 facing upstream. This run was approximately 20 m long and ended in a small pool.

Figure 43. VRR1 facing downstream. The pool that was considered the end of VRR1 sample site is in the center of this photo.

Figure 44. VRR1 facing downstream. Downstream flow appeared to be continuous from here through VRR2.

Figure 45. VRR2 facing downstream. Flow appeared to be continuous from here nearly to the Old Stage Road crossing (SRC) at the property border.

Figure 46. VRR2 facing upstream. The bank area on the inside of this bend showed signs of heavy use by cattle. Two *O. mykiss* were observed in this reach, but only one was captured.

Figure 47. The barrier downstream of the Old Stage Road crossing (SRC). The height of the retaining wall was approximately 0.98 m (3.2 feet). Flows sufficient to allow for passage over this structure were not observed during 2020 or 2021 sampling (see Figure 19 for an overhead view during a period of flow).

Figure 48. Spring in the Gabilan Mountains above the creek.

Conclusions

The Gabilan Creek watershed is considered critical habitat for federally threatened SCCC DPS steelhead (NMFS 2005). Therefore, understanding the potential for steelhead to move throughout the system, mapping the extent of suitable spawning and rearing habitat, and confirming the contemporary or historic occurrence of migratory fish in the system is of great significance for informing and guiding management and recovery actions in the watershed and across the DPS. Assessments of flow and migration barriers, eDNA monitoring, and habitat surveys conducted in 2020 and 2021 provide additional insight into the spatial extent of the contemporary Gabilan *O. mykiss* population and the potential for a spawning steelhead population in the watershed. Although collected data is insufficient to confidently confirm or refute the presence of migratory *O. mykiss* in Gabilan Creek and its tributaries, these surveys have improved understanding of the availability of perennial habitat in the upper watershed and identified several significant impediments to fish migration in the system. Under consideration of the rarity of historical observations of steelhead in the watershed, the presence of several significant passage barriers as well as impaired water quality, and the lack of any anecdotal observations of steelhead by local landowners indicate that this stream does not currently have the potential to support steelhead.

Detection of *O. mykiss* eDNA in sample sites upstream of the city of Salinas is likely attributable to the presence of resident *O. mykiss* in the upper watershed, which were confirmed during upper watershed habitat surveys. However, the detection of *O. mykiss* eDNA at sites downstream of the city of Salinas in the Reclamation Ditch and at the San Jon Road crossing in 2020, and in Tembladero Slough and the Old Salinas River in 2021 raise the possibility that adult *O. mykiss* may be attempting to migrate upstream in the system. However, multiple obstacles constitute partial or complete barriers to upstream migrating fish (Figure 49). First, the grade control structure at Little River Drive presents a significant impediment to upstream movement and may only be passable during very high flows. Notably, the only confirmed observation of an anadromous *O. mykiss* in the system occurred just below this barrier, which has also precluded passage of upstream migrating stray adult Chinook salmon. Second, the 6.32-km-long stretch of Gabilan Creek between the Little River Drive grade control structure and the Hebert Road crossing is subject to rapid drying, and even large precipitation events may only provide very short periods of flow conditions suitable for passage. Third, the barrier at the Boronda Road crossing, which is the highest of all the barrier in the stream, may also be impassable under most flow conditions. Although another barrier exists at the Hebert Road crossing, it represents a lesser impediment to migration (if conditions were suitable for passage downstream, the Hebert Road barrier would likely be passable as well). Fourth, an old retaining wall and pipeline immediately downstream of the Old Stage Road crossing forms a 0.98 m (3.2 foot) barrier. At no point during eDNA and water quality sample collection in 2020 and 2021 did this barrier appear passable, largely because majority of water in the stream flows underneath the wall rather than over it. This barrier apparently serves no functional purpose and may be the easiest to remediate. Finally, an additional barrier of concrete rip rap was identified on the Vierra Ranch property during upper watershed surveys. No flow was present at the time of the survey, and the flow conditions required for this barrier to be passable to adult steelhead remain unknown. This barrier may also represent a good opportunity for removal and reconnection, as it also does not appear to serve any functional purpose.

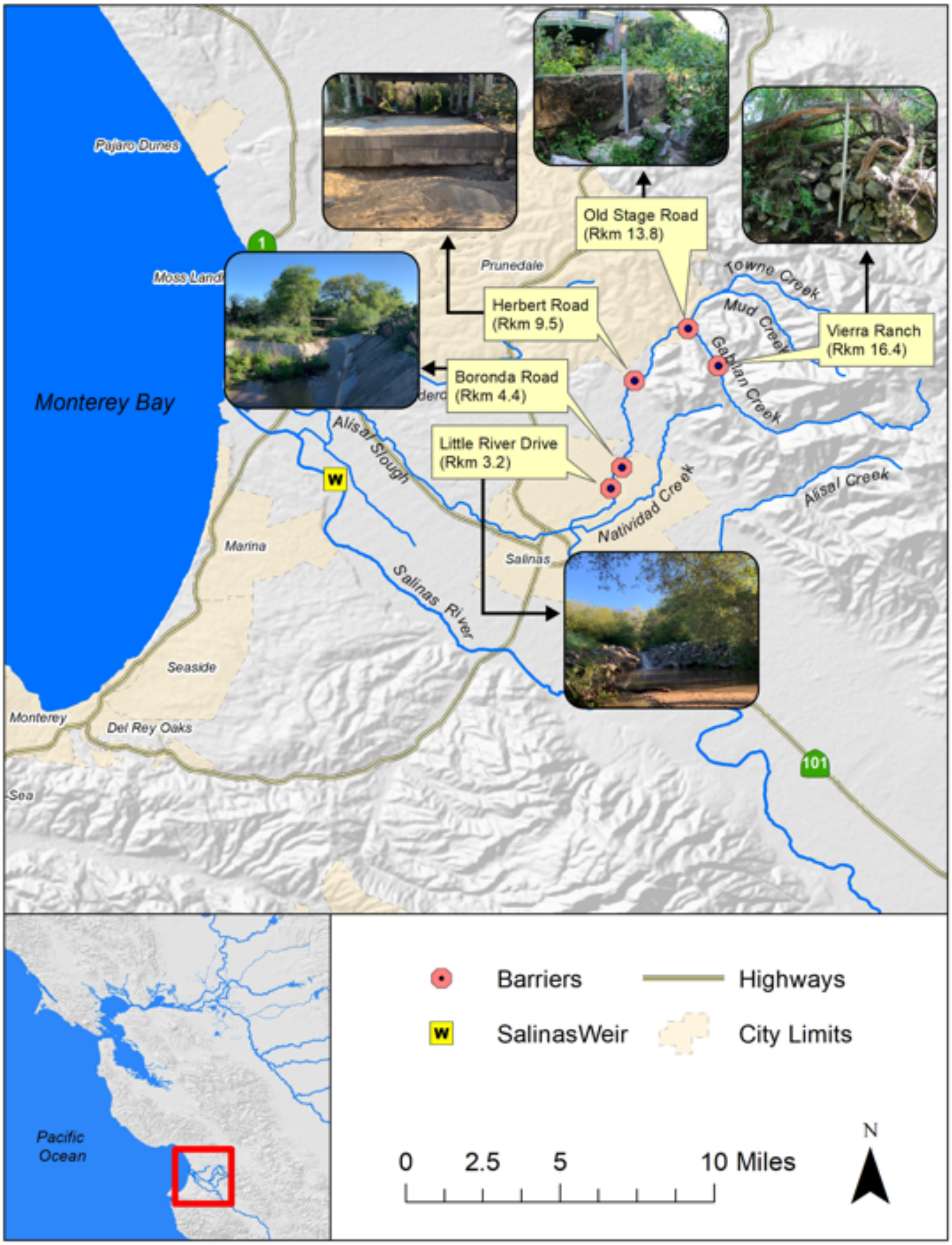

Figure 49. Map depicting the locations of instream structures observed during this study which may function as complete or partial barriers to anadromy.

In addition to these anthropogenic barriers throughout the watershed, the Gabilan system is characterized by extremely flashy flows and rapid drying. The Mediterranean climate, high-relief topography of the upper watershed, and porous nature of the soil all play a role in limiting flow connectivity, even after substantial rain events. It is likely that even under historical, unimpaired conditions, connectivity and the potential for fish migration may have been limited to only wet or very wet years. Conservative estimates based on collected flow data and visual observations of barriers under different flow conditions indicate that passage by adult steelhead may have been possible for only 2-3 days over the past two years. However, both years of monitoring (2020 and 2021) have been characterized by low levels of precipitation, with the late winter and spring of 2021 being the fifth driest and 2020 being the 16th driest since 1980 (Figure 50). Further, even when average total precipitation does occur in the spring, the specific duration, frequencies, and total rainfall of precipitation events are likely important for determining when sufficient flow may be present to allow for steelhead passage in the watershed. For example, although the spring of 2020 was only the 16th direst observed since 1980, no rain fell for the entire month of February (Figure 51), and as such passage was not possible for a significant portion of the peak migratory period. Given the relatively low precipitation in 2020 and 2021, these years may not have been representative of the wet or very wet conditions necessary for steelhead passage.

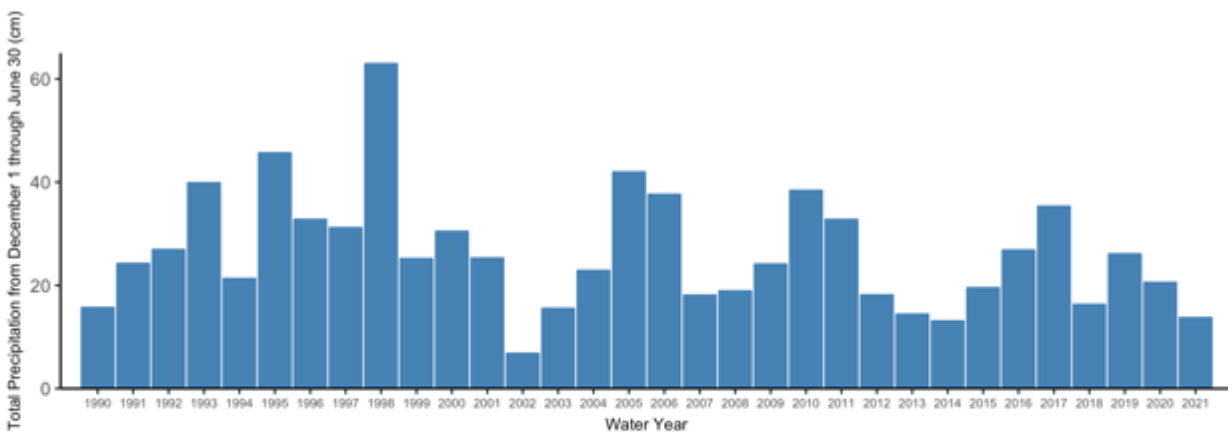

Figure 50. Summary of total precipitation (in centimeters) falling between December 1 and June 30 from 1990 through 2021, as measured at the Salinas Airport (source: NOAA National Centers for Environmental Information, available at https://www.ncdc.noaa.gov/).

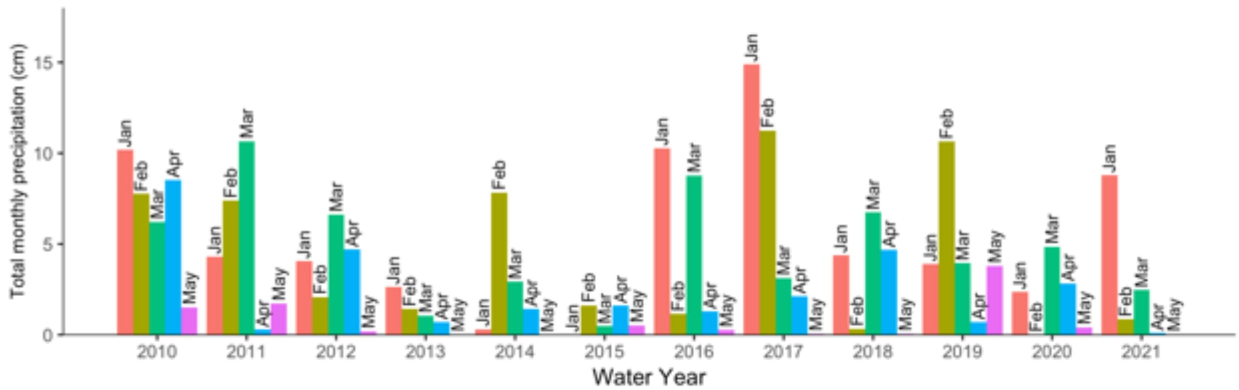

Figure 51. Summary of total monthly precipitation (in centimeters) falling from January through May from 2010 to 2021, as measured at the Salinas Airport (source: NOAA National Centers for Environmental Information, available at https://www.ncdc.noaa.gov/).

The upper watershed of Gabilan Creek does appear to contain suitable habitat for steelhead spawning and rearing, and the presence *O. mykiss* in the wetted sections of the creek, as well as anecdotal accounts indicate a persistent resident population. Based on fish lengths, the majority of the population appears to have been born during 2020, which was a historically dry year in the Salinas Basin. This would suggest that spawning occurs in the watershed during most years, even under unfavorable conditions. However, the aforementioned challenges to fish movement throughout the middle and lower watershed suggest it is unlikely steelhead were able to reach these upper watershed habitats in the past two years. It is possible steelhead are able to reach the upper watershed in years when flow conditions are more suitable to migration, but the current and historic frequencies at which this may occur remain unclear.

Recommended Studies

The past two years of monitoring have provided several important insights into the Gabilan watershed and the *O. mykiss* population residing therein. First, habitat suitable for steelhead spawning and rearing is present in the upper watershed. Second, populations of resident *O. mykiss* exist in the upper watershed and appear to occur in most wetted sections of the stream above the Old Stage Road crossing. Third, conditions appeared generally unsuitable for fish passage in 2020 and 2021. Finally, detections of *O. mykiss* eDNA in the lower river suggest adult steelhead may occasionally be present in the system.

Several questions, however, remain unanswered. The most fundamental unknown is whether there is currently or historically has been a migratory component of the Gabilan *O. mykiss* population, and, if so, what contribution this component makes to the population in the watershed. The single most informative study that could be conducted would be microchemical analysis of otolith samples collected from *O. mykiss* in the upper watershed. Analysis of otoliths not only provides information on the age and life history of the individual, but can also be used to determine maternal life history (Zimmerman et al. 2009). If migratory steelhead are reaching the upper watershed and successfully reproducing, there is potential to find evidence of this in the otoliths of juvenile *O. mykiss*. It is important to consider that the collection of otoliths requires lethal sampling, however the detection of numerous *O. mykiss* trapped in drying pools on the Vierra Ranch property during habitat surveys suggests that natural mortalities may be opportunistically recovered as the streambed dries in the late spring.

Alternatively, non-lethal genetic analysis of scale or tissue samples (e.g., fin clips) could provide insight into the migratory potential of the population. A chromosomal rearrangement on the *Omy5* chromosome in *O. mykiss* resulted in two different forms, one of which is associated with migratory behavior and one of which is associated with expression of a resident life history (Pearse et al. 2019). These forms, or genotypes, are not deterministic, meaning that individuals with a resident genotype may express migratory behavior and vice versa (Kelson et al. 2019). However, the relative proportion of migratory genotypes among *O. mykiss* in the upper watershed may provide insight into the migratory potential of the population. For example, a high occurrence of migratory genotypes would provide strong support for the hypothesis that steelhead are actively contributing to the population in the system, and even a low occurrence of migratory genotypes may suggest the population historically had a migratory component, as genetic variation associated with anadromy may persist in populations cut-off from the ocean for decades or centuries (Hecht et al. 2012; Phillis et al. 2016). A failure to detect any migratory genotypes would not definitively rule out the possibility that the *O. mykiss* population in the upper watershed may produce individuals that express a migratory life history, but detection of migratory genotypes would provide significant support for contemporary migratory potential.

One final approach to answering the question of whether the upper watershed *O. mykiss* population produces migratory individuals would be a combined PIT tagging and PIT antenna monitoring effort. Backpack electrofishing could be used to capture and tag juvenile *O. mykiss* in perennial pools (i.e., over summer holding habitats) in the spring. Not only would this allow for the accumulation of many tagged individuals to increase the likelihood of detecting outmigration, but if repeated would also allow for estimation of population abundance and density in upper watershed habitats through analysis of mark-recapture data. Further, the capture of individuals for tagging would also provide an opportunity for collection of tissue samples for genetic analysis, which could provide insight into the occurrence of migratory genotypes in the population. In conjunction with a concerted tagging effort, a PIT antenna installed at the Old Stage Road crossing to detect any juveniles that move downstream. The confined nature of the channel and existing infrastructure at that road crossing make it well-suited for an antenna that could be custom designed to achieve maximum detection range.

Each of these studies would provide valuable information if conducted alone, but a strategically designed combination of all three would maximize efficiency and increase the probability of confirming any migratory behavior that may be occurring in the system.

Literature Cited

Becker, G.S., Smetak, K.M., and Asbury, D.A. 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, CA.

Boughton, D. A., Adams, P.B., Anderson, E., Fusaro, C., Keller, E., Lentsch, L., Nielsen, J., Perry, K., Regan, H., Smith, J., Swift, C., Thompson, L., and Watson, F. 2006. Steelhead of the south-central/southern California Coast: population characterization for recovery planning. Technical memorandum NMFS-SWFSC-394. National Marine Fisheries Service, Southwest Fisheries Science Center, Santa Cruz, California.

Boughton, D.A., and Goslin, M. 2006. Potential steelhead oversummering habitat in the South-Central/Southern California Coast recovery domain: Maps based on the envelope method. NOAA Technical Memorandum NMFS-SWFSC-391. NOAA Fisheries, US Dept. of Commerce, Washington DC.

California State Water Resources Control Board. 2016. Final 2014 and 2016 Integrated Report (CWA Section 303(d) List / 305(b).

Casagrande, J., Hager, J., Watson, F., and Angelo, M. 2003. Fish Species Distribution and habitat quality for selected streams of the Salinas watershed; Summer/Fall 2002. Central Coast Watershed Studies. Available at http://ccows.csumb.edu/pubs/reports/CCoWS_SalFishHabReport_030529_600dpi.pdf

Casagrande, J., and Watson, F. 2006. Reclamation Ditch Watershed Assessment and Management Strategy: Part A - Watershed Assessment. Monterey County Water Resources Agency and The Watershed Institute, California State University Monterey Bay, 283 pp.

Casagrande, J.M. 2010. Historic and current status of steelhead and barriers to migration in the Gabilan watershed. The Watershed Institute, California State University Monterey Bay, 16 pp.

Deiner, K., and Altermatt, F. 2014. Transport distance of invertebrate environmental DNA in a natural river. *PloS one*. 9(2): e88786.

Flosi, G., Downie, S., Hopelain, J., Bird, M., Coey, R., and Collins, B. 2010. California Salmonid Stream Habitat Restoration Manual, Fourth Edition. California Department of Fish and Game, Wildlife and Fisheries Division. Sacramento, CA.

Haas, D. 2017. Standard operating procedure for critical riffle analysis for fish passage in California. California Department of Fish and Wildlife Instream Flow Program Standard Operating Procedure CDFW-IFP-001.

Hager, J. 2001. An evaluation of steelhead habitat and population in the Gabilan Creek Watershed, Senior Thesis, Department of Earth, Systems, Science, and Policy, California State University of Monterey Bay, 38 pp. plus appendix. Available at http://science.csumb.edu/~ccows/ccows/pubs/capstones/JHager_FinalThesis.pdf

Hecht, B. C., Thrower, F. P., Hale, M. C., Miller, M. R., Nichols, K. M. 2012. Genetic architecture of migration-related traits in rainbow and steelhead trout, *Oncorhynchus mykiss*. G3 (Bethesda). 2(9):1113-1127.

Kelson S. J., Miller, M. R., Thompson, T. Q., O'Rourke, S. M., Carlson, S. M. 2019. Do genomics and sex predict migration in a partially migratory salmonid fish, *Oncorhynchus mykiss*? Can J Fish Aquat Sci. 76(11):2080-2088.

Kendall, N. W., McMillan, J. R., Sloat, M. R., Buehrens, T. W., Quinn, T. P., Pess, G. R., Kuzischin, K. V., McClure, M. M., and Zabel, R. W. 2015. 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(3): 319-342.

Moyle, P. B. 2002. *Inland fishes of California: revised and expanded*. Univ of California Press.

National Marine Fisheries Service. 1997. Endangered and threatened species: Listing of several evolutionary significant units (ESUs) of West Coast steelhead. Federal Register. 62(159): 43937-43954.

National Marine Fisheries Service. 2001. Guidelines for salmonid passage at stream crossings.

National Marine Fisheries Service. 2005. Endangered and threatened species; Designation of critical habitat for seven evolutionarily significant units of Pacific salmon and steelhead in California. Federal Register. 70: 52488- 52627.

National Marine Fisheries Service. 2013. South-central California steelhead recovery plan. West Coast Region, National Marine Fisheries Service Long Beach, CA

Pearse, D. E., Barson, N. J., Nome, T., Gao, G., Campbell, M. A., Abadía-Cardoso, A., Anderson, E. C., et al. 2019. Sex-dependent dominance maintains migration supergene in rainbow trout. *Nature Ecology & Evolution*. 3(12): 1731-1742.

Phillis, C. C., Moore, J. W., Buoro, M., Hayes, S. A., Garza, J. C., Pearse, D. E. 2016. Shifting thresholds: Rapid evolution of migratory life histories in steelhead/rainbow trout, *Oncorhynchus mykiss*. J Hered. 107(1):51-60.

Pilliod, D. S., Goldberg, C. S., Arkle, R. S., and Waits, L. P. 2014. Factors influencing detection of eDNA from a stream-dwelling amphibian. *Molecular Ecology Resources*. 14(1): 109-116.

Robison, E. G., Mirati, A. H., and Allen, M. 2000. Oregon road/stream crossing restoration guide. National Marine Fisheries Service.

Wilcox, T. M., McKelvey, K. S., Young, M. K., Sepulveda, A. J., Shepard, B. B., Jane, S. F., Whiteley, A. R., Lowe, W. H., and Schwartz, M. K. 2016. Understanding environmental DNA detection probabilities: A case study using a stream-dwelling char Salvelinus fontinalis. *Biological Conservation*. 194: 209-216.

Wipfli, M. S., Richardson, J. S., and Naiman, R. J. 2007. Ecological linkages between headwaters and downstream ecosystems: Transport of organic matter, invertebrates, and wood down headwater channels 1. *Journal of the American Water Resources Association*. 43(1): 72-85.

Zimmerman, C.E., Edwards, G. W., and Perry, K. 2009. Maternal origin and migratory history of steelhead and rainbow trout captured in rivers of the Central Valley, California. *Transactions of the American Fisheries Society*. 138:280-291.

Appendix

Table A1. Additional sites sampled during initial survey on January 9, 2020. These sites were not revisited for the remainder of the study period.

Code	Site	Coordinates
GAB	Gabilan Creek ~20' upstream of confluence with Natividad and Alisal creeks	36.686703, -121.641108
REC	Gabilan Creek ~30' downstream of confluence with Natividad and Alisal creeks	36.686436, -121.641236
MUD	Mud Creek (tributary in the upper Gabilan watershed)	36.795603, -121.574961
TWN	Towne Creek (tributary in the upper Gabilan watershed)	36.801200, -121.565722

Table A2. Sample ID number, location, date, time, volume, and *O. mykiss* detection status of all 100 eDNA samples that were collected during the 2020 monitoring season and submitted for analysis. Note that an additional 21 samples that were collected but not submitted for analysis are currently archived in cold storage.

Sample ID	Site	Date	Time	Volume Filtered (L)	*O. mykiss* Detected (Y/N)
001	REC	1/9/2020	8:26	1	N
002	GAB	1/9/2020	8:40	0.52	Y
003	GAB	1/9/2020	8:50	0.56	Y
004	LRD	1/9/2020	10:25	1.03	N
005	HEB	1/9/2020	11:15	0.51	Y
006	HEB	1/9/2020	11:40	0.5	N
007	SRC	1/9/2020	12:20	2.07	N
008	TWN	1/9/2020	14:06	2.1	N
009	MUD	1/9/2020	14:20	0.42	N
010	MUD	1/9/2020	14:20	0.59	N
011	SJR	1/9/2020	15:30	0.5	N
012	SJR	1/9/2020	15:40	0.5	N
013	TEM	1/9/2020	16:00	0.44	N
014	TEM	1/9/2020	16:00	1.56	N
016	OSR	1/9/2020	16:15	0.64	N
017	OSR	1/17/2020	8:07	0.51	N
018	OSR	1/17/2020	8:33	0.5	N
019	TEM	1/17/2020	8:55	0.51	N
020	TEM	1/17/2020	9:03	0.49	N

022	SJR	1/17/2020	9:43	0.6	N
023	LRD	1/17/2020	10:22	1.5	N
026	HEB	1/17/2020	11:21	2	N
027	SRC	1/17/2020	11:47	1.08	Y
028	OSR	1/20/2020	7:48	1.26	N
029	TEM	1/20/2020	8:04	1.31	N
030	SJR	1/20/2020	8:42	0.51	N
031	SJR	1/20/2020	8:50	0.5	N
033	OSR	1/24/2020	7:43	0.52	N
034	OSR	1/24/2020	7:54	1.02	N
035	TEM	1/24/2020	8:12	1.38	N
036	SJR	1/24/2020	8:47	0.51	N
037	SJR	1/24/2020	8:57	0.49	N
040	OSR	1/27/2020	7:43	0.52	N
041	OSR	1/27/2020	8:00	0.4	N
042	TEM	1/27/2020	8:31	1.01	N
043	SJR	1/27/2020	9:07	0.5	N
044	SJR	1/27/2020	9:15	0.51	N
046	OSR	1/31/2020	7:37	0.51	N
047	OSR	1/31/2020	7:46	0.53	N
048	TEM	1/31/2020	8:06	1.03	N
049	SJR	1/31/2020	8:32	1.59	N
051	SRC	1/31/2020	9:30	2.2	Y
052	OSR	2/3/2020	7:41	1.59	N
053	TEM	2/3/2020	7:57	1.54	N
054	SJR	2/3/2020	8:37	2.06	N
056	OSR	2/7/2020	7:46	1.52	N
057	TEM	2/7/2020	8:02	0.7	N
059	SJR	2/7/2020	8:42	0.4	N
060	SJR	2/7/2020	8:50	0.61	N
063	OSR	2/10/2020	7:48	0.56	N
064	OSR	2/10/2020	7:53	0.52	N
065	TEM	2/10/2020	8:14	0.52	N
066	TEM	2/10/2020	8:19	0.51	N
067	SJR	2/10/2020	8:50	1	N
069	OSR	2/14/2020	7:47	1.02	N
070	TEM	2/14/2020	8:04	0.49	N
071	TEM	2/14/2020	8:14	0.53	N
072	SJR	2/14/2020	8:45	1.05	N

074	SRC	2/14/2020	10:10	3.04	Y
075	OSR	2/17/2020	7:54	1.11	N
076	TEM	2/17/2020	8:13	1.25	N
077	SJR	2/17/2020	8:41	1.11	N
078	OSR	2/26/2020	7:53	0.51	N
079	OSR	2/26/2020	8:00	0.67	N
082	SJR	2/26/2020	9:13	1	N
083	OSR	3/4/2020	7:46	1.04	N
084	TEM	3/4/2020	8:07	1.18	N
085	SJR	3/4/2020	8:36	1.09	N
086	OSR	3/9/2020	8:05	1.01	N
087	TEM	3/9/2020	8:24	1.03	N
088	SJR	3/9/2020	8:56	1	N
090	OSR	3/13/2020	7:43	1	N
091	TEM	3/13/2020	8:00	1.1	N
092	SJR	3/13/2020	8:25	2.02	N
093	OSR	3/16/2020	7:47	1.3	N
095	TEM	3/16/2020	8:20	0.4	N
096	SJR	3/16/2020	8:53	1.01	N
097	LRD	3/16/2020	9:45	0.47	N
099	LRU	3/16/2020	10:15	1	N
100	HEB	3/16/2020	10:41	0.6	Y
101	OSR	3/24/2020	8:55	1.04	N
102	TEM	3/24/2020	9:17	1.01	N
103	SJR	3/24/2020	9:56	0.5	N
104	SJR	3/24/2020	10:08	0.51	N
105	LRD	3/24/2020	10:50	0.72	N
106	OSR	3/31/2020	7:29	1	N
107	TEM	3/31/2020	7:56	0.4	N
108	TEM	3/31/2020	8:09	0.4	N
109	SJR	3/31/2020	9:01	1	Y
110	OSR	4/7/2020	6:42	2.02	N
111	TEM	4/7/2020	7:02	1.04	N
112	SJR	4/7/2020	7:36	1	N
113	LRD	4/7/2020	8:17	1.01	Y
114	LRU	4/7/2020	8:33	1.5	Y
115	HEB	4/7/2020	9:07	0.4	Y
117	SRC	4/7/2020	9:45	1.36	Y
118	OSR	4/10/2020	6:58	1	N

119	TEM	4/10/2020	7:27	1.03	N
120	SJR	4/10/2020	7:52	1.02	N
121	HEB	4/10/2020	8:56	1.01	Y

Table A3. Sample ID number, location, date, time, volume, and *O. mykiss* detection status of all 29 eDNA samples that were collected during the 2021 monitoring season. Note that an additional 10 samples that were collected but not submitted for analysis are currently archived in cold storage.

Sample ID	Site	Date	Time	Volume Filtered (L)	*O. mykiss* Detected (Y/N)
201	SAL	12/17/2020	10:50	0.38	N
202	TEM	12/17/2020	11:35	1.04	N
204	SJR	12/17/2020	12:45	0.41	N
205	SAL	12/23/2020	10:35	1	N
206	TEM	12/23/2020	10:55	1.01	N
207	SJR	12/23/2020	11:25	1.01	N
209	SAL	12/29/2020	11:40	1.01	N
210	TEM	12/29/2020	12:36	1.01	N
212	SJR	12/29/2020	1:30	1.02	N
213	SAL	1/5/2021	8:43	1.01	N
214	TEM	1/5/2021	9:12	1.01	N
215	SJR	1/5/2021	9:52	1.06	N
217	SAL	1/12/2021	10:15	1.05	Y
218	TEM	1/12/2021	10:48	1.07	Y
219	SJR	1/12/2021	11:29	1	N
220	SAL	1/18/2021	9:44	1.01	Y
221	TEM	1/18/2021	10:19	1.09	N
222	SJR	1/18/2021	10:50	1.02	N
223	SAL	1/25/2021	10:49	1.02	N
224	TEM	1/25/2021	11:15	1.28	N
225	SJR	1/25/2021	11:52	1.06	N
226	SAL	1/29/2021	10:10	1.01	N
227	SJR	1/29/2021	1:52	0.5	N
230	SAL	2/1/2021	10:15	1.03	N
231	TEM	2/1/2021	10:47	1.21	N
232	SJR	2/1/2021	11:18	1.04	N
234	SAL	2/8/2021	10:55	0.78	N
235	TEM	2/8/2021	11:26	0.96	N
236	SJR	2/8/2021	12:21	0.23	N

Table A4. Water quality measurements from sample sites on Vierra Ranch property.

Date	Time	Site Code	Pool Depth (m)	Pool Width (m)	Pool Length (m)	Temp (˚C)	DO (mg/L)	SpC (µS/cm)	C (µS/cm)	TDS	Salinity (ppt)
4/5/21	13:51	SRP1	0.18	2.3	2.6	13.6	1.31	735	565	478	0.36
4/5/21	14:12	SRP2	0.15	1.83	2.74	13.9	3.72	736	580	479	0.36
4/5/21	14:28	SRP3	0.18	2.74	2.44	15.3	1.37	702	572	461	0.35
4/5/21	15:15	SRR1	0.25	-	-	15.6	6.59	694	570	453	0.34
4/5/21	16:22	SRR2	0.15	-	-	15.3	7.81	722	586	469	0.35