# NATIONAL FISH AND WILDLIFE FOUNDATION FINAL REPORT

**for**

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# INTRODUCTION

*Oncorhynchus mykiss* is a Pacific salmon species native to North America. Often referred to as coastal rainbow or steelhead trout, *O. mykiss* exhibit two main life history strategies (resident and anadromous, respectively). These strategies allow *O. mykiss* to persist in diverse habitats and through large environmental events. However, despite these varied life history strategies, *O. mykiss* in southern California are listed under the Federal Endangered Species Act. The National Marine Fisheries Service has identified a number of recovery actions necessary for *O. mykiss* to be delisted in southern California (NMFS 2012). Underlying these actions is the need for a thorough understanding of *O. mykiss* ecology, including trout abundance, movement, and habitat use. In this report, we describe the use of National Fish and Wildlife Foundation funding (Pacific States Marine Fisheries Commission Grant #1056.16) for monitoring of *O. mykiss* in the Ventura River Basin from March 2016 to March 2017. With this funding, we 1) designed and built stationary passive integrated transponder (PIT) tag arrays to monitor *Oncorhynchus mykiss* movement throughout the Ventura River watershed, 2) estimated *O. mykiss* population abundance in Upper North Fork Matilija Creek, 3) examined fine-scale *O. mykiss* movement in Upper North Fork Matilija, and 4) examined trout cover availability and use in Upper North Fork Matilija. This was an initial effort of a larger monitoring project focused on both developing a PIT tagging program and estimating population abundance in a watershed designated as high priority for steelhead recovery.

# SUMMARY

## Stationary PIT Tag Arrays in the Ventura River Watershed

We designed AC- and solar-powered stationary PIT tag arrays to monitor *O. mykiss* movement throughout the Ventura River Basin at three locations: the Ventura mainstem (VEN), the confluence of North Fork Matilija and Matilija Creeks (NFM), and the confluence of San Antonio Creek and Ventura River (SAN). We built an AC-powered array with pass-through antennas at VEN. We test deployed the array during a storm on January 19, 2017, which led to design alterations to strengthen the durability of the array during high flow events. These alterations will be implemented when flow subsides. For the NFM array, we obtained landowner access, but were not able to begin building due to a storm system on February 17, 2017 that created unsafe conditions. We will install the NFM array as soon as the site is safe for construction. We hope to install the NFM array and rebuild the VEN array in April or May 2017. The SAN array site was dry from the start of the grant period to January 2017 and threatened by a large debris load located upstream of the site. We therefore delayed building the array until flows returned and the debris was cleared. A series of storms in January and February 2017 significantly improved flow, but did not clear the debris. Installation at SAN will commence once conditions allow.

## Population Abundance Estimation of *Oncorhynchus mykiss* in Upper North Fork Matilija in 2016

We estimated the trout population abundance of Upper North Fork Matilija Creek in winter 2016. We chose to sample a non-anadromous creek due to permitting restrictions. Using habitat typing, snorkeling, and electrofishing surveys, we estimated that 274 ± 124 trout (± 95% CI) populate the shallow units of Upper North Fork Creek. This is a much lower estimate than was found in 2015 in a similar sampling effort. However, we believe that the 2016 estimate presented in this report is more reliable. We are currently listed as principal investigators in a Section 10(a)(1)(a) permit application submitted by the California Department of Fish and Wildlife, which we hope will be approved by spring 2017. This permit will allow for further abundance estimations in anadromous reaches of the Ventura River watershed. Our priority at that time will be to sample North Fork Matilija Creek from June to December 2017.

## Fine-Scale *O. mykiss* Movement in Upper North Fork Matilija

We PIT tagged trout in Upper North Fork Matilija Creek across two sampling efforts, including during the abundance estimation survey described above. Following tagging, we conducted a PIT tag scanning survey to examine fine-scale trout movement. Using a mobile reader and antenna pole, we scanned the entire sampled section of Upper North Fork Matilija Creek for PIT tags implanted into trout. We detected 54 of 62 (87%) deployed PIT tags. Of these, eight tags moved either up- or downstream from the habitat unit in which tagged trout were caught, tagged, and released. The greatest distance a tag moved was 0.1 mile upstream. These results suggest that trout do not move greatly between habitat units, which may be explained in part by low flow conditions. However, this hypothesis assumes that tags were retained within live trout, which we could not confirm in most cases. Due to the limitations of this pilot study, further surveys are required. We could not complete additional surveys prior to submission of this report due to a series of storm events that resulted in high, fast flow. We will continue scanning to track fine-scale *O. mykiss* movement as soon as conditions allow.

## Observed *O. mykiss* Cover Availability and Use in Upper North Fork Matilija

During snorkeling surveys used to estimate population abundance in Upper North Fork Matilija Creek, we also collected data regarding *O. mykiss* cover availability and use. We found that boulders comprised the greatest percentage of available cover in habitat units, and that trout were most often observed in association with boulders. As we could not account for the differential observation probabilities between cover types, we could not conclude that trout used boulder more so than other cover types. Nevertheless, our results demonstrate that boulders are both prevalent and frequently used by trout in a southern California stream system, where data regarding fine-scale habitat use are limited. We will continue to collect cover data during snorkel surveys of other streams, which will expand our scope of inference and strengthen our findings.

## ACKNOWLEDGEMENTS

Funding for this work was provided by the National Fish and Wildlife Foundation. Many individuals contributed to and collaborated on this project, including: Tom van Meeuwen, Shannon Mueller, Stan Allen, Amy Roberts, Mark Zuspan, Brennan Helwig, Brian Poxon, Samuel Bankston, and Kyle Evans (Pacific States Marine Fisheries Commission), David Boughton, Ann-Marie Osterback, Emerson Kanawi, David Rundio, Colin Nicol, and Lea Bond (National Oceanic and Atmospheric Administration, NOAA), Philip Taylor, James Garcia, Lillian Del Beccaro, and Manny Garcia (California Conservation Corps/NOAA Veterans Corps Fisheries Program), and Benjamin Lakish, Emma Moffitt, Tanielle Redman, Alexa Mutti, Katherine McLaughlin, Dana McCanne, and Mary Larson (California Department of Fish and Wildlife). We gratefully thank these contributors for their hard work and patience. Additionally, we gratefully acknowledge the landowners, whose cooperation and willingness to work with our staff made this project possible.

## Stationary Passive Integrated Transponder (PIT) Tag Arrays in the Ventura River Basin

Prepared by Yi-Jiun Tsai

### INTRODUCTION

To monitor the wide-scale movement of *Oncorhynchus mykiss* throughout the Ventura River Basin, we designed, built, and test deployed stationary arrays that detect passive integrated transponder (PIT) tags implanted within juvenile trout. These arrays detect and record PIT tags encrypted with individually-unique identification codes when tagged trout swim past. We selected three locations at which to monitor the passage of migrating trout using PIT tag arrays: the mainstem Ventura River, the confluence of Matilija and North Fork Matilija Creeks, and the confluence of San Antonio Creek and Ventura River (Figure 1). These locations were chosen because they would allow us to identify the source creeks of out-going smolts and the spawning creeks of incoming anadromous adults. Such information is critical to the monitoring of steelhead.

### ARRAY LOCATIONS

The confluence of Matilija and North Fork Matilija Creeks (site hereafter referred to as NFM) and the confluence of San Antonio Creek and Ventura River (site hereafter referred to as SAN) are key locations for monitoring migrating trout. North Fork Matilija and San Antonio Creeks provide some of the most important spawning and rearing habitat available to steelhead in the Ventura River Basin (summarized by Walter 2015), and extensive ongoing monitoring efforts focus on these tributaries.

*North Fork Matilija (NFM) Confluence*

For NFM, we identified an ideal array location at the Camino del Cielo bridge, just downstream of the confluence. We applied for permits to build an array using the bridge as an anchoring structure, but were denied permission. We then approached private landowners of parcels adjacent to the bridge and obtained permission from a friendly landowner to build an array just upstream of the bridge on February 21, 2017 (Figure 1). All necessary equipment was purchased and prepared for installing the NFM array after we secured landowner permission. However, a storm event on February 17, 2017 created extremely high and fast flow (21,000+ cfs; USGS 2017), which remained unsafe for array installation as of February 28, 2017 (Figure 2). We will commence building the NFM array once conditions become safe for construction, which is projected to be in April or May 2017.

*San Antonio (SAN) Confluence*

For SAN, we had an existing agreement with Rancho Arnaz to build an array just upstream of the confluence (Figure 1). However, due to persistent, extreme drought conditions, the proposed array location stayed dry with intermittent low flow conditions, and the trout population in San Antonio Creek was severely depressed for most of the grant period (March 2016–January 2017). Furthermore, a large debris accumulation (approximately 10 ft by 150 ft) located upstream of the array site was thought to pose a danger to our planned array. We therefore delayed building at this location until flow conditions improved such that trout could migrate through the area and until the debris pile could be flushed downstream of the site. On January 19, 2017 and February 17, 2017, two storm events significantly improved flow conditions at SAN for the first time since 2014. However, the upstream debris pile was not completely cleared by these storms. We therefore will wait until the debris load is cleared before installing an array at this location. All necessary equipment has been purchased and prepared for installation of the SAN array, whenever conditions allow.

*Ventura River Mainstem (VEN)*

The array located on the mainstem of Ventura River (location hereafter referred to as VEN) is approximately 5 miles upstream of the Ventura Estuary (Figure 1). Although the Ventura Estuary is a preferable location for detecting incoming spawning adults, the upstream location was ultimately chosen due to 1) its security, 2) existing monitoring efforts and infrastructure, and 3) river hydrology. Due to the site's location on gated property owned by Ojai Valley Sanitary District, VEN is more secure and less accessible to the public than areas further downstream. This site is shared with an ongoing monitoring project using underwater sonar cameras (dual-frequency identification sonar, DIDSON). The existing relationship between the DIDSON project and the Ojai Valley Sanitary District facilitated landowner access for this project and resulted in free AC power for the array. Furthermore, positioning our PIT tag arrays next to these cameras provides the unique opportunity to directly pair PIT tag detections with trout observations on sonar. In conjunction with the DIDSON project and redd surveys conducted throughout the Ventura River watershed, the addition of a PIT tag array at the VEN establishes this location as a life-cycle monitoring station, an essential tool in steelhead monitoring (Adams et al. 2011). Finally, the river is more constricted, but protected, at this location than in areas further downstream. The narrower channel width enabled us to build an array with antennas of manageable size and good tag read range.

## ARRAY DESIGN

With substantial help from National Oceanic and Atmospheric Administration (NOAA) and Pacific States Marine Fisheries Commission (PSMFC) collaborators, we designed AC- and solar-powered PIT tag arrays, the use of which was based on the availability of resources at each array location. We chose to use half-duplex (HDX) tags and readers instead of full-duplex tags/readers because 1) we could build larger antennas with greater read range, 2) we could more easily repair and replace antennas after large storm events due to its simpler antenna design, 3) the nearest PIT tagging project to our location (Malibu and Topanga, CA) uses HDX, 4) NOAA collaborators use HDX, and 5) recently-available 12mm HDX tags allow for smaller trout to be tagged than was previously available in HDX.

*Basic Array Components*

A PIT tag array is composed of four main components: a power source, a reader, tuner boxes, and antenna wire, all wired in sequence (Figure 3 & 4). When operational, the antenna loop emits an electromagnetic field. When a PIT tag (e.g. within a trout) passes within the field, the tag is energized and relays a signal back to the antenna, which passes the information to the reader. For each signal received, the reader translates and records the PIT tag code information and time of detection.

Antennas can be configured as either pass-over (also known as pass-by) or pass-through designs. When the antenna loop is laid flat along the substrate, trout are detected as they pass over the array (pass-over). When the antenna loop is maintained upright, perpendicular to flow, trout are detected as they pass through the loop (pass-through; Figure 3). Both designs feature advantages and disadvantages that guide their use at specific sites. A pass-over array is entirely secured to the substrate, which reduces the probability of antenna displacement during high flow events and from associated debris. However, pass-over antennas are limited in vertical detection range. In contrast, pass-through antennas are more susceptible to displacement, but have greater tag detection range spanning the water column, because one end of the antenna is vertically suspended across the channel. We used a combination of pass-over and pass-through antennas, depending on the hydrological and geographical specifications of each site; we generally chose pass-through antennas in relatively protected areas or with stable substrate, and pass-over antennas in areas of extremely fast flow or unstable substrate (e.g. sand). A list of the specific equipment used for each component is listed in Table 1.

Based on the remoteness and infrastructure available at each site, we chose either AC or solar power to run our arrays. SAN and NFM sites do not have available AC power, so we chose a solar design for these locations. At VEN, we were granted access to an AC outlet by the Ojai Sanitation District. Each array included modifications to the basic design outlined above to reduce electrical interference and thereby increase our tag detection range. The designs for each type of array are described below and illustrated in Figures 3 and 4. A great deal of input and support from Mark Zuspan and Brian Poxon (PSMFC) and Ann-Marie Osterback, David Boughton, and Emerson Kanawi (NOAA) contributed to these designs and the successful installation of these arrays.

*Solar-Powered Arrays on North Fork Matilija and San Antonio Creeks*

Our solar-powered arrays (Figure 3) will consist of a 140W solar panel connected to a solar charge controller with MC4 solar cable. The charge controller, which regulates power from the solar panel, will also be wired to two 12V batteries wired in parallel and the PIT tag reader. A fuse will be wired between the charge controller and battery on the positive lead. The reader will then be connected to two tuner boxes, which are also connected to antenna wire. Both the solar panel and jobox will be grounded using a copper grounding rod, 6 AWG copper wire, and copper lugs.

*AC-Powered Array on the Ventura Mainstem*

Our AC-powered array (Figure 4) consists of a GFCI plug connected to an extension cord, which is then plugged into a surge protector. A timer and a battery charger are plugged into the surge protector. A relay is wired to the battery charger, timer, a PIT tag reader, and batteries, all of which are housed within a jobox (Figure 5). The relay is wired to the timer such that power can be routed along two different paths at 12-hour intervals. At any given time, one battery powers the reader while the other is charging, with the batteries switching every 12 hours. This allows for battery charging to be isolated from powering of the reader. This relay feature was designed by Ann-Marie Osterback (NOAA). The PIT tag reader is connected with twinaxial cables to two tuner boxes mounted on t-posts. The tuner boxes (Figure 6) are then connected to antenna wire looped across the stream channel. One antenna is upstream and one antenna downstream of two DIDSON cameras. Both antennas are configured as pass-throughs, using t-posts and truck rope to suspend the wire (Figure 7). The antenna wire is also encased in PVC piping along the bottom edge and anchored to the stream substrate using duckbill earth anchors and hose clamps. Both antennas span the entire wetted width of the stream at the time of installation and measure approximately 20’ x 3’. When tested, tag read range was approximately 2–3 feet (Figure 8), and was not affected by the use of duckbills, t-posts, or simultaneous operation of two DIDSON cameras. All site installation was completed with a great deal of help from Mark Zuspan (PSMFC) and Philip Taylor and Jimmy Garcia (California Conservation Corps/NOAA Veterans Corps Fisheries Program).

While a reader could be more directly powered by AC power using an AC-DC adapter, we chose to use batteries as an intermediary. This design produces the least electrical noise (i.e. interferes the least with the reader). By the same token, we used a relay to isolate the charger from the reader and prevent the battery charging from creating interference with the reader.

**ARRAY TEST DEPLOYMENT ON VENTURA MAINSTEM**

At the first opportunity, we test deployed the VEN array to evaluate its durability and identify any weaknesses in design. Our PIT tag array deployment coincided with DIDSON deployment. The timing of deployment was based on an open connection between the Ventura River Estuary and the ocean, as well as flow conditions that allowed for trout passage. Based on the experience of other PIT tag projects further north, we expected that we might lose the antenna wire and tuner boxes during any given deployment. Such equipment is often carried away by high, fast flow and associated debris during storm events.

Due to permitting restrictions, we could not PIT tag trout below an impassable fish barrier (Matilija Dam) prior to test deployment. As there was little to no chance of detecting a tagged, outgoing juvenile, we decided not to risk the loss or damage of the more expensive equipment, such as the tuner boxes and reader. We therefore deployed only antenna wire, truck rope, t-posts, and the wooden boards on which tuner boxes were normally mounted prior to the storm event.

We deployed our array on January 19, 2017, before the first major storm series of 2016-2017. During this event, flow in the Ventura River mainstem rose to around 4000 cfs (USGS 2017). This was the largest event that had occurred within the Ventura River Basin since 2012 and resulted in extremely high and fast flow conditions. These conditions resulted in the following damage to our array: 1) all t-posts being bent and twisted out of shape, 2) all t-posts on river-right being removed from the substrate, and 3) PVC pipe (securing antenna wire to the substrate) being pulled out of the substrate (Figure 9). Upon close inspection, it appeared that the PVC pipe had detached from the substrate due to breaks in the duckbill earth anchor wires (Figure 10). All displaced equipment, including dislodged t-posts, antenna wire, truck rope, and PVC piping, remained attached to t-posts on river-left, and were recovered after the storm.

Based on the test deployment, we will make several modifications to the site set up: 1) installing stronger duckbill earth anchor wires or larger earth anchors, 2) changing the upstream antenna from a pass-through to a pass-over design, and 3) using a large, already-present boulder to anchor the downstream pass-through antenna on river-right. The PVC piping was ripped from the substrate due to breaks in earth anchor wires, so we will install either larger earth anchors, or earth anchors with thicker gauge steel wires, depending on the feasibility of installation. Specifically, the stream substrate at the VEN site contains many large cobbles and boulders, which may limit the size of earth anchor that can be installed. To protect the DIDSON cameras from being damage by dislodged PIT tag array equipment, we will modify the upstream antenna from a pass-through to a pass-over design. This should provide a more stable system that prevents floating or dangling debris from hitting or dragging on the camera. For the downstream pass-through antenna, an already-present boulder may better anchor the antenna wire and prevent antenna loss. All modifications will be implemented as soon as flow conditions allow. This is projected for April or May 2017.

## TABLES AND FIGURES

**Table 1.** Brand, specifications, and site at which each component of a solar- or AC-powered array was used.

| Component | Details | Brand | Site |
|---|---|---|---|
| Reader | HDX, multi-antenna | OregonRFID | VEN, NFM, SAN |
| Battery Charger | 12v, gel-cel compatible | Samlex | VEN |
| Battery | deep cycle, gel-cel, 110 AH, 12V | Deka | VEN, NFM, SAN |
| Solar panel | 140W, 12V | Solarland | NFM, SAN |
| Solar charge controller | 15A, MPPT | Morningstar | NFM, SAN |
| Solar cable | MC4, 10 AWG | Four Star | NFM, SAN |
| Tuning board | standard | OregonRFID | VEN, NFM, SAN |
| Twinaxial cable | 100 Ohm | Belden | VEN, NFM, SAN |
| Antenna wire | marine 10-2 gauge | Ancor | VEN, NFM, SAN |

**Figure 1.** Map of Ventura River Basin showing stationary PIT tag array locations (green circles). Locations are: Ventura River mainstem (VEN), confluence of North Fork Matilija and Matilija Creeks (NFM), and confluence of San Antonio Creek and Ventura River (SAN).

**Figure 2.** Camino del Cielo bridge just downstream of the planned NFM array. Pictures of the bridge before (a) and after (b) the February 17, 2017 storm event, which created flows of over 21,000 cfs (USGS 2017). High flows have delayed building the SAN and NFM arrays until conditions become safe.

a)

b)

**Figure 3.** Diagram of planned solar-powered arrays for NFM and SAN sites. A solar panel is connected to a MPPT charge controller with 10 AWG, MC4 solar cable (a). The charge controller is connected to both the batteries and the reader. Batteries are connected to each other (wired in parallel) and the charge controller using 10 AWG stranded copper wire (b). The charge controller is wired to the reader using 16 AWG (or thicker) stranded copper wire (c). The reader is connected to the tuner boxes using twinaxial, 100 ohm wire (d). The tuner boxes are connected to loops of 10-2 marine antenna wire (e) configured as either pass-over or pass-through antennas. Both the solar panel and the jobox are grounded using 6 AWG copper wire (f) connected to a buried grounding rod.

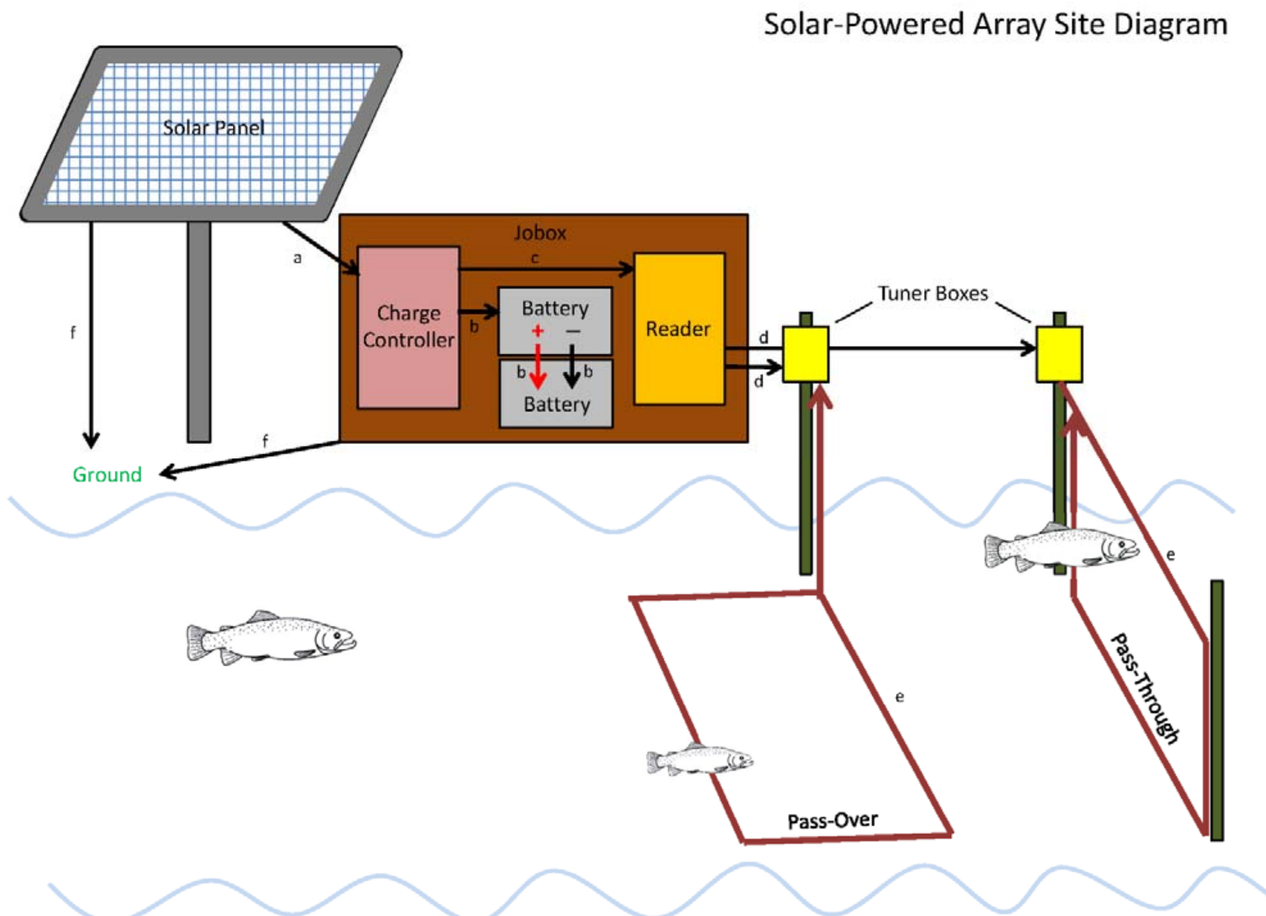


**Figure 4.** Diagram of the Ventura mainstem stationary, AC-powered PIT tag array, including antenna placement up- and downstream of two DIDSON cameras. A GFCI is plugged into an AC outlet, which is connected to a surge protector by an extension cord (a). A timer and battery charger are plugged into the surge protector. The timer is wired into the relay using a 120V feed (i.e. spliced 16/2 cord, b). The battery charger and two batteries are wired to the relay using 10 AWG stranded copper wire (c). The reader is also wired to the relay with a 16 AWG stranded copper wire. The relay allows for one battery to be charging, while the other battery is powering the reader. The reader is connected to two tuner boxes via twinaxial, 100 ohm cable (e). The tuner boxes are connected to loops of 10-2 marine antenna wire (f).

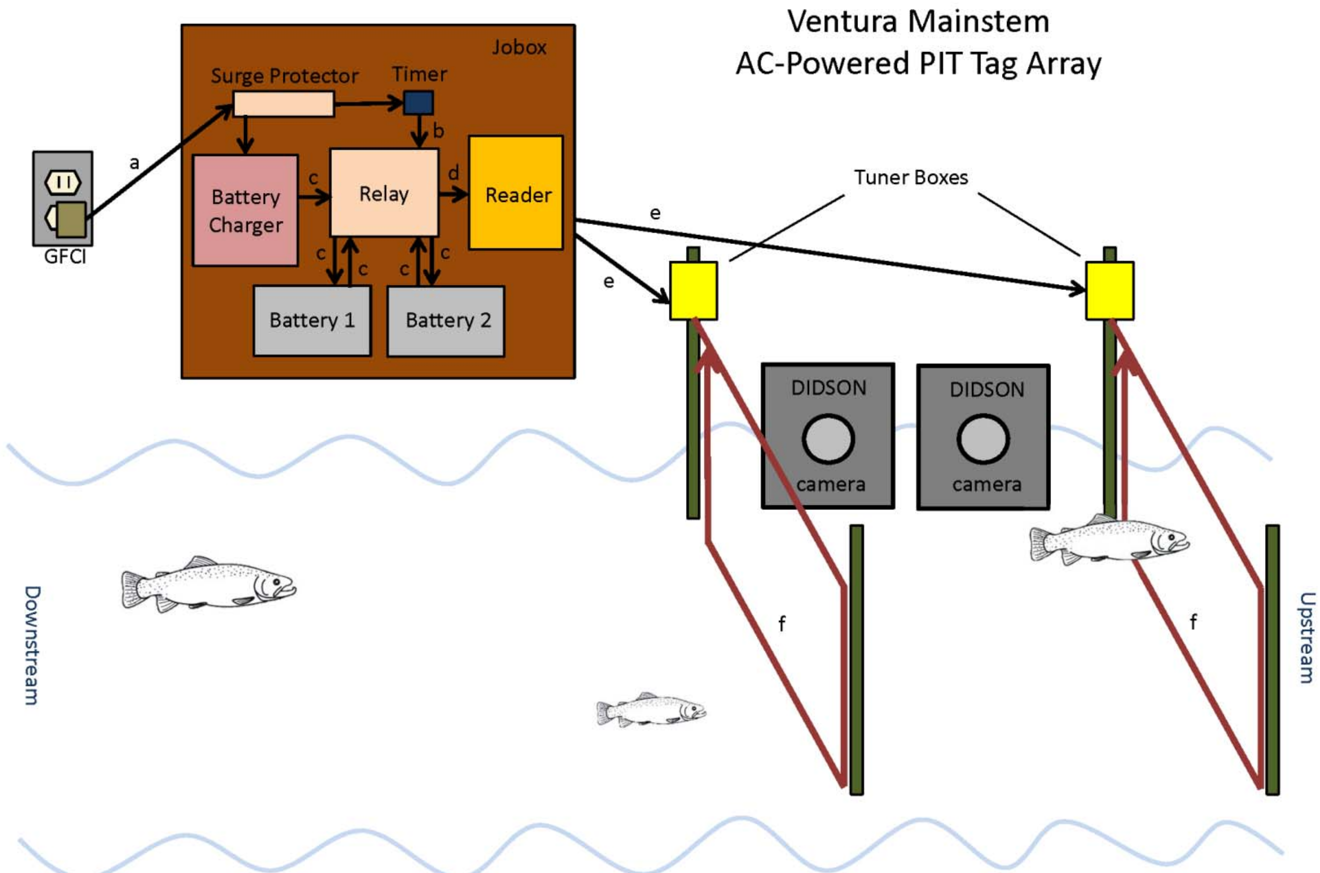


**Figure 5.** Picture of the surge protector, battery charger, relay, and PIT tag reader contained within a protective jobox at the AC-powered Ventura mainstem PIT tag array site. Two batteries are contained beneath the wooden board.

**Figure 6.** Picture of a tuner box screwed onto a wooden board mounted on a t-post at the Ventura River mainstem PIT tag array site.

**Figure 7.** Picture of two pass-through antennas installed at the Ventura River mainstem PIT tag array site. Perspective is from upstream looking downstream.

**Figure 8.** Picture of Kathryn Carmody (Fisheries Technician, PSMFC) testing the read range of the installed array at the Ventura mainstem. On the right is a tuner box mounted on a t-post with a wooden board and connected to twinaxial cable (black, thick cable on top) and antenna wire (black and red cables on right side and bottom).

**Figure 9.** Picture of bent t-posts, dangling truck rope and antenna wire, broken debris panels (for DIDSON), and upended tuner box t-posts after the January 19, 2017 storm event. T-posts held by Samuel Bankston (Fisheries Biologist, PSMFC).

**Figure 10.** Picture of PVC pipe dislodged from earth anchor wire breakage during January 19, 2017 storm event. Shown from wide-angle (a) and close-up (b) perspectives.

a)

b)

**Population abundance estimation of *Oncorhynchus mykiss* in Upper North Fork Matilija Creek in 2016**

Prepared by Yi-Jiun Tsai

## INTRODUCTION

In this study, we estimated *O. mykiss* abundance in Upper North Fork Matilija Creek in the winter of 2016. We used a double sampling design modified from Hankin & Reeves (1988) and described by McCanne & Reisberger (2005), which included habitat typing, snorkeling, and electrofishing shallow habitat units. This report describes an initial effort of a larger monitoring project focused on estimating population abundance in a watershed designated as high priority for steelhead recovery.

## METHODS

*Upper North Fork Matilija Creek*

Due to permitting limitations, we surveyed a creek above Matilija Dam, and therefore above anadromy. While the dam prevents the movement of anadromous *O. mykiss* into these reaches, resident *O. mykiss* in these creeks are still important to recovery efforts as relatively protected population that could contribute to the production of anadromous *O. mykiss* (Clemento et al. 2008).

Upper North Fork Matilija Creek is a tributary to Matilija Creek within the Ventura River Basin, a watershed designated as high priority for steelhead recovery in the Monte Arido Highlands (Core 1; NMFS 2012). Upper North Fork Matilija Creek is a fourth-order stream that drains a watershed of 12.45 square miles. Elevations range from approximately 1600 to 5700 feet. Lower montane mixed chaparral dominates the watershed (CALVEG 2015). Most of Upper North Fork Matilija occurs on federally-protected United States Forest Service and agricultural properties.

*Field Methods*

Field data were collected from October 3, 2016 to December 1, 2016 by Pacific States Marine Fisheries Commission, with help from the California Department of Fish and Wildlife and the California Conservation Corps/NOAA Veterans Corps Fisheries Program. Upper North Fork Matilija Creek was surveyed for 4,996 feet, starting at the first wetted unit (34.51011°N, -119.38307°W) upstream of the confluence of Matilija and Upper North Fork Matilija Creeks. The endpoint (34.51606°N, -119.37746°W) was the start of a long dry section.

Field sampling was comprised of three parts: habitat typing, snorkeling, and electrofishing. For habitat typing, the stream was delineated into discrete habitat units, which were categorized by habitat type and measured for dimensions. Based on these dimensions, units were determined to be snorkelable or unsnorkelable. For unsnorkelable units, a subset was electrofished. For snorkelable units, all units were snorkeled if conditions were appropriate. A subset of snorkelable units was then electrofished. Habitat typing, snorkeling, and electrofishing of a given unit were completed within the same week.

Habitat Typing

For habitat typing, we delineated the wetted stream channel into discrete, natural units of similar habitat (Hankin 1984). Habitat units were then classified as riffles, pools, or flatwaters based on characteristics outlined in the California Salmonid Stream Habitat Restoration Manual (Flosi et al. 1998). Riffles were generally characterized by a medium to high gradient, a large amount of exposed substrate, and rapidly flowing water. Pools were areas where water collected with bowl-shaped edges. Flatwaters contained slow-moving water with a smooth or calm surface.

Although these habitat unit types were generally defined by Flosi et al. (1998), there were important deviations from these definitions. Specifically, each run or pool was considered to be a separate unit, even if it would traditionally be considered as part of a larger step-pool or step-run in the Habitat Restoration Manual. This was to prevent inflation of unit size in abundance estimation. Additionally, unit dimensions had no bearing on unit type.

In a few cases, flatwaters transitioned into pools with no apparent break in connectivity; in other words, these units were effectively one connected unit, in which trout could pass freely. These units were thought to function as, and were therefore categorized as, pools.

Once categorized by habitat type, units were then measured for length, mean width, mean depth, and maximum depth. Based on these dimensions, units were considered to be either snorkelable or unsnorkelable and electrofishable or unelectrofishable. Units selected for later snorkeling and electrofishing were flagged. All units less than 0.3 feet in mean depth, greater than three feet in mean depth, greater than 3.5 feet maximum depth, or less than six feet in length were excluded from sampling, as they could not be snorkeled or electrofished properly.

Snorkeling

Units were considered to be snorkelable if mean depth was greater than or equal to 0.7 feet (minimum depth at which snorkelers can reliably count fish; O'Neal 2007) and there were no barriers to snorkeling, such as poor water clarity or the presence of hazards. Only pools and flatwaters were considered to be snorkelable (McCanne & Reisberger 2005), as riffles were generally too shallow to be adequately snorkeled (one of 51 riffles sampled had a mean depth of 0.7 feet or greater). All units that could be snorkeled were snorkeled.

For snorkeling, a single diver was used for units in which visibility was clear bank-to-bank, which generally included units less than or equal to 15 feet wide and units without complex cover. For larger or more complex units, two divers were used. Divers entered the unit from the downstream end, taking care to minimize disturbance to the water, avoid scaring any trout present, and avoid stirring up substrate sediment. Once under water, the diver counted the number of trout visually observed while moving towards the upstream end of the unit. Each unit was snorkeled once.

Depletion Electrofishing

We used depletion electrofishing to calibrate snorkel counts. Depletion electrofishing assumes that each successive electrofishing pass catches fewer fish than in the previous pass (White et al. 1982). Additional passes are required if this assumption is violated (McCanne & Reisberger 2005). This method further assumes that trout do not move between units and that equal effort is used across passes (Raleigh & Short 1981, White et al. 1982, McCanne & Reisberger 2005). Each of these assumptions was address in the methodology described below.

A final assumption of depletion electrofishing is that all individuals (trout) have an equal probability of being caught (Raleigh & Short 1981, White et al. 1982). This assumption was unlikely to be strictly true in this study, given the general size biases of electrofishing (Temple & Pearsons 2007). We did not correct for these possible biases in our analyses due to time constraints.

Units were electrofishable if 1) there were no undercuts greater than three feet (length of the electrofishing pole), 2) there were no barriers to electrofishing, such as structures preventing effective netting or use of the electrofishing anode, and 3) the water cleared of disturbed sediment within one hour between passes.

Units were selected to be electrofished using pseudo-stratified random sampling within each habitat type. Electrofishable units were first grouped by habitat type, ordered from downstream to upstream, and then divided into groups of four. Within each group of four, one unit was randomly selected to be electrofished. This sampling selection was conservative and was chosen to minimize unnecessary electrofishing trauma to *O. mykiss*.

For electrofishing, we placed block nets at the upstream and downstream ends of the unit to prevent trout passage between units. Water temperature and conductivity were measured prior to each pass. Due to concern for trout health, units were only electrofished if the temperature was below 69°F (21°C). We used Smith-Root LR-24 electrofishers set at 120 volts, 35 hertz, and 30% duty cycle.

For a given pass, electrofishers and netters worked from the downstream end of the unit, shocking the water and netting trout affected by the electrical field while moving upstream. One electrofisher was used unless habitat units were of odd shape, wider than ten feet, or contained complex cover, in which case two electrofishers were used. We recorded the number of trout captured and the time spent electrofishing for each pass. Approximately equal effort was spent on each pass of a given unit. We held captured trout in buckets of stream water (with aerators) until all passes were complete. Buckets were refreshed with water every 10–20 minutes.

Electrofishing of a unit was complete if no trout were caught in the first two passes (minimum of two passes) or if 25% or less trout were caught in the last pass than in the previous pass. For example, if eight trout were caught in the second pass, and more than two trout (>25% of eight trout) were caught in the third pass, then a fourth pass was required. If two or less trout (≤25% of eight trout) were caught in the third pass, then no further passes were required. A maximum of five electrofishing passes was allowed per unit (D. McCanne, California Department of Fish and Wildlife).

All electrofishing was conducted under supervision of the California Department of Fish and Wildlife.

### Abundance Estimation

Trout abundance estimate for Upper North Fork Matilija was applicable only to shallow units (mean depth 0.3 to 3 feet and maximum depth less than or equal to 3.5 feet). We included unit length as an auxiliary variable in our calculations (Hankin 1984; McCanne & Reisberger 2005). The equations used to calculate abundances are described in Appendix I.

To estimate trout abundance, we excluded units that did not meet the above outlined parameters for sampling, snorkeling, and electrofishing. We calculated a jackknife estimator for each electrofished unit using Equation 1 (Appendix I). These jackknife estimators were then included in subsequent calculations of trout abundance.

We estimated abundance for snorkelable and unsnorkelable units separately, using Equations 2 and 3 (Appendix I), respectively. Due to low sample size of electrofished units within each habitat type, we pooled habitat types in calculations of snorkelable and unsnorkelable abundances (see McCanne & Reisberger 2005).

The total within-reach trout abundance estimated was calculated as the sum of the snorkelable and unsnorkelable abundance estimates. Within-reach variance was calculated as the sum of snorkelable and unsnorkelable variances. Ninety-five percent confidence intervals were calculated based on these variances (Equation 4, Appendix I).

All data management and calculations were completed using R (version 3.3.1, R Core Team 2016) and RStudio (version 0.99.903, RStudio, Inc. 2016).

**RESULTS**

A total of 235 units were habitat typed. Of these, 193 units met the criteria for sampling. Fifty-eight units were categorized as flatwaters, 84 as pools, and 51 as riffles. Of 68 snorkelable units, 52 units were snorkeled, 15 units were both snorkeled and electrofished, and one unit was neither snorkeled nor electrofished. Of 125 unsnorkelable units, 27 units were electrofished (Table 1).

The estimated trout abundance of shallow units in Upper North Fork Matilija Creek was 274 ± 124 trout (± 95% CI; Table 1).

**DISCUSSION**

We estimated that there were 274 ± 124 trout (± 95% CI) within shallow units of Upper North Fork Creek. Although this abundance estimate alone is not informative, it contributes to an ongoing monitoring project, in which we will compare estimates across reaches and over time. Additionally, the field methods (snorkeling and electrofishing) used to estimate abundances has aided and been adopted in part by the California Department of Fish and Wildlife in southern California. Furthermore, we will estimate the abundance of anadromous streams in the near future, which will help inform current monitoring of *O. mykiss* populations.

A previous trout abundance estimation conducted in 2015 reported 475 ± 355 trout (± 95% CI) in Upper North Fork Matilija (Bankston et al. 2015). This previous sampling effort was a pilot study, and was used to shape the protocols in this report. We believe that several factors contributed to the greater reliability and lower estimate of the 2016 estimation. First, our selection of sampleable units was more stringent; some deeper pools that could not be adequately electrofished to depletion were included in the 2015 sampling, but excluded from our 2016 study. These deeper pools are likely to hold higher densities of trout, and may have inflated estimates in 2015. Second, snorkeling followed electrofishing in 2015, whereas snorkeling preceded electrofishing in 2016. The order of sampling methods is important, given that abundance estimation relies on the assumption that trout do not move between units. We believe that electrofishing is likely to disturb trout more so than snorkeling, and may lead to greater movement between units. Furthermore, our sample size of snorkeled and electrofished units was greater in 2016 than in 2015. Thus, we believe that the 2016 estimation of abundance is more reliable than the 2015 estimate. Finally, following the 2015-2016 winter season, southern California entered its fifth year of drought, with Ventura County continuing to experience lower-than-average precipitation levels. These conditions led to drying of previously-wetted sections of stream. Specifically, 1,466 feet of stream was wet in 2015 but dry in 2016. Thus, the stresses of the drought on the trout population combined with the smaller area sampled likely contributed to the lower abundance estimate in 2016.

Our abundance estimation of Upper North Fork Matilija was limited to the shallow units that could be sampled; we could not snorkel or electrofish deeper pools and therefore could not make inferences about trout abundance within these pools. However, there were only three deep pools that were excluded from sampling, and estimates of abundance for shallow units still provide important data for comparisons across reaches and time. Future research could explore alternative estimation methods for *O. mykiss* in deep or complex habitats.

Future estimations of abundance may be improved by increasing the sample size of electrofished units. We conservatively electrofished 22% of snorkelable and unsnorkelable units. Increased effort in electrofishing may provide less variable abundance estimates, although this must be balanced carefully with electrofishing mortality concerns and limitations in time and available effort.

Although we were restricted to sampling a non-anadromous stream in this case, we are listed as principal investigators in a Section 10(a)(1)(a) permit application submitted by the California Department of Fish and Wildlife, which we hope will be approved by spring of 2017. This will allow us to sample anadromous reaches in the Ventura River watershed. Our priority at that time will be to sample North Fork Matilija Creek from June to December 2017.

## TABLES

**Table 1.** Habitat unit sample sizes and estimated trout abundance for Upper North Fork Matilija Creek in 2016. Unit sample sizes include the total number of units that could be sampled (Total # Hab. Units), the total number of snorkelable and unsnorkelable units, and the number of units that were snorkeled, electrofished, or both. A subset of unsnorkelable units was electrofished. All snorkelable units that could be snorkeled were snorkeled. A subset of snorkeled units was also electrofished.

| Total # Hab. Units | Snorkelable Units | | | Unsnorkelable Units | | Est. Trout Abund. |
|---|---|---|---|---|---|---|
| | Total # | # Snorkeled | # Snorkeled + E-fished | Total # | # E-fished | |
| 193 | 68 | 52 | 15 | 125 | 27 | 274 ± 124 |

## APPENDIX I. EQUATIONS USED TO ESTIMATE ABUNDANCE ESTIMATION

### Equation 1. Electrofishing Jackknife Estimation

Jackknife estimation for electrofishing data, where the total number fish ($\hat{y}_i$) and sampling variance ($\hat{V}(\hat{y}_i)$) in unit *I* are estimated by (modified from Pollock & Otto 1983, as notated in McCanne & Reisberger 2005):

$$\hat{y}_i = \sum_{j=1}^{r_i-1} c_{i\cdot j} + r_i c_{r_i}$$

$$\hat{V}(\hat{y}_i) = r_i(r_i - 1)c_{r_i}$$

where

$r_i$ = number of electrofishing passes conducted in the *i*th habitat unit,
$c_{r_i}$ = number of fish captured in the *r*th (last) pass in the *i*th habitat unit, and
$c_{i\cdot j}$ = number of fish captured in the *j*th pass in the *i*th habitat unit.

**Equation 2. Abundance Estimation for Snorkelable Units**
Snorkelable units included pools and flatwaters that were 0.7–3 feet in mean depth and ≤3.5 feet in maximum depth. A subset of snorkelable units was snorkeled (referred to as phase one sampling in McCanne & Reisberger 2005). A further subset of these snorkeled units were also electrofished and were denoted below as snorkeled + e-fished units (referred to as phase two sampling in McCanne & Reisberger 2005). We used unit length as an auxiliary variable (McCanne & Reisberger 2005; Hankin 1984).

The total number of fish in snorkelable units ($\hat{T}_S$) and sampling variance ($\hat{V}(\hat{T}_S)$) are estimated by (modified from Särndal et al. 1992, as notated in McCanne & Reisberger 2005):

$$\hat{T}_S = N\bar{\hat{y}}_2\left(\frac{\bar{x}_1}{\bar{x}_2} + \frac{\bar{L} - \bar{l}_1}{\bar{l}_2}\right)$$

$$\hat{V}(\hat{T}_S) \approx N^2\left(1 - \frac{n_1}{N}\right)\left(\frac{\bar{L}}{\bar{l}_1}\right)\frac{s_{y|l}^2}{n_1} + N^2\left(1 - \frac{n_2}{n_1}\right)\left(\frac{\bar{x}_1}{\bar{x}_2}\right)^2\frac{s_{y|x}^2}{n_2}$$

$$s_{y|l}^2 = \frac{1}{n_2 - 1}\sum_{i=1}^{n_2}\left(\hat{y}_i - \bar{\hat{y}}_2\frac{l_i}{\bar{l}_2}\right)^2$$

$$s_{y|x}^2 = \frac{1}{n_2 - 1}\sum_{i=1}^{n_2}\left(\hat{y}_i - \bar{\hat{y}}_2\frac{x_i}{\bar{x}_2}\right)^2$$

where

$N$ = total number of snorkelable units,
$\hat{y}_i$ = jackknife estimate of the true number of fish in the $i^{th}$ unit (within snorkeled + e-fished units; calculated from Equation 1),
$\bar{\hat{y}}_2$ = mean jackknife estimate of the true number of fish in all snorkeled + e-fished units,
$x_i$ = observed number of fish counted during snorkeling the $i^{th}$ unit (within snorkeled + e-fished units),
$\bar{x}_1$ = mean number of fish counted during snorkeling in units that were snorkeled,
$\bar{x}_2$ = mean number of fish counted during snorkeling in units that were snorkeled + e-fished,
$\bar{L}$ = mean length of all snorkelable units,
$l_i$ = length of the $i^{th}$ unit (within snorkeled + e-fished units),
$\bar{l}_1$ = mean length of units that were snorkeled,
$\bar{l}_2$ = mean length of units that were snorkeled + e-fished,
$n_1$ = number of units that were snorkeled, and
$n_2$ = number of units that were snorkeled + e-fished.

**Equation 3. Abundance Estimation for Unsnorkelable Units**

Unsnorkelable units were those 0.3–3 feet in mean depth and ≤3.5 feet in maximum depth. These units included all riffles, as well as pools and flatwaters <0.7 in mean depth. A subset of unsnorkelable units was electrofished.

The total number of fish in unsnorkelable units ($\hat{T}_U$) and sampling variance ($\hat{V}\left(\hat{T}_U\right)$) are estimated by (modified from Hankin 1984 and Cochran 1977, as notated in McCanne & Reisberger 2005):

$$\hat{T}_U = N\bar{\hat{y}}\left(\frac{\bar{L}}{\bar{l}}\right)$$

$$\hat{V}\left(\hat{T}_U\right) \cong N^2\left(1-\frac{n}{N}\right)\frac{s^2_{\hat{y}|l}}{n} + \frac{N}{n}\sum_{i=1}^{n}\hat{V}\left(\hat{y}_i\right)$$

$$s^2_{\hat{y}|l} = \frac{1}{n-1}\sum_{i=1}^{n}\left(\hat{y}_i - \bar{\hat{y}}\frac{l_i}{\bar{l}}\right)^2$$

where

$N$ = total number of unsnorkelable units,
$n$ = number of electrofished units,
$\hat{y}_i$ = jackknife estimate of the true number of fish in the *i*th unit (within electrofished units; calculated from Equation 1),
$\bar{\hat{y}}$ = mean jackknife estimate of the number of fish across all electrofished units,
$\bar{L}$ = mean length of all unsnorkelable units,
$l_i$ = length of the *i*th unit (within electrofished units), and
$\bar{l}$ = mean length of electrofished units.

**Equation 4. Within-Reach Estimation**
Within reach estimates ($\hat{T}_{reach}$) were calculated as the sum of individual estimates (Hankin 1984):

$$\hat{T}_{reach} = \sum_{hab=1}^{j} \hat{T}_{hab}$$

where
*j* = the total number of strata, and
*hab* = habitat type group.

The total sampling variance across($\hat{V}\left(\hat{T}_{reach}\right)$) was estimated by the sum of individual variances (Hankin 1984):

$$\hat{V}\left(\hat{T}_{reach}\right) = \sum_{hab=1}^{j} \hat{V}\left(\hat{T}_{hab}\right)$$

where
*j* = the total number of strata, and
*hab* = habitat type group.

Ninety-five percent confidence intervals were calculated by (Cochran 1977, as notated in McCanne & Reisberger 2005):

$$\hat{T}_{reach} \pm t_{0.025,n-1}\sqrt{\hat{V}\left(\hat{T}_{reach}\right)}$$

approximated by

$$\hat{T}_{reach} \pm 2\sqrt{\hat{V}\left(\hat{T}_{reach}\right)}$$

## Fine-scale *Oncorhynchus mykiss* Movement in Upper North Fork Matilija Creek

Prepared by Yi-Jiun Tsai

### INTRODUCTION

In this pilot study, we examined fine-scale *Oncorhynchus mykiss* movement in Upper North Fork Matilija Creek using passive integrated transponder (PIT) tag scanning surveys. This report describes an initial effort of a larger monitoring project focused on monitoring tagged trout in a watershed designated as high priority for steelhead recovery.

### METHODS

*Upper North Fork Matilija Creek*

Due to permitting limitations, we surveyed a creek above Matilija Dam, and therefore above anadromy. While the dam prevents the movement of anadromous *O. mykiss* into these reaches, resident *O. mykiss* in these creeks are still important to recovery efforts as relatively protected population that could contribute to the production of anadromous *O. mykiss* (Clemento et al. 2008).

Upper North Fork Matilija Creek is a tributary to Matilija Creek within the Ventura River Basin, a watershed designated as high priority for steelhead recovery in the Monte Arido Highlands (Core 1; NMFS 2012). Upper North Fork Matilija Creek is a fourth-order stream that drains a watershed of 12.45 square miles. Elevations range from approximately 1600 to 5700 feet. Lower montane mixed chaparral dominates the watershed (CALVEG 2015). Most of Upper North Fork Matilija occurs on federally-protected United States Forest Service and agricultural properties.

*Field Methods*

Field data were collected from October 3, 2016 to December 20, 2016 by Pacific States Marine Fisheries Commission, with help from the California Department of Fish and Wildlife and the California Conservation Corps/NOAA Veterans Corps Fisheries Program. Upper North Fork Matilija Creek was surveyed for 4,996 feet, starting at the first wetted unit (34.51011°N, -119.38307°W) upstream of the confluence of Upper North Fork Matilija and Matilija Creeks. The endpoint (34.51606°N, -119.37746°W) was the start of a long dry section.

For this study, we capitalized on previous survey efforts, in which we delineated the reach into discrete habitat units, electrofished sub-selected units, and PIT tagged trout captured during electrofishing. After tagging, we returned to the surveyed reach and scanned for PIT tags, recording any movement within and between habitat units. While we briefly summarize all stages of field methods here, further details regarding habitat typing and electrofishing can be found in the Abundance Estimation Chapter of this report.

Habitat Typing

For habitat typing, we delineated the wetted stream channel into discrete, natural units of similar habitat (Hankin 1984). These units were numbered sequentially, starting from the downstream-most end of the reach. A subset of units were flagged and photographed so that trout movement could be tracked relative to landmarked units throughout the year. These units were distinctive, deeper, and generally larger habitat units, with mean depths of one foot or greater.

### Electrofishing Sampling Efforts

Trout were PIT tagged during two separate electrofishing efforts. The first was during an abundance estimation effort, in which units were electrofished to depletion. Units were sub-selected and then electrofished if they were 1) 0.3–3 feet in mean depth, 2) greater than 6 feet in length, 3) less than or equal to 3.5 feet in maximum depth, 4) without undercuts greater than three feet, 5) without barriers to electrofishing, and 6) cleared of sediment within one hour between passes. Units were selected to be electrofished using pseudo-stratified random sampling within habitat type. Each unit was electrofished using at least two, but no more than five, passes. One electrofisher was used unless habitat units were of odd shape, wider than 10 feet, or contained complex cover, in which case two electrofishers were used. See Abundance Estimation Chapter for further details.

The second electrofishing survey was an effort to increase the number of PIT tagged trout. During this second effort, we targeted habitat units that were greater than or equal to one foot in mean depth at the time of habitat typing. This was a haphazardly-chosen criterion that resulted in the electrofishing of a manageable number of deeper units. All units were only electrofished if they met the six, previously-describe criteria. Our focus was to tag as many trout as possible in areas of high trout densities, excluding any units that were previously sampled during the abundance estimation effort. In this case, we electrofished each unit once (single pass). Given the size and depth of the units being sampled, we used two electrofishers to sample all units.

### Electrofishing

Across both electrofishing efforts, we measured dissolved oxygen levels and temperature prior to electrofishing. Electrofishing was only permitted if water temperature was less than 69°F (21°C) prior to starting a unit. We used Smith-Root LR-24 electrofishers set at 120V, 30Hz, and 30% duty cycle.

Beginning from the downstream end of the unit and working upstream, one to two electrofishers moved through the unit with the anode, using an up-down, side-to-side sweeping movement such that the entire water column and the entire surface area of the unit was covered. Sampling the entire area of the unit once was considered a single pass. As the electrofisher(s) moved through the system, one to two netters followed each electrofisher and netted any stunned trout. Captured trout were immediately transferred from the net to a holding bucket.

Captured trout were held in buckets filled approximately 2/3 full with stream water. Buckets were kept in shade and refreshed with water every 10–20 minutes for the entire duration that trout were held. No more than four large or seven small trout were held within a bucket at any given time to prevent overcrowding and suffocation

### PIT Tagging

Prior to PIT tagging wild trout in Upper North Fork Matilija, we conducted PIT tag training at Filmore Hatchery, in which we practiced PIT tagging techniques and retained the trout for four weeks to determine our tagging efficiency. We tagged 42 hatchery trout, all of which were alive and retained their PIT tags after four weeks.

In the field, following electrofishing, captured wild trout were measured and PIT tagged. Trout less than or equal to than 80 mm were measured without anesthetization and then immediately released. Trout greater than 80 mm were anesthetized, measured, PIT tagged, and then released. All trout were released into the same unit in which they were captured.

All trout were measured for fork length to the nearest millimeter, photographed, and visually assessed for the presence of black spot disease (dark pigmented spots indicating parasitic infection).

Trout greater than 80 mm were PIT tagged if they appeared to be in good health, showed normal behavior, and did not already carry a PIT tag. Tagging entailed anesthetization, tag implantation, recovery, and release. We prepared tagging equipment by soaking the scalpel, injector syringe, and PIT tag in ethanol for at least 30 seconds. For anesthetization, we added Alka Seltzer Gold to a bucket of stream water at the lowest concentration that permitted safe trout handling, approximately 1–2 tablets per gallon of fresh river water, depending on fish size and water temperature. Anesthesia was administered by placing trout into the anesthesia bucket one at a time. To minimize stress and potential mortalities, anesthesia was administered only for as long as necessary to achieve temporary immobilization. Anesthesia was administered until loss of equilibrium was achieved but operculum movement was still present.

After trout were immobilized, we implanted PIT tags into the trout body cavity. We implanted 12mm 134.2 kHz ISO half-duplex (HDX) PIT tags into trout 80 to 175mm in fork length, and 23mm tags into trout greater than 175 mm. To implant the tag, we created an incision between the pectoral fin and pelvic girdle on the abdomen to the right or left of the mid-ventral line of the trout using a disinfected scalpel (PTAGIS 2014). The tag was inserted into the incision using a blunted implanter syringe, with the bevel pointed toward the trout (down). The syringe and scalpel were disinfected between each trout by soaking in ethanol for at least 30 seconds.

Once the tag was inserted, trout were released into recovery buckets containing an aerator and stream water. Recovery buckets were refreshed with stream water every 10–20 minutes. Recovering trout were monitored for the return of normal behavior. Following full recovery, trout were released.

All electrofishing and tagging was conducted under the supervision of the California Department of Fish and Wildlife.

Scanning

Scanning surveys began at the downstream end of the reach, working upstream. The entire wetted area of the stream was scanned using an HDX portable backpack reader (OregonRFID), pole antenna, Piezo buzzer, and linked Meazura PDA. We scanned the water as systematically as possible by walking in a zig-zag-like-pattern across the stream width, while moving the pole antenna in slow, wide, lateral sweeping arcs (Figure 1). The antenna was maintained approximately four to six inches above the substrate, including in deeper pools. In deep pools, all areas up to approximately five feet deep (pole antenna length when taking antenna buoyancy into consideration) were scanned. Care was taken to scan areas in which trout are likely to hide, including crevices alongside boulders, and the margins of habitat units. All units of the reach were scanned, even if units were not previously electrofished.

All detected tags and their GPS locations were recorded. Additionally, a diagram of the unit in which the tag was detected, including prominent boulders or trees, was sketched and the exact location of the tag relative to these habitat features was recorded. This allowed for tracking of within-unit movement between surveys. Finally, using the flagged, deeper units as landmarks, tags detected more than 200 feet up- or downstream from its previous location (e.g. unit in which trout were captured, tagged, and released) were measured using a hip chain whenever possible to determine the exact distance the tag had traveled.

No assumptions were made regarding whether the tag remained implanted within trout or whether the tag had been shed, unless there was clear indication otherwise. Evidence for tags being detected within live trout included visual observation of trout in the location where a tag was detected using a snorkel mask and active movement of the tag during scanning of a given unit.

**RESULTS**

We habitat typed, electrofished, and PIT tagged trout from October 3, 2016 to December 7, 2016. During this time, we PIT tagged a total of 62 trout. Of these, 61 trout were implanted with 12mm HDX tags and one with a 23mm HDX tag.

One scanning survey was conducted December 19–20, 2017. Although surveys were planned as monthly events, high and fast flow resulting from a series of storm events prevented any further surveys as of February 28, 2017.

We detected 45 of 62 (73%) total tags. Of these, eight (18%) tags had moved since trout were tagged. Three of these tags were detected downstream and five tags upstream from where tagged trout were released. The greatest distance a tag moved was 0.1 miles upstream. In this particular case, we tagged the trout during the first electrofishing event, recaptured the trout 0.1 miles upstream during the second electrofishing effort, and detected the tag in the same upstream pool during scanning.

In eight cases, we found that the tag detected was in live trout, confirmed either by visual observation or movement of the tag during scanning.

Tags that were implanted from October 13 through December 7 were detected during scanning, with no obvious bias towards a given tagging date or period.

**DISCUSSION**

We detected 45 of 62 total tags within the surveyed reach. Our recapture rate of 73% was comparable to other studies of trout species in northern systems. Studies of Atlantic salmon, brown trout, and steelhead trout have reported PIT tag detection efficiencies ranging from 25 to 96% when blocked sections of creek were scanned within eight days of tagging (Roussel 2000; Cucherousett 2005; Hill 2006), and 39–43% efficiency when an unblocked study reach was scanned repeatedly for up to four months after tagging (Cucherousett 2010). Our recapture rate is high when considering that we used almost entirely 12 mm tags (smaller read range than 23 mm tags), that our study reach was unblocked, and that tags were implanted 12 days to two months prior to scanning. It is likely that the low flow conditions and the small size of Upper North Fork Matilija Creek contributed to successful scanning (Figure 2).

Of the 45 tags that were detected during scanning, only eight had moved either up- or downstream from the unit in which trout were tagged and released. This result suggests that these *O. mykiss* did not often move between habitat units. Low flow conditions may have constrained trout movement, although the 0.1-mile, upstream movement of one individual from its release location suggests that trout may be able to overcome these low flow conditions, to some degree. However, this hypothesis assumes that tags have been retained within trout, and our scope of inference was limited. Additional scanning surveys in Upper North Fork Matilija across a longer time span will help us better determine the extent to which trout move between units. Surveys conducted across different stream systems will help us evaluate the extent to which our findings apply to *O. mykiss* throughout southern California.

We confirmed that eight tags were detected in live trout at the time of scanning, using visual observation and deduction from tag movement. We often found it difficult to visually observe trout where tags were detected; tags were often detected in inaccessibly deep boulder crevices. We therefore determined that attempting to visually observe trout was not an effective use of time for future scanning surveys. In contrast, tag movement during scanning of a habitat unit was a clear and easy method of determining that tags were retained in live trout. However, this occurred infrequently. Previous studies have reported that trout remain immobile during scanning activity, and suggest that stationary tag reads should not be discounted (Hill et al. 2006). Even so, our study suggests that scanning was not an effective means of estimating tag retention rates, although its effectiveness is likely to improve with repeated scanning surveys.

We could not continue our scanning surveys in January and February due to a series of storm systems that created heavy, fast flow. These systems connected the entirety of the Ventura River watershed. We plan to continue scanning once conditions allow, and predict that our re-detection rate will be substantially reduced, due to trout movement out of the Upper North Fork Matilija, tag loss, or trout mortality as a result of these storms and associated flow.

For little additional time and monetary cost, we were able to capitalize on existing monitoring efforts to conduct a scanning survey of PIT-tagged trout in Upper North Fork Matilija. Scanning is minimally invasive and is not thought to greatly disturb trout (Hill et al. 2006). The high recapture rate of tags in this pilot study suggests that for the relatively small, low flow systems of southern California, PIT tag scanning surveys could be an effective method of tracking fine-scale trout movement and an important tool in the monitoring of *O. mykiss*. This is especially important in southern California, where relatively little is understood regarding *O. mykiss* habitat use and movement.

## FIGURES

**Figure 1.** Scanning surveys were conducted by walking upstream in a zig-zag-like pattern, bank-to-bank (dotted line arrows). The solid arrows indicate the use of the pole antenna, using both wide, sweeping, lateral arcs and focused scanning along boulder edges and other potential trout hiding spots. The pole antenna was held no more than four to six inches above the substrate, whenever possible. This method ensured that the entirety of the wetted stream was scanned. Illustration by Kathryn Carmody & Yi-Jiun Tsai (PSMFC).

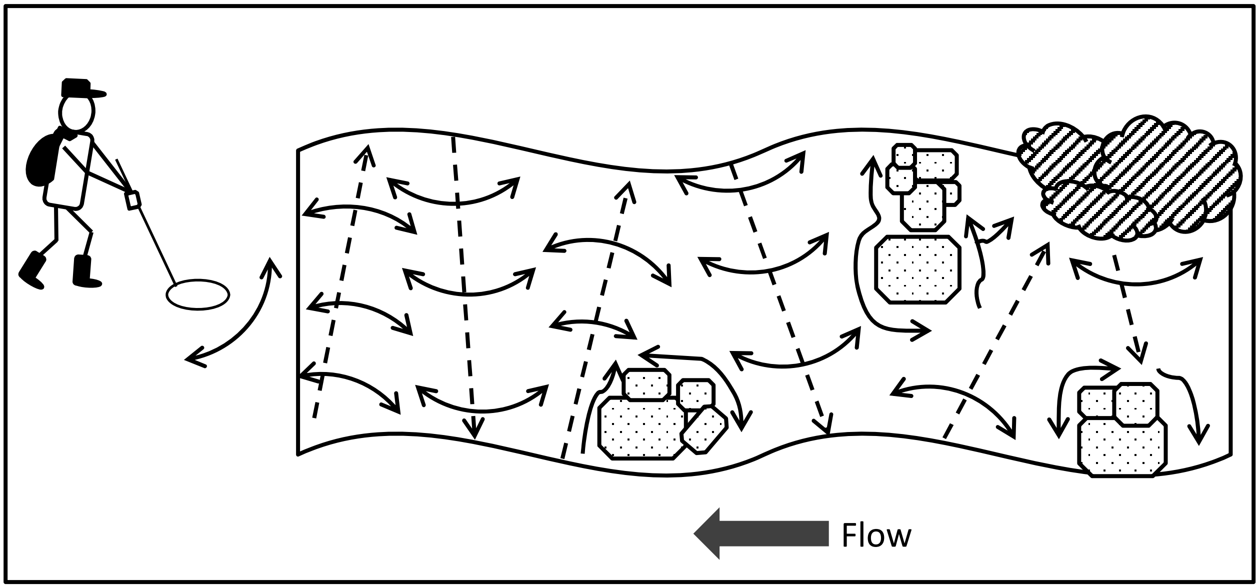


**Figure 2.** Picture of Yi-Jiun Tsai (Fisheries Biologist, Pacific States Marine Fisheries Commission) PIT tag scanning a habitat unit in Upper North Fork Matilija.

**Observed *Oncorhynchus mykiss* cover availability and use in Upper North Fork Matilija Creek**

Prepared by Kathryn Carmody & Yi-Jiun Tsai

## INTRODUCTION

In this study, we examined fine-scale *Oncorhynchus mykiss* habitat use in Upper North Fork Matilija Creek. Specifically, we estimated cover availability and use by trout during snorkel surveys. This report describes an initial effort of a larger monitoring project within the Ventura River Basin.

## METHODS

*Upper North Fork Matilija Creek*

Due to permitting limitations, we surveyed a creek above Matilija Dam, and therefore above anadromy. While the dam prevents movement of anadromous *O. mykiss* into these reaches, resident *O. mykiss* in these creeks are still important to recovery efforts as a relatively protected population that could contribute to the production of anadromous *O. mykiss* (Clemento et al. 2008).

Upper North Fork Matilija Creek is a tributary to Matilija Creek within the Ventura River Basin, a watershed designated as high priority for steelhead recovery in the Monte Arido Highlands (Core 1; NMFS 2012). Upper North Fork Matilija Creek is a fourth-order stream that drains a watershed of 12.45 square miles. Elevations range from approximately 1600 to 5700 feet. Lower montane mixed chaparral dominates the watershed (CALVEG 2015). Most of Upper North Fork Matilija occurs on federally-protected United States Forest Service and agricultural properties.

*Sampling Methods*

Field data were collected from October 4, 2016 to November 30, 2016 by Pacific States Marine Fisheries Commission, with help from the California Conservation Corps/NOAA Veterans Corps Fisheries Program. Upper North Fork Matilija Creek was surveyed for 4,996 feet, starting at the first wetted unit (34.51011°N, -119.38307°W) upstream of the confluence of Upper North Fork Matilija and Matilija Creeks. The endpoint (34.51606°N, -119.37746°W) was the start of a long dry section.

We delineated the wetted stream channel into discrete, natural units of similar habitat (Hankin 1984). Habitat units were classified as riffles, pools, or flatwaters based on characteristics outlined in the California Salmonid Stream Habitat Restoration Manual (Flosi et al. 1998). Riffles were characterized by relatively fast flowing water, usually shallow depths, medium to high gradient, and a large amount of exposed substrate. Flatwater units contained slow moving water with a smooth surface. Pools generally were bowl-shaped with collected, still water. See Abundance Estimation Chapter of this report for further details regarding habitat typing.

Once classified by type, units were measured for: (1) length, usually measured from inflow to outflow along the thalweg, (2) mean width, usually measured perpendicular to length, (3) maximum depth, and (4) mean depth.

All units that were snorkelable were snorkeled. Snorkelable units were defined as units: (1) 0.7–3 feet in mean depth, (2) categorized as flatwaters or pools (no riffles), (3) without hazards to divers, and (4) with good visibility. Trout could not be reliably counted at very low or very high depths, so riffles (usually very shallow), very shallow units (less than 0.7 feet in mean depth; O'Neal 2007), and very deep units (greater than 3 feet in mean depth or greater than 3.5 feet in maximum depth) were excluded.

Snorkel surveys were conducted using one to two snorkelers. Each snorkeler was equipped with a mask and snorkel, wetsuit, neoprene booties, wading boots, underwater dive light, and wrist slate with a graphite pencil. Snorkeler(s) approached each unit from the downstream end and moved in a zig-zag like pattern upstream (Thurow 1994; O'Neal 2007), ensuring that the entire unit was surveyed and avoiding disturbance to the substrate. Snorkeler(s) examined every available hiding space for trout.

A single diver was used for units with clear visibility bank-to-bank, which generally included units less than or equal to 15 feet wide and units without complex cover. For larger or more complex units, two divers were used. When two snorkelers sampled the same unit, they conducted the survey adjacent to one another and proceeding upstream in tandem (Thurow 1994).

For each trout observed, snorkeler(s) recorded the size class and cover type in which the trout was found on the wrist slate. Size classes consisted of two-inch bins (e.g. 0–1.99 in, 2–3.99 in, etc.). Cover types were boulder, bedrock, small woody debris (SWD), large woody debris (LWD), root mass, soil undercut, bubble curtain, aquatic vegetation, terrestrial vegetation, and open water (described in Table 1).

Snorkeler(s) visually estimated the total percent of the wetted unit's surface area comprised of possible trout cover. Snorkeler(s) also visually estimated the percentage of each cover type that comprised the total trout cover available within a unit. For example, a given unit could contain 40% trout cover and 60% open. Within the 40% of trout cover, 75% of the total available cover could comprise of boulders and 25% of small woody debris.

To ensure that trout sizes were estimated accurately, snorkeler(s) calibrated their visual size estimation when they completed snorkeling of the first unit and every tenth unit thereafter. Calibration consisted of a non-diver placing three haphazardly-chosen PVC pipes of varying lengths (ranging from 2–16 inches) into the unit. Snorkeler(s) then estimated the size of each pipe underwater. If any estimated size was not within two inches of the pipe's true size, then snorkeler(s) repeated the calibration until all three pipe sizes were estimated accurately.

*Data Analysis*

Habitat Assessment

To examine the overall habitat structure of the sampled reach in Upper North Fork Matilija, we calculated the percentage of pools, flatwaters, and riffles comprising the entire sampled reach. We then examined the number and percentage of pools and flatwaters that were snorkeled. We also calculated the mean unit dimensions of snorkeled units, including length, mean width, mean depth, and maximum depth.

Relative Trout Abundance

We examined trout density in two ways: the number of trout per unit and the mean density of trout per $ft^2$. The number of trout per unit was calculated as the total number of trout divided by total number of units in which trout were observed. Mean density of trout per $ft^2$ was calculated by dividing the number of trout observed in a given unit by the unit's surface area for units in which trout were observed. Then we took the mean of all densities. Surface area was calculated as unit length multiplied by mean width.

To evaluate the diversity of trout life stages observed, we examined the number and percentage of trout observed by size class.

Trout Cover Availability and Use

To examine the availability of potential trout cover, we calculated the mean percentage of trout cover available across all units snorkeled. We also calculated the mean percentage of each cover type comprising the total available cover across all units snorkeled. We then examined trout cover use by calculating the percentage of total trout observed using each cover type.

Due to the low number of flatwaters that could be sampled, we could not further examine trout density, trout cover availability, or trout cover use in relationship to habitat type.

## RESULTS

### *Habitat Assessment*

The surveyed reach was delineated into 235 natural habitat units, of which 12 were excluded from analysis. Of the remaining 223 units, 83 (37.2%) were pools, 77 (34.5%) were riffles, and 63 (28.3%) were flatwaters. Of these, we snorkeled 61 units, eight (13.1%) flatwaters and 53 (86.9%) pools (Figure 1).

The mean dimensions of snorkeled units were: length of 23.5 ± 2.1 ft, mean width of 11.0 ± 0.5 ft, mean depth of 0.9 ± 0.03 ft, maximum depth of 1.7 ± 0.07 ft, and area of 279.3 ± 31.0 (Table 2).

### *Trout Relative Abundance*

A total of 81 trout were observed within 61 snorkeled units. 69 (85.2%) trout were observed in pools and 12 (14.8%) trout were observed in flatwaters. We calculated a total density of 1.33 trout per unit snorkeled and a mean density of 0.0047 ± 0.00085 trout per $ft^2$ (mean ± SE). Of 81 trout observed, 33 (40.9%) were 2–3.99 inches, 35 (43.2%) were 4–5.99 inches, 11 (13.6%) were 6–7.99 inches, 1 (1.1%) was 8–9.99 inches, and 1 (1.1%) was 12–13.99 inches (Figure 2).

### *Trout Cover Availability and Use*

The mean percentage of available trout cover was 42% ± 2.2% (mean ± SE; range = 15%–90%). This was primarily composed of boulder (38.7 ± 2.6%) and small woody debris (25.3 ± 2.0%). Root mass and aquatic vegetation comprised 14.1% ± 1.6% and 12.1% ± 2.1% of total cover, respectively. All other cover types comprised less than 10% of the total available cover (Figure 3a).

76 trout of 81 total trout (93.8%) were observed using cover; five trout (6.1%) were observed in open water. Of these, 59 (72.8%) were observed under boulder, 12 (14.8%) were

observed under bedrock, 3 (3.7%) in root mass, and 2 (2.5%) in small woody debris. No trout were observed in aquatic vegetation, terrestrial vegetation, bubble curtain, large woody debris, or soil undercut (Figure 3b).

## DISCUSSION

*Habitat Assessment*

We found that Upper North Fork Matilija Creek was comprised mostly of pools and riffles and slightly less dominated by flatwater units. Our mean unit measurements are representative of the small scale stream network in the upper Ventura watershed. Rain-fed systems like Ventura are commonly intermittent with reduced natural water storage and low average flow (Boughton 2009). During the 2015-2016 winter season, southern California experienced record low levels of precipitation and elevated temperatures compounding the dry watershed conditions (Ventura River Watershed Council 2017). Such conditions have increased areas of intermittency and reduced minimal flow.

*Trout Relative Abundance*

We observed a total of 81 trout within Upper North Fork Matilija, at a mean density of 0.0047 trout per $ft^2$. The majority of trout observed fell in the 2–3.99 inch (40.9%) and 4–5.99 inch (43.2%) size classes. These results are consistent with a 2015 study conducted in Upper North Fork, which found that 64 of the 119 (53.8%) trout sampled were 2–3.99 inches long (Bankston et al. 2015).

*Trout Cover Availability and Use*

As far as we are aware, this is the first examination of fine-scale *O. mykiss* cover use within the Ventura River watershed. We found that boulders comprised the greatest percentage of all cover available (42%) and that nearly 73% of all trout were observed in association with boulders. In contrast, no trout were observed under aquatic vegetation, which made up 12 percent of cover available. Notably, there was a complete lack of large woody debris (LWD) observed in all units surveyed. Our results are consistent with habitat studies of southern California streams, which found that these systems are typically dominated by bedrock outcrops and in-stream boulders (Boughton 2009; Keller, Bean, & Best 2015). The availability of boulders in our reaches, combined with the lack of LWD, may correspond to the prevalence of boulder use as cover by trout.

While these results suggest that trout predominantly use boulder for cover in Upper North Fork Matilija, there are several caveats that must be considered carefully. In particular, we could not make any assumptions regarding cover type preference without further experimental tests. Additionally, our observations were biased by differential trout observation probabilities between cover types. For instance, trout can be spotted easily when using shallow bedrock ledges as cover. However, aquatic vegetation can grow in thick patches that make it difficult for snorkelers to search or observe hiding trout. Thus, we could not directly compare cover use between cover types.

While additional data are needed to support our findings, our results clearly show that boulders are both prevalent and frequently used by trout in a southern California stream system. The survey methods detailed in this report will be used to sample additional reaches within the Ventura watershed, including anadromous waters, for continued monitoring efforts of the native *O. mykiss* populations.

**TABLES AND FIGURES**

**Table 1.** Table of the definitions used to characterize each cover type. We counted the number of trout in association with each cover type, as well as the percentage of total available cover comprised by each cover type.

| Cover Type | Description |
|---|---|
| Open/No cover | Percentage of the unit that is open and without trout cover. Trout are not hiding, instead milling or swimming in an open area of the unit. |
| Cobble/Boulder | Rocks less than the size of a Volkswagen Beetle. This category includes instances in which trout hide in the crevices of a boulder cluster and underneath the ledge of the boulder. |
| Small Woody Debris | Fallen (dead) twigs, leaves, tree-related debris, and logs less than 1 foot in diameter or less than 6 feet long that is in the water and capable of providing cover to at least a 3-inch fish. |
| Large Woody Debris | Logs at least 1 foot in diameter **and** at least 6 feet long touching the water and capable of providing cover to at least a 3-inch fish. |
| Bedrock Ledge | Rocks larger than a Volkswagen Beetle that overhang the water such that a 3-inch trout could hide underneath (approximately 6-inch deep). |
| Terrestrial Vegetation | Any live, terrestrial vegetation touching or overhanging within a foot of the water's surface that is large or complex enough to hide a 3-inch trout. |
| Aquatic Vegetation | Any live, aquatic vegetation that is large or complex enough to hide a 3-inch trout. |
| Bubble Curtain | Bubbles created by flow that could provide cover a 3-inch trout. |
| Root Mass | A mat or cluster of live roots (e.g. willow mats or thick roots from a large tree) that could provide cover to a 3-inch trout. |
| Soil Undercut | An area along the margins of the unit comprised mostly of soil that has eroded only underneath the surface to create a ledge. This undercut should be able to hide a 3-inch trout (approximately 6 inches deep). |

**Table 2.** Mean unit measurements (± SE) calculated for all units snorkeled in Upper North Fork Matilija Creek. Each unit surveyed was measured for a length (ft), mean width (ft), mean depth (ft), and maximum depth (ft). Area ($ft^2$) for each unit was calculated by multiplying the length by mean width.

| Length (ft) | Mean Width (ft) | Max Depth (ft) | Mean Depth (ft) | Area ($ft^2$) |
|---|---|---|---|---|
| 23.5 ± 2.1 | 11.0 ± 0.5 | 1.7 ± 0.07 | 0.9 ±0.04 | 279.3 ± 31.0 |

**Figure 1.** Bar graph of the number of habitat units classified as riffles, flatwaters, or pools within Upper North Fork Matilija Creek. The proportion of units snorkeled is shown in blue. Sample sizes are shown within each bar.

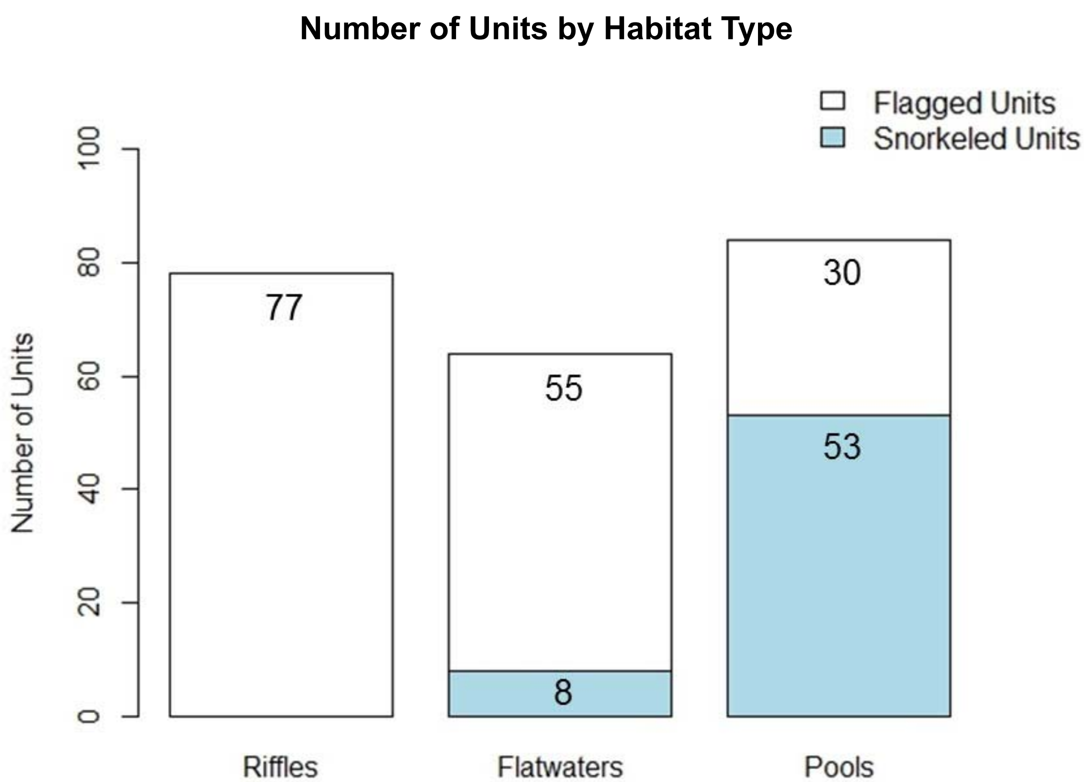


**Figure 2**. Bar graph of the number of trout observed in each two-inch size class. Number of trout observed is indicated above each bar.

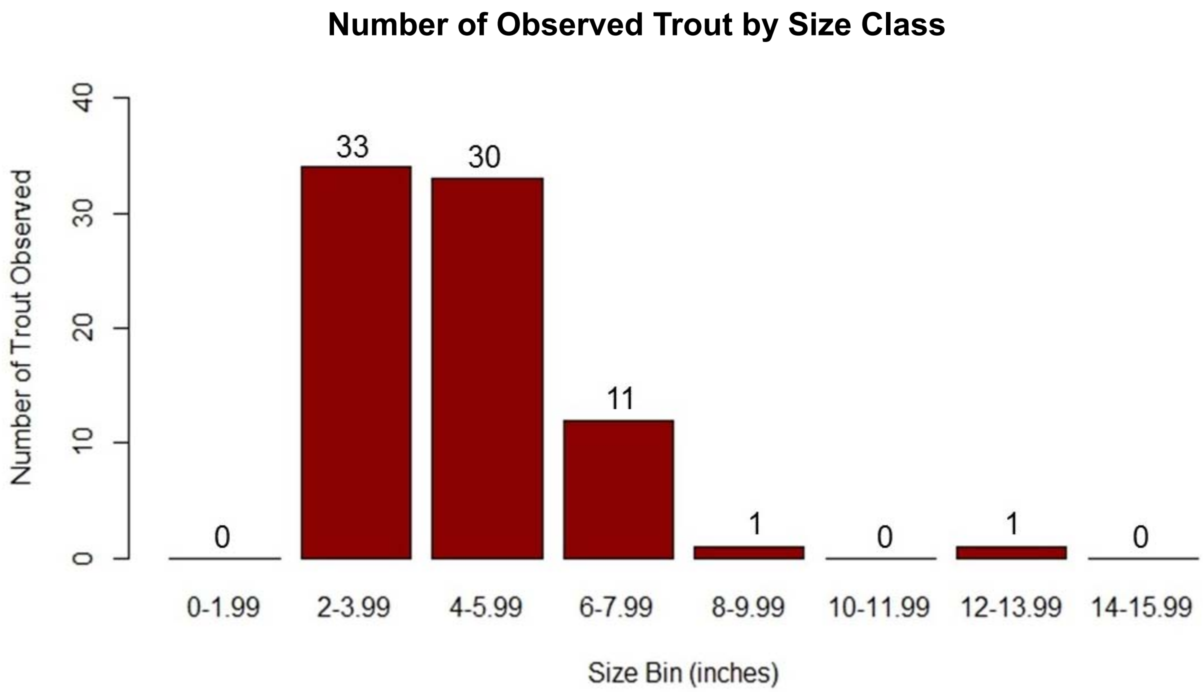
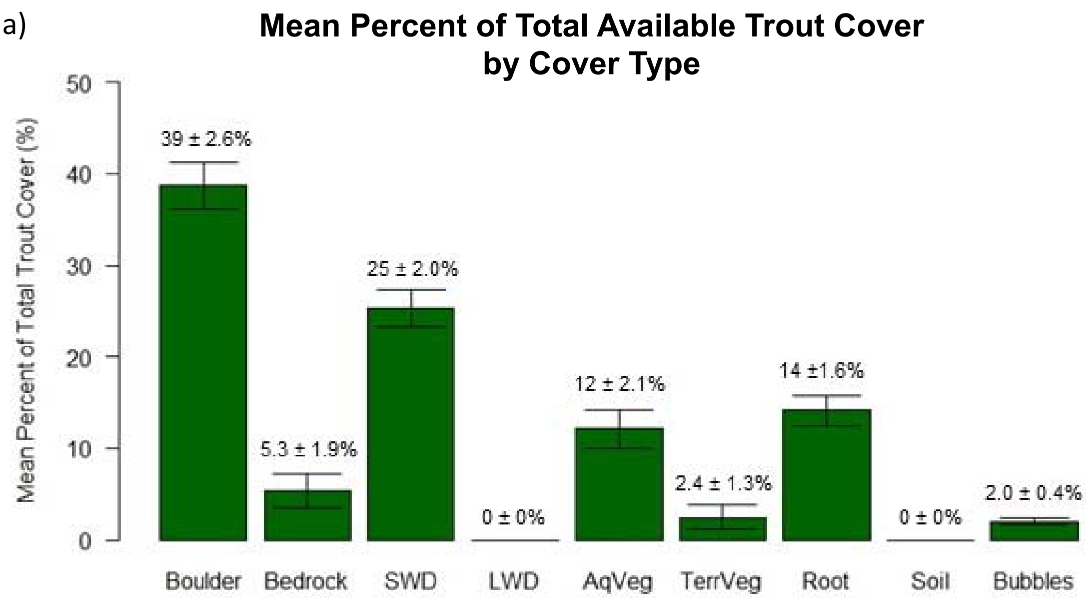
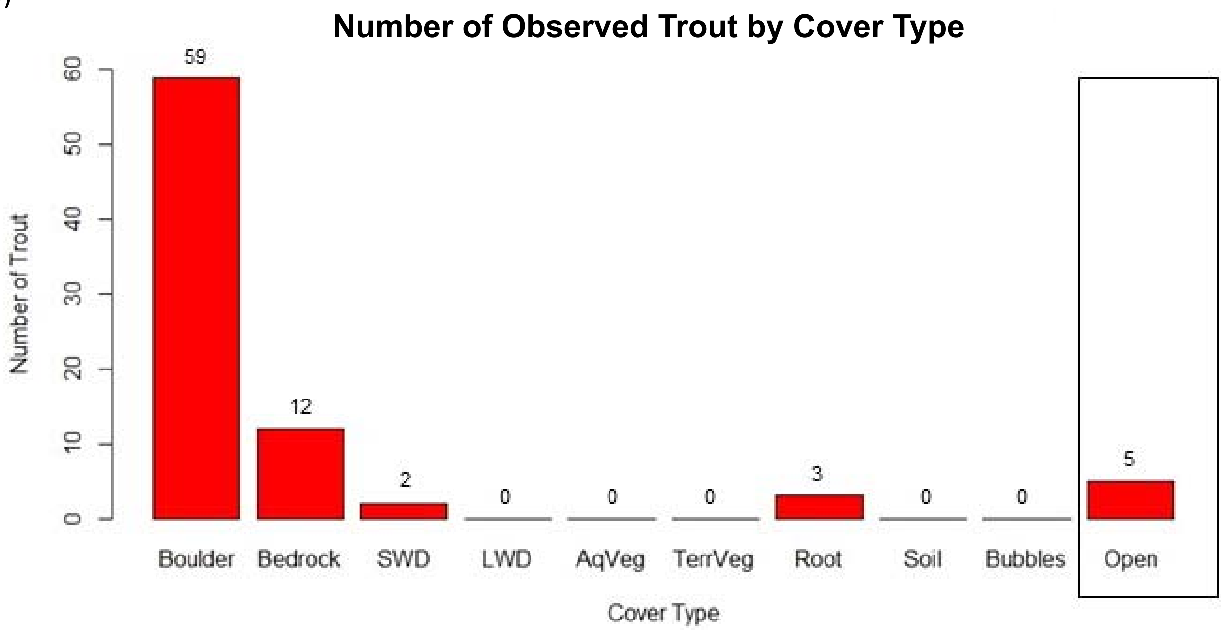


**Figure 3.** Graph (a) shows the mean percent of total available trout cover by cover type. Graph (b) shows the total number of trout observed in association with each cover type. Cover types are: cobble/boulder (Boulder), bedrock, small woody debris (SWD), large woody debris (LWD), aquatic vegetation (AqVeg), terrestrial vegetation (TerrVeg), root mass (Root), soil undercut (Soil), and bubble curtain (Bubble). For graph (b), an additional cover type was open water (Open). Mean percent ± standard error is indicated above each bar for graph (a). Number of trout observed is indicated above each bar in graph (b).

a)




b)



## LITERATURE CITED


Bankston, S. B., Y.-J. Tsai, K. Evans, T. van Meeuwen, M. Morse, P. Saldana, P. Hunter. 2015. Southern California steelhead monitoring: Santa Ynez River, Conception Coast, and Ventura River 2013–2015. Report for California Department of Fish and Wildlife. Fisheries Restoration Grant Program (Project Number: P1250012).

Cochran, W. G. 1977. Sampling Techniques, 3rd edition. John Wiley and Sons, New York.

Classification and Assessment with LANDSAT of Visible Ecological Groupings (CALVEG). Accessible: http://www.fs.usda.gov/detail/r5/landmanagement/resourcemanagement/?cid=stelprdb5365219. U.S. Forest Service. (November 2015).

Clemento, A. J., E. C. Anderson, D. Boughton, D. Girman, and J. C. Garza. 2008. Population genetic structure and ancestry of *Oncorhynchus mykiss* populations above and below dams in south-central California. Conservation Genetics 10: 1321–1336.

Flosi, G., S. Downie, J. Hopelain, M. Bird, R. Coey, and B. Collins. 1998. California salmonid stream habitat restoration manual, 3rd edition. California Department of Fish and Game.

Hankin, D. G. 1984. Multistage sampling designs in fisheries research: applications in small streams. Canadian Journal of Fisheries and Aquatic Sciences 41: 1575–1591.

Hankin, D. G. and G. H. Reeve. 1988. Estimating total fish abundance and total habitat area in small streams based on visual estimation methods. Canadian Journal of Fisheries and Aquatic Sciences 45: 834–844.

Hill, M. S., G. B. Zydlewski, J. D. Zydlewski, and J. M. Gasvoda. 2006. Development and evaluation of portable PIT tag detection units: PITpacks. Fisheries Research 77: 102–109.

McCanne, D. M. and B. Reisberger. 2005. Regional abundance survey for the Mad River-Redwood Creek hydrologic unit. Agreement No.: CAWIP-NR-29, Final Report.

National Marine Fisheries Commission (NMFS). 2012. Southern California steelhead recovery plan. Southwest Region, Protected Resources Division, Long Beach, California.

O’Neal, J. S. 2007. Snorkel Surveys. Pages 325–340 *in* D. H. Johnson, B. M. Shrier, J. S. O’Neal, J. A. Knutzen, X. Augerot, T. A. O’Neil, and T. N. Pearsons, editors. Salmonid Field Protocols Handbook: Techniques for assessing status and trends in salmon and trout populations. American Fisheries Society, Bethesda, Maryland.

Pollock, K. H. and M. C. Otto. 1983. Robust estimation of population size in closed animal populations from capture-recapture experiments. Biometrics 39: 1035–1049.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

RStudio Team. 2016. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. http://www.rstudio.com/.

Raleigh, R. F. and C. Short. 1981. Depletion sampling in stream ecosystems: assumptions and techniques. Progressive Fish-Culturist 43: 115–120.

Särndal, C.-E., B. Swensson, and J. Wretman. 1992. Model assisted survey sampling. Springer-Verlag, New York.

Temple, G. M. and T. N. Pearsons. 2007. Electrofishing: Backpack and drift boat. Pages 95–132 *in* D. H. Johnson, B. M. Shrier, J. S. O'Neal, J. A. Knutzen, X. Augerot, T. A. O'Neil, and T. N. Pearsons, editors. Salmonid Field Protocols Handbook: Techniques for assessing status and trends in salmon and trout populations. American Fisheries Society, Bethesda, Maryland.

US Geological Survey (USGS). Accessed March 2, 2017. USGS Current Conditions for the Nation. https://waterdata.usgs.gov/nwis/uv?.

Walter, L. 2015. Ventura River watershed management plan. Ventura River Watershed Council. Pp. 797.

White, G. C., D. R. Anderson, K. P. Burnham, and D. L. Otis. 1982. Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory, LA-8787-NERP, Los Alamos, New Mexico.