# 2016 Steelhead Survival Study

## Tagging, Release, and Mobile Monitoring

Denise Barnard, Pat Brandes, David LaPlante;
U.S. Fish and Wildlife Service; Lodi, California

September 15, 2016

## Introduction

The Lodi U.S. Fish and Wildlife Service (USFWS) office was responsible for the tagging and release of steelhead *Oncorhynchus mykiss* during the 2016 Steelhead Survival Study, the sixth and final year of the study. The following report includes the methods and results for the tagging, release, tag retention tests, and mobile monitoring components of the study. This report is intended to act as a preliminary supplement to the final, comprehensive report combining all aspects of the study, including the fish tagging, release, and health examination; receiver deployment and the survival models and statistical analysis.

## Study Design and Methods

### Study Fish

Juvenile steelhead were used as study fish and obtained from the Mokelumne River Hatchery (MKR). Fish were held at the MKR in ambient Delta water in outdoor raceways. Prior to tagging, fish were sorted such that they were between 13.0 g (~110 mm forklength [FL]) and 199.9 g (~285 mm FL), though all fish used in the study were between 30.0 g (~145 mm FL) and 199.9 g. Tagged study fish averaged 144.6 g (SD=28.1 g) and 248 mm FL (SD=17.3 mm). Fish were taken off feed 24 hours prior to surgery.

### Tags

Juvenile steelhead were tagged with both VEMCO V5 180 kHz acoustic transmitters and Biomark FDX-B HPT12 Passive Integrated Transponder (PIT) tags. Both tags were inserted into the body cavity through the surgical incision of each fish, with the PIT tag inserted first and the acoustic tag inserted next, such that acoustic tag rested on the PIT tag. The percentage of tag weight to body weight of tagged study fish averaged 0.6% (SD=0.2%) and all fell within 0.4% and 2.6%. The tag weight to body weight percentage was calculated using the measured weight of each acoustic transmitter corresponding to each fish, plus the average PIT tag weight.

#### Acoustic Transmitters

The VEMCO V5 180 kHz acoustic transmitters were 12.7 mm long, 4.3 mm in height, and 5.6 mm wide (http://vemco.com/products/v4-v5-180khz/; accessed June 29, 2016). Each study transmitter was weighed to the nearest 0.001 mm, and weighed on average 0.673 g in air (SD=0.004 g). The average was calculated only using study tags and did not include transmitters used for tag life or spares.

Tags were custom programmed with two separate codes; a traditional Pulse Position Modulation (PPM) style coding and a hybrid PPM/High Residence (HR) coding. The HR component of the coding allowed for detection at high residence receivers. High residence receivers were placed where tag signal collisions (i.e., many tags emitting signals at the same time to the same receiver) were anticipated (e.g., Central Valley Project [CVP] and Clifton Court Forebay). The transmission of the PPM identification code was followed by a 25–35 second delay, followed by the PPM/HR code, followed by a 25–35 second delay, and then back to the PPM code, etc. The PPM code consisted of eight pings approximately every 1.2–1.5 seconds. The PPM/HR code consisted of one PPM code and eight HR codes (all the same for each individual fish) with eight pings approximately every 1.2–1.5 seconds. The VEMCO V5 180 kHz tag specifications listed the 95% and 50% estimated battery life of the transmitters as 48 and 58 days, respectively.

Tags were soaked in distilled water (the first week of tagging) or saline water (the second and third weeks of tagging) for at least 12 to 24 hours prior to tag activation. Tags were activated using a VEMCO Tag Activator-180k-200 approximately 24 hours prior to tag implantation. The tag activation time was identified to the nearest minute.

### PIT Tags

PIT tags were implanted in the study fish in order to provide additional data for a concurrent experiment conducted by the National Oceanic Atmospheric Administration (NOAA), which aimed to test the detection rate of two PIT tag arrays on the San Joaquin River. PIT tag arrays also existed in the release pipes at the State Water Project (SWP) release sites located at Horseshoe Bend on the Sacramento River side of Sherman Island and Curtis Landing on the San Joaquin side of Sherman Island. Tags detected at the SWP release pipes represent study fish or fish that predated on study fish that were captured during salvage at the SWP facility, transported to one of the SWP release sites, and released into the river through the release pipe. PIT tags were 12.5 mm long and 2.1 mm in diameter (http://www.biomark.com/catalog/tags/; accessed June 29, 2016). A subsample of 69 study PIT tags weighed on average 0.113 g (SD=0.001 g).

## Tagging Training

Surgical tag training occurred between February 16 and February 19, 2016, at the MKR. The training was conducted by staff from the U.S. Geological Survey (USGS)'s Columbia River Research Laboratory (CRRL) and the USFWS. The training week was used to refine standard operating procedures (SOP), train support staff, establish consistent necropsy assessment criteria between surgeons and release site crews, and to refresh surgeons on acoustic transmitter implantation methods (based on Liedtke 2012; Liedtke et al. 2012). All taggers were returning after tagging during the preceding year of the study (2015), and were required to tag 60 live fish with subsequent necropsies to evaluate their surgical technique. Each tagger also held 20 tagged fish overnight to assess fish recovery 24 hours after tagging. Training concluded with a mock tagging session to simulate the logistical and support procedures of tagging operations.

## Tagging

Groups of steelhead were tagged at the MKR with VEMCO V5 tags over three, 3-day periods—February 23–25, March 15–17, and April 26–28, 2016 (Table 1). Each day was further divided into three sessions each corresponding to one transport truck. The fish for each session were divided between three surgeons, and each surgeon was paired with an assistant. Three additional support staff (runners) helped to move fish into and out of the tagging operation.

The surgical tagging SOP (Appendix A) was developed by the CRRL and the USFWS and directed all aspects of the tagging operation. The SOP was based on Adams et al. (1998), Martinelli et al. (1998), and Liedtke et al. (2012); and was modified as needed during the training week. To begin the surgical procedure, each fish was first anesthetized with either 3.0 or 3.5 mL of Aqui-S 20E/10 L water (33.8 mg/L or 39.4 mg/L, respectively). After 30–60 seconds of losing equilibrium, the fish was weighed to the nearest 0.1 g, FL was measured to the nearest mm (Figure 1a), and the level of descaling was assessed. If the fish was descaled by 20% or more, the fish was rejected from tagging (Appendix A). If the fish did not have any obvious abnormalities and met the size criteria, the fish was retained for tagging. The fish was placed onto a tagging platform of closed cell EVA grade foam for the surgical procedure. A gravity feed of surgical tubing placed into the fish's mouth delivered a solution of 1.0 mL Aqui-S 20E/10 L water (11.2 mg/L) from a carboy that ran over the gills to keep the fish anesthetized (Figure 1b). A PIT and acoustic tag were then surgically implanted into the fish's body cavity following the procedure outlined in Appendix A. After successful completion of the surgery, the fish was placed into a recovery bucket containing water saturated with between 130–150% dissolved oxygen (DO), and allowed to recover from the anesthesia for at least 10 minutes (Figure 2a–b; Figure 3). The duration of each surgery was measured from the time the fish was removed from the initial anesthetization bath to the time the fish was placed into the recovery bucket. The duration of each surgery was between 1:10 min and 4:30 min and averaged 2:18 min (SD=25 s). Each recovery bucket contained 1 or 2 fish. Recovery buckets were covered with lids at all times to minimize escape and stress experienced by study fish.

### Quality Assurance and Quality Control Tagging Inspection

Each tagging day, we performed a quality assurance and quality control (QA/QC) tagging inspection based on Liedtke (2012). The QA/QC inspection consisted of ensuring that water quality parameters were within the limits set by the SOP and that important aspects of the SOP were consistently followed. It consisted of 16 parameters, defined in Table 2, that were previously provided by Theresa [Marty] Liedtke, USGS CRRL, through personal communication. A YSI ProODO meter was used to measure the water temperature and DO for each QA/QC inspection.

The QA/QC was typically performed under conditions most likely to result in parameters falling outside of the limits of the SOP. In order to ensure that the study fish experienced a gradual increase in water temperatures, we aimed to keep all water temperatures within 2°C from the water source during each tagging session (Liedtke 2012; Liedtke et al. 2012). We considered the water source as where fish were held before being anesthetized (Figure 4). Temperatures in buckets and gravity feed carboys were measured during the hottest part of the day to test conditions most likely to produce the highest increase in water temperatures, which was usually during the last tagging session. Water temperatures were also measured right before water in anesthesia buckets and gravity feed carboys was discarded to be replaced with fresh, cooler water. Water was changed about every 15–25 minutes per tagging

session depending on air temperature (see Appendix A for more information regarding water changes). When any procedural issue was noticed that was outside of compliance with the SOP, it was immediately evaluated and corrected.

## Transmitter Validation

While fish were recovering from the anesthesia in the recovery buckets, two VEMCO High Residency Receiver prototypes (VRHR), each with a 180 kHz hydrophone (Figure 5), were used to verify the PPM and the HR tag codes emitting from the fish during the tagging sessions. Fish containing tags that were unable to be verified were replaced with a new fish and a new tag.

## Transport to Release Site

After the transmitter validation and 10 minutes of recovery each group of 2 to 3 recovery buckets was combined into a 68 L (18 gal) perforated tote (Figure 2c), and then loaded into a transport tank (Figure 6) mounted on the back of a flatbed truck. Each tote was perforated with 1.0 or 1.27 cm diameter holes, contained no more than three fish, and was covered by a lid at all times. Immediately prior to loading, all fish were visually inspected for mortality or signs of poor recovery from tagging (e.g., erratic swimming behavior). Fish that did not recover from surgery were replaced with a new tagged fish.

In order to minimize the stress associated with fish transport and to track small groups of individually tagged fish, three specially designed transport tanks were used to move steelhead from the tagging site at the hatchery to the holding site. The transport tanks were designed to securely hold twenty-four 68-L perforated totes. Tanks had an internal frame that held totes in individual compartments to minimize contact between containers and to prevent tipping (Figure 6). Totes were covered in the transport tanks with stretched cargo nets to ensure that the totes did not tip over or their lids did not come off. Each transport tank was mounted on the bed of an 8 m flatbed truck that was equipped with an oxygen tank and hosing to deliver oxygen to the tank during transport. One trip for each truck to the release site was made each tagging day, for a total of three trips per day (Table 1). The holding site was located at Durham Ferry in Manteca (Figure 7). The trip from the MKH to Durham Ferry took approximately 75minutes.

Water temperature and DO was measured using a YSI 85 or ProDO meter in the transport tanks prior to loading totes, after loading totes into transport tanks but before leaving the MKR, and at the release site after transport prior to unloading totes. The water temperature and DO were also measured in the river at the holding site prior to moving the fish into containers in the river.

## Transfer to Holding Containers

Once the transport truck arrived at the release site, the fish were moved from the transport tank to the river (Figure 8). Soon after the transport truck arrived, the river's water temperature and DO levels were measured; and approximately ten 68-L non-perforated totes ("sleeves") were placed into the back of a pickup truck and filled halfway with river water. The pickup was then driven from the river's edge to where the transport truck was parked on the levee road and positioned alongside the transport truck. The perforated totes were unloaded from the transport truck into the sleeves in the pickup truck. Transporting the perforated totes in sleeves allowed the water level of the totes to rise

above the tote perforations, providing fish with more access to water during transport outside of the transport truck. The pickup truck was then driven back to the river's edge about 100 m away; and the perforated totes were separated from the sleeves, unloaded from the pickup truck, and carried to the river. It took two or three trips between the transport truck and the river before transfer of all totes to the river's edge was complete.

Once at the river's edge, the tagged steelhead were transferred from the totes into 166 L (44 gal) plastic garbage cans ("holding cans") held in the river. Each holding can was perforated with 1.27 cm diameter holes. Generally, four totes containing three fish each were emptied into each perforated holding can. Each holding can was labeled to ensure that fish in each labeled tote were loaded into the correct holding can for later release at the correct time.

## Fish Releases

Tagged study fish were held in the perforated holding cans for approximately 24 hours prior to release, and were then transported downstream by boat to the release location. The release location was located in the middle of the channel approximately 200 m downstream of the holding cans. Just prior to moving fish downstream for release, the perforated holding cans were placed into non-perforated sleeves. We used sleeves and released the fish downstream of the holding site to potentially reduce initial predation of tagged fish immediately after release. We were concerned that predators may congregate near the holding location and follow the smell of the water from within the perforated holding cans as the holding cans were moved downstream, resulting in high initial mortality from predation. Releases were made every four hours after the 24 hour holding period; at approximately 1500, 1900, and 2300 hours (the day after tagging); and 0300, 0700, and 1100 hours (two days after tagging; Table 1).

Immediately prior to release, each holding can was checked for any dead or impaired fish. At the release time the wingnuts holding the lid in place were unscrewed and the lid was removed from the holding can and pulled partially out of the water to look for mortalities. The can was then inverted to allow the fish to be released into the river. After the holding can was inverted, the time was recorded. As the holding cans were flipped back over, they were inspected to make sure that none of the released fish swam back into the can.

Once the release was completed, the date and release time for any dead fish was recorded and the tags were removed. The tags were returned to the tagging location or office to have the individual tag identified.

## Dummy-Tagged Fish

In order to evaluate the effects of tagging and transport on the survival of study fish, several groups of steelhead were implanted with inactive, or "dummy", transmitters. During the 2016 field season surgeons did not know if the transmitter that they were implanting into each fish was an active tag or a dummy tag. This "blind dummy" system was intended to reduce bias in the surgeon's treatment of tagged or dummy-tagged fish. For each day of tagging and transport, 12 or 24 fish were implanted with dummy transmitters and included in the tagging process (Table 1). Groups of dummy transmitters (consisting of 3 fish each) were randomly interspersed into the tagging order for each release group across one week. The order was then repeated for subsequent tagging weeks.

Procedures for tagging these fish, transporting them to the release site, and holding them at the release site were the same as for fish with active transmitters. However, unlike the tagged fish, the dummy-tagged fish were not released into the river after the holding period.

Dummy-tagged fish were held in the holding cans for approximately 48 hours, after which they were evaluated for mortality and condition (Table 3). Just prior to condition assessments, holding cans containing dummy-tagged fish were placed into a holding can sleeve and transported downstream by boat, halfway to the release site and back, to mimic the release process experienced by tagged study fish. Water temperature and DO were taken in the perforated holding can after returning from the mimicked boat transport in the river.

Two groups of dummy-tagged fish from each week (tagged on day 1 and day 2; Table 1) were then examined to determine if any fish had died during the holding period or during transport of the can. After this initial examination, dummy-tagged fish were euthanized with tricaine methanesulfonate (MS-222) and assessed for condition, percent scale loss, body color, fin hemorrhaging, eye quality, and gill coloration (Table 3).

After the mortality and condition examination, a necropsy was performed on each dummy-tagged fish to assess the internal and external aspects of the surgery. A composite score (0–12) was calculated from seven parameters to consider possible tagging effects that could affect survival of study fish (T. Liedtke, personal communication; Table 4). The remaining dummy-tagged fish (one group of 24 fish per week; tagged on day 3) were retained for assessment of pathogens and disease by the USFWS's CA-NV Fish Health Center (CNFHC).

## Tag Retention Test

On February 22, 2016, each of the three surgeons tagged 16 or 17 steelhead with PIT tags and acoustic dummy tags to assess tag retention, recovery, and mortality of tagged fish after 30 and 60 days. A total of 50 fish were tagged following the same surgical SOP as study fish (Appendix A), and held in a raceway at the MKR. After 30 days, each surgeon identified five of his or her tagged fish using PIT tag codes. After a fish was identified, it was euthanized and assessed for growth, appearance, and internal and external recovery at the surgery site. The parameters assessed on the tag retention fish included the same initial and composite parameters assessed on the dummy-tagged fish, except that scales were assessed categorically as "normal" (0–5% scale loss; 0), "partial" (6–19% scale loss; 1), or "descaled" (>19% scale loss; 2) for the retention fish instead of estimating the specific numerical percent scale loss (Table 3 and Table 4). Additionally, the fish's final weight, and the internal acoustic and PIT tag positions were assessed (directly over, anterior to, or posterior to the incision site; 0=no, 1=yes). The assessments were repeated for the remaining 11 or 12 fish per surgeon after 60 days. The average and SD was calculated for each parameter separately for the 30 or 60 day retention tests.

## Mobile Telemetry Monitoring

After completion of all three weeks of fish releases, we conducted mobile telemetry monitoring surveys to locate some of the acoustic transmitters that were still active in the survey area. We conducted four mobile monitoring surveys, each spaced about 1 week apart (Figure 9). The first survey was conducted on May 11, 2016, started about 1.0 km upstream from the Durham Ferry fish release site, and followed the San Joaquin River downstream for 10.5 km. The second survey was conducted on

May 18 between the end of the first survey and the head of Old River barrier, for about 15.5 km downstream on the San Joaquin River. The third survey was conducted on May 25 between the head of Old River barrier to the head of Fourteen Mile Slough, for about 30.5 km downstream on the San Joaquin River. The fourth survey was conducted on June 2, started about 3.5 km upstream of Fourteen Mile Slough, followed the San Joaquin River for about 19.5 km downstream, and ended at Prisoner's Point. We did not conduct any mobile monitoring in Old River, near the trashracks of the CVP or outside or inside of Clifton Court Forebay.

Surveys were conducted by using a VEMCO VRHR, connected to a 180 kHz hydrophone hanging over the side of a boat about 10–30 mm under the surface of the water, to detect active transmitters in the survey area. The boat remained mostly in neutral and was allowed to drift downstream with the current in the middle of the channel. Whenever a code or cluster of codes was detected; the codes, time, and GPS coordinates were linked and recorded. After completion of the surveys, the data from the VRHR was downloaded and checked against the recorded data to ensure that all codes detected by the receiver were documented.

Mobile monitoring data was also provided by the USFWS Anadromous Fish Restoration Program (AFRP). The AFRP mobile monitoring was conducted earlier in the season than the 2016 South Delta Study mobile monitoring, intermittently between March 1 and May 1, 2016. The AFRP staff followed a similar protocol to that followed by the 2016 South Delta Study, except that they used a VEMCO VR100 with a VH165 hydrophone (50–85 kHz) to detect tags. It is uncertain how effective the VH165 hydrophone is for detecting 180 kHz transmitters, though it did successfully detect several 2016 Steelhead Survival Study transmitters.

# Results and Discussion

## Quality Assurance and Quality Control Tagging Inspection

All parameters tested during the QA/QC tagging inspection fell within limits set by the SOP, except for the DO in five pre-surgery recovery buckets (Appendix B). These buckets were saturated with between 152 and 161% DO. The DO omitted by the oxygen tanks was shown to consistently increase throughout the day, even though the number of seconds used to oxygenate buckets remained consistent. We suspect that this was caused by increasing air temperatures increasing the PSI in the oxygen tanks over the course of the day. When buckets were measured to have more than 150% DO, we checked all the remaining recovery buckets and replaced any buckets that were out of compliance. Since we knew this was a recurring issue, we consistently checked the DO of the pre-surgery recovery buckets throughout each tagging session each day to ensure that they were within compliance, even if we were not performing a QA/QC inspection. However, we usually only encountered issues during the third truck, when the QA/QC inspection was being performed.

On the first day of the study (February 23, 2016), one post-surgery recovery bucket was shown to have a temperature more than 2°C higher than the raceway where fish were held before being tagged (Appendix B). This was observed after the QA/QC inspection was complete, when a bucket felt warm to the touch and so the water temperature in the bucket was checked. This was likely caused by the corresponding tagging station being in direct sunlight towards the end of the day, while the rest of

the tagging operation remained in the shade. After this was observed, a shade structure was constructed for this tagging station and was employed throughout the remainder of the study. No other temperatures fell outside of compliance of the SOP. There were no other issues encountered during the QA/QC inspections.

## Transport to Release Site

There were no mortalities observed after transport to the holding site at Durham Ferry (Table 5). Water temperature in the river at the holding site ranged from 14.4°C to 17.3°C, with an average during the first week of 15.9°C, an average during the second week of 15.8°C, and an average during the third week of 15.3°C DO levels ranged between 6.97 and 12.01 mg/L for all measurements in the transport tanks or in the river, with the lowest DO observed during the March releases. During transport, water temperatures increased by at most 2.2°C (Table 5; Appendix C). There were two instances when water temperatures actually decreased during transport.

Over the course of the season, a total of seven fish escaped from their totes into the transport tank during transport from the tagging site to the holding site due to their tote lids coming off during transport. After all totes were unloaded, these fish were netted from the transport tank and placed into the correct tote and holding can, as usually only one tote was missing its lid. In one case, when multiple fish from two totes escaped into the transport truck, both affected totes were to be loaded into cans for release at the same time. Thus, fish were returned to one of the two totes without regard to which tote they originated from.

There were also two instances when lids and tote numbers did not match once fish were delivered to the release site. When this occurred, we used the PIT tag reader to identify which fish belonged in which tote and holding can. In the first instance, the PIT tag reader malfunctioned. We assumed that the tote, and not the lid, had the correct label based on the way in which fish were loaded at the tagging site. This assumption was supported by the second instance, in which the tote was verified to correspond to the PIT tag codes. However, it is possible that the six fish from the first two switched totes were loaded into the incorrect cans and their release times were switched.

## Fish Releases

There were no study fish mortalities that occurred after holding and prior to release in the 2016 Steelhead Survival Study (Table 6).

## Dummy-Tagged Fish

None of the 144 dummy-tagged steelhead were found dead when evaluated after being held for 48 hours (Table 6). Of the 72 dummy-tagged fish assessed at the holding site for healing and recovery (the remainder were assessed for fish health), three fish had light body color and none had bulging eyes or light gill color (Table 6). All remaining fish were found swimming vigorously, with no fin hemorrhaging. Mean scale loss for each group of fish assessed ranged from 4.5–8.9% (Table 6). Mean FL of the six groups of dummy-tagged fish ranged from 233.1–259.3 mm.

The mean composite score (0–12) of the six groups dummy-tagged steelhead ranged between 1.1 and 1.5 (Table 7), indicating that these fish had only a few instances of inferior tagging and healing characteristics. There were no signs of tag expulsion or fungus, and only three fish had anterior or

posterior sutures loose or untied, three fish had poor incision apposition, and two fish displayed signs of organ damage. Three of the 72 examined fish were found to have stitched organs. Stitched organs did not appear to affect short-term survival of the fish since those fish with stitched organs survived the duration of the holding period (48 hours). Almost all the dummy-tagged fish had poor peritoneal apposition; with the peritoneal cavity only partially closed or open (Table 7). Poor peritoneal apposition might suggest that these fish are more prone to internal irritation at the tagging site or longer healing times. The composite score was calculated to consider possible effects of tagging on the survival of study fish. It is not clear that poor peritoneal apposition would affect survival of study fish, but may suggest an avenue for later tag expulsion. However, tag retention studies dispute this potential connection (see Tag Retention Test discussion below). These data indicate that the steelhead used in 2016 study likely did not die from the tagging and transport processes.

## Tag Retention Test

No mortalities occurred among the 50 dummy-tagged retention fish held for 30 or 60 days, and all fish appeared to be swimming vigorously before the retention assessments. All 50 fish displayed normal body and gill color and eyes, and did not show signs of fin hemorrhaging. One fish expelled its tag during the retention test and no other fish expelled their tags or showed signs of tag expulsion.

### 30 Day Retention

The 15 dummy-tagged fish assessed after 30 days showed an average increase in growth of 25.15 g (SD=4.94 g) and 13.20 mm (SD=3.19 mm; Table 8). The amount of descaling lessened between day 0 (mean=0.33; SD=0.47) and day 30 (mean=0.20; SD=0.40), suggesting the steelhead recovered from any descaling that occurred initially. In one fish the acoustic tag resided posterior to the suture, and in the remaining 14 fish the acoustic tag resided directly over the incision (Table 8). The PIT tag position displayed more variability, with seven tags remaining directly over the incision, six tags migrating anterior to the incision, and two tags migrating posterior to the incision. It is possible that tag migration is a preliminary component of tag expulsion, and might indicate that these fish are more likely to undergo later tag expulsion. Eight fish were calculated to have a composite score (0–12) of 0, four fish had a composite score of 1, and three fish had a composite score of 2 (Table 9). None of the fish displayed signs of tag expulsion. The anterior and posterior sutures of all 15 fish remained present. Four of the 15 fish displayed gape or skin overlap in the incision and 5 fish exhibited a partially open peritoneum (Table 9). None of the 15 fish displayed visible organ damage, and only 1 fish displayed fungus which resided on the sutures.

### 60 Day Retention

The 35 dummy-tagged steelhead assessed after 60 days showed an average increase in growth of 111.50 g (SD=28.65 g) and 41.63 mm (SD=6.16 mm) (Table 10). The scale assessment of the 60 day retention fish showed an average of 0.37 (SD=0.48) at day 0, and an average of 0.69 (SD=0.52) at day 60, indicating that the longer holding period resulted in more scale loss.

One of the 35 steelhead was found without its acoustic transmitter present. Other than the missing presence of the transmitter, the fish did not show any scarring or signs related to tag expulsion (Figure 10; fish #17 in Table 11), which may indicate that the tag was expelled within the first few days after tagging. This steelhead was also the smallest fish of the study (67.5 g at day 0, compared to an

average [SD] of 131.2 g [28.3 g] among all 50 retention fish). This fish was also missing its posterior suture, which may have contributed to the tag expulsion. However, scarring related to the posterior suture was visible, so the suture might have been only recently lost. Of the remaining 34 fish, the acoustic tag of 1 fish migrated anterior to the suture, 11 migrated posterior to the suture, and 22 remained directly over the incision. The PIT tags of 2 fish migrated anterior to the incision, 12 migrated posterior to the incision, and 22 remained directly over the incision (Table 11).

Of the 35 dummy-tagged fish, the anterior suture of one fish and the posterior sutures of three fish were missing after 60 days (Table 11). In one of these fish, both the anterior and posterior sutures were missing, but the tags were still present and the surgical site appeared to have mostly healed (Figure 11; fish #26 in Table 11). Nine of the 35 fish displayed gape or skin overlap in the incision and all fish displayed good peritoneal apposition (Table 11). One fish displayed visible organ damage, where it appeared that the pyloric caeca were captured by the suture (Figure 12; fish #49 in Table 11). Two fish displayed fungus which resided on the sutures Table 11. Twenty-two fish were calculated to have a composite score of 0, nine fish had a composite score of 1, one fish had a composite score of 2, one fish had a composite score of 3, and two fish had a composite score of 4 ((average [SD] of 35 fish=0.0.63 [1.07]; Table 11).

## Mobile Telemetry Monitoring

There were a total of 63 individual tag codes detected during mobile monitoring (Figure 13–Figure 16). Sixteen study tag codes were detected during the AFRP surveys between April 6 and April 19, 2016. One of these codes was detected again after 33 days by the 2016 Steelhead Survival Study during mobile monitoring survey 2, approximately 180 m downstream from the first detection (Figure 14). Since it is unlikely that a live, migrating steelhead would not move more than 180 m after a month, this detection likely represents a stationary tag on the bottom of the river that is no longer in a live study fish. Of the 47 tags detected solely by the 2016 Steelhead Survival Study, 1 tag was detected a second time after 14 days during survey 3, approximately 19 km downstream from its first detection during survey 1 (Figure 15). This tag could have been in the digestive tract of a predator; or could have been in a live study fish that was actively migrating but remained in the system longer than typically observed.

Mobile monitoring can only be used to verify the presence of a tag at a specific location, but it cannot be used to verify the absence of a tag. Study tags present in the survey area during mobile monitoring may not have been emitting a code, or the codes they were emitting may not have been detected by the hydrophone. The batteries in the transmitters from the first and second releases of steelhead likely were expired before we conducted our first mobile monitoring survey. Starting mobile monitoring surveys within the range of the first releases' study tag battery lives could provide data on tags from earlier releases. Even if a tag still had a functional battery, it is possible that tags were missed during a mobile monitoring survey, because tags can become buried in the sand and not detected by the hydrophone.

Also, mobile monitoring can only provide information regarding the specific areas where surveys were conducted. For example, the CVP trashracks and Clifton Court Forebay have been shown to be places of high tag density in the past (Buchanan et al. 2015). However, since these locations were not included in the 2016 mobile monitoring surveys, no information was collected regarding post-study tag

presence around these facilities (though data will still be provided by stationary receivers at these two specific locations).

Even when mobile monitoring data provides a positive confirmation of the location of a tag code, what this implies regarding the fate of the corresponding study fish is open to interpretation. It is possible that tag detections are actually false positives. This is especially a potential concern with the AFRP hydrophone that was not set to pick up 180 kHz tags. Additionally, a detected tag may not be in a live study fish but lying on the bottom of the river, either from being shed from a live study fish or from being defecated by a predator. This scenario is likely if a transmitter was detected near the same location across multiple mobile monitoring dates. If a transmitter was detected at different points across the same or different mobile monitoring dates, this movement could indicate that the transmitter was in the digestive tract of a live predator or in a study fish that remained in the system longer than the expected time frame. It could also indicate some movement of the tag itself along the river bottom, depending on how far the distance between detections. In the future, visiting the same locations repeatedly over multiple surveys could provide more information regarding the last location a tag was detected. The more detections provided for a single tag may make interpretations regarding the ultimate fate of the tag less subjective, especially if the tag is shown to remain in the same location over multiple surveys.

Despite its limitations, mobile monitoring data can provide useful information, especially when used in conjunction with receiver detection data. It can be used to give more detailed detection data between receivers, which can be used to better pinpoint potential areas of high mortality.

## PIT Tag Detections

Thirty-eight study fish with PIT tags were detected by the arrays at the SWP release pipes located at Horseshoe Bend or Curtis Landing between February 29 and June 7, 2016 (Figure 9). Since these are tag detections and not visual confirmations of live fish, similar assumptions and interpretations must be considered as those encountered during mobile monitoring or from receiver detections. The association of these tags with a group of fish transported from the fish salvage facility implies that these tags were in live fish. Therefore, these detections likely represent either live study fish or fish that predated study fish. This detection data could be useful as a supplement to data from the receiver array, especially in potentially confirming which fish were trucked and where they were released and in estimating survival from the SWP release sites to Chipps Island.

## Recapture Confirmations

One live study fish was captured by the California Department of Fish and Wildlife (CDFW) Region 4 during sampling at Mossdale Trawl on April 30, 2016, and 17 live study fish were captured in the CVP tank during salvage between March 2 and March 31, 2016 (Figure 9). Each of these steelhead was verified to be a 2016 Steelhead Survival Study fish based on the identification of the tag code. Since these fish could be visually confirmed as alive, these fish validate that any detections of their tags up to this point came from live study fish. The fish captured by the Mossdale Trawl was released back into the river alive at the trawl location. The study fish recaptured in the CVP holding tanks were captured during regular sampling at the facility, and after detection were put back into the CVP holding tanks and trucked to the federal release locations. They were not detected at the release sites because there was

no pit tag array on the CVP release pipes.  This data can be used to supplement the receiver data in the same way as detections from the PIT tag array, except that the fish captured by the CVP were confirmed as alive while the fish associated with the PIT tag detections were not. Additionally, it is important to consider recaptures of study fish because the experience of being captured and handled might have a negative impact on the subsequent survival of these fish.

# References

Adams, N. S., D. W. Rondorf, S. D. Evans, and J. E. Kelly. 1998. Effects of surgically and gastrically implanted radio transmitters on growth and feeding behavior of juvenile Chinook Salmon. Transactions of the American Fisheries Society 127(1):128–136.

Buchanan, R., P. Brandes, M. Marshall, J. S. Foott, J. Ingram, D. LaPlante, and J. Israel. 2015. 2012 South Delta Chinook Salmon Survival Study. Edited by P. Brandes. U.S. Fish and Wildlife Service. Lodi, California.

Liedtke, T. 2012. 2012 South Delta Study tagger training and QA-QC summary. Prepared by T. Liedtke, U.S. Geological Survey, Western Fisheries Research Center, Columbia River Research Laboratory, Cook, Washington; for J. Israel, U.S. Bureau of Reclamation, Bay-Delta Office, Sacramento, California.

Liedtke, T. L., J. W. Beeman, and L. P. Gee. 2012. A standard operating procedure for the surgical implantation of transmitters in juvenile salmonids. U.S. Geological Survey Open-File Report 2012-1267.

Martinelli, T. L., H. C. Hansel, and R. S. Shively. 1998. Growth and physiological responses to surgical and gastric radio transmitter implantation techniques in subyearling Chinook Salmon (Oncorhynchus tshawytscha). Hydrobiologia 371/372:79–87.

## Acknowledgements

Funding for the project came from the U.S. Bureau of Reclamation. Several individuals from a variety of agencies made this project possible. Those agencies and individuals who participated in the tagging and release components of the project were—USFWS: David Bridgman, Emma Davidson, Dustin Dinh, David Dominguez, Duncan Fry, Garrett Giannetta, Patrick Hapgood, Maddelyn Harden, Christopher Hart, Cheyenne Hefley, Jack Ingram, Inji Isaac, Carlie Jackson, Katherine Jardine, Zachary Jesse, Allison Lane, David LaPlante, Jerrica Lewis, Mike Marshall, Louanne McMartin, Graham Mytton, Brynn Perales, William Powell, Lori Smith, Ronald Smith, Christopher Thomas, and Sarah Trujillo; California Department of Water Resources: Rachel August; and USBR: Josh Israel and Raymond Bark. USGS provided training for the surgeons (Theresa [Marty] Liedtke).

# Figures

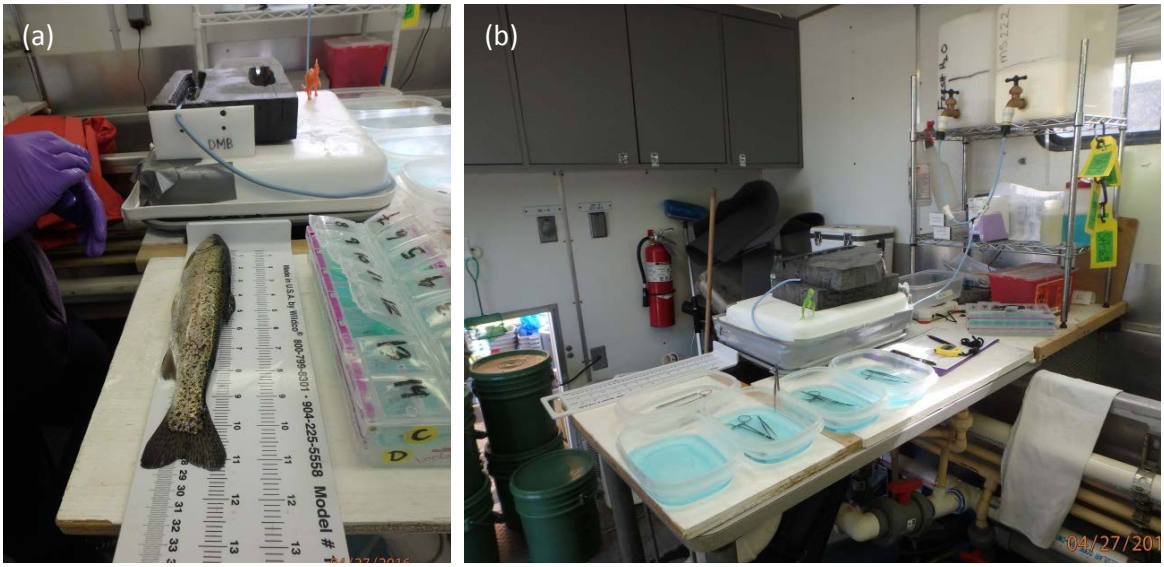


**Figure 1. A steelhead being measured immediately prior to surgery (a) and a tagging station (b) during the 2016 Steelhead Survival Study at the Mokelumne River Hatchery. Photo credit: Denise Barnard/USFWS**


**Figure 2. The recovery bucket staging area at the Mokelumne River Hatchery during the 2016 Steelhead Survival Study (a). Recovery buckets, which each contained 1–2 tagged fish, were placed at the left side of the staging area immediately after surgery. Their tags were validated, and the buckets were moved to the right side of the staging area to await completion of 10 minutes in the recovery buckets (b). After recovery, 2–3 buckets were combined into a perforated tote in a sleeve of water (c). A tote never contained more than three steelhead. Photo credits: Denise Barnard/USFWS (a–b) and Cheryl Strong/USFWS (c)**

**Figure 3. Recovery buckets were aerated to 130–150% dissolved oxygen during the 2016 Steelhead Survival Study. Photo credit: Denise Barnard/USFWS**


**Figure 4. Steelhead were held in perforated garbage cans in the raceway before surgery during the 2016 Steelhead Survival Study. The surgical procedure began by netting a fish from a holding can into an anesthesia bucket. Photo credit: Denise Barnard/USFWS**

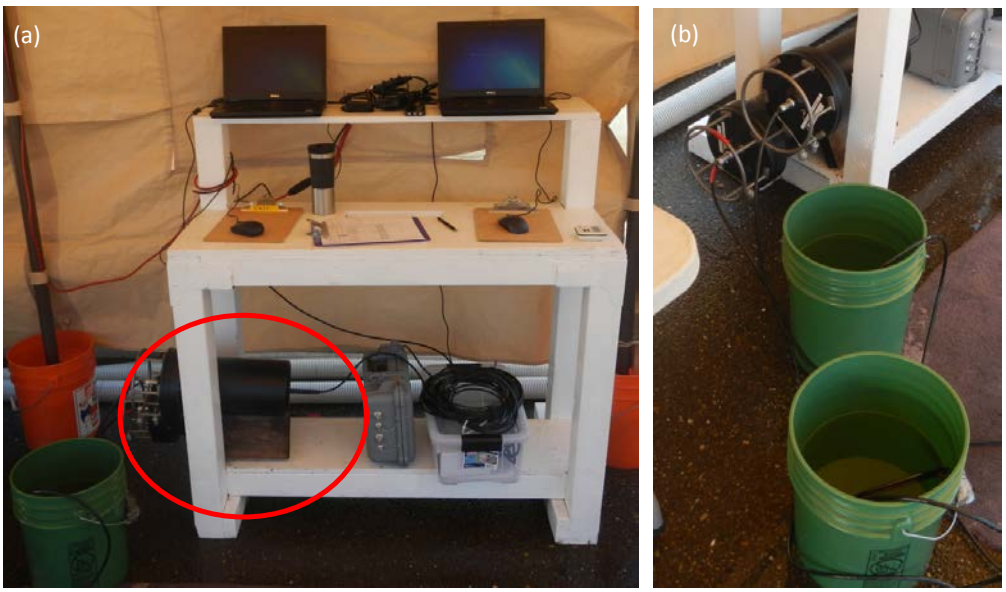


**Figure 5. The transmitter validation station (a) at the Mokelumne River Hatchery during the 2016 Steelhead Survival Study consisted of two VEMCO High Residency Receiver prototypes (circled), each attached to a 180 kHz hydrophone. The hydrophones were placed into recovery buckets to verify the tag codes emitted by tagged steelhead while fish recovered from surgery (b). Photo credit: Pat Brandes/USFWS**


**Figure 6. A transport tank is filled with perforated totes of tagged steelhead during tagging operations of the 2016 Steelhead Survival Study (a). At the end of a tagging session, the transport tank is filled with totes and ready to transport to the holding site on the bed of a flatbed truck (b). Photo credits: Cheryl Strong/USFWS (a) and Denise Barnard/USFWS (b)**

**Figure 7. The Durham Ferry holding site of the 2016 Steelhead Study. Tagged steelhead were held in holding cans in the river for at least 24 hours, before being transported by boat approximately 200 km downstream from the holding site for release. Photo credit: Pat Brandes/USFWS**


**Figure 8. At the Durham Ferry holding site of the 2016 Steelhead Survival Study, totes containing tagged steelhead were removed from the transport truck and placed in sleeves in the back of a pickup truck (a). The totes were driven to the river's edge, removed from the pickup truck (b), and carried to the holding cans in the river (c). Tagged steelhead were then transferred from the tote to the holding can. Photo credits: Cheryl Strong/USFWS**


**Figure 9. The area in the south Delta covered by four mobile monitoring events during the 2016 Steelhead Survival Study. Survey 1 (green) was conducted on May 11, started about 1 km upstream from the Durham Ferry release site, and followed the San Joaquin River downstream for 10.5 km. Survey 2 (white) was conducted on May 18 between the end of the first survey and the head of Old River (HOR) barrier, for about 15.5 km downstream on the San Joaquin River. Survey 3 (blue) occurred on May 25 between the HOR barrier to the head of Fourteen Mile Slough, for about 30.5 km downstream on the San Joaquin River. Survey 4 (yellow) occurred on June 2, started about 3.5 km upstream of Fourteen Mile Slough, followed the San Joaquin River for about 19.5 km downstream, and ended at Prisoner's Point. The location of the SWP (State Water Project) and CVP (Central Valley Project) fish salvage facilities, and SWP release sites are shown but mobile monitoring was not conducted at these locations. However, seventeen study fish were recaptured during salvage at the CVP and 38 study fish with PIT tags were detected by arrays at the SWP release sites located at Horseshoe Bend on the Sacramento River side of Sherman Island and Curtis Landing on the San Joaquin side of Sherman Island. One tagged study fish was recaptured at the Mossdale Trawl by the California Department of Fish and Wildlife, Region 4.**

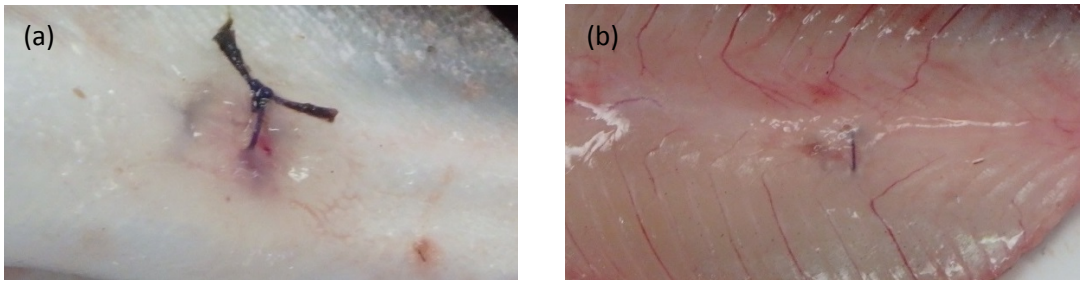


**Figure 10. The external (a) and internal (b) appearance of the surgical site on the only steelhead of 50 to expel its tag after the 30 or 60 day retention tests. Photo credit: USFWS**

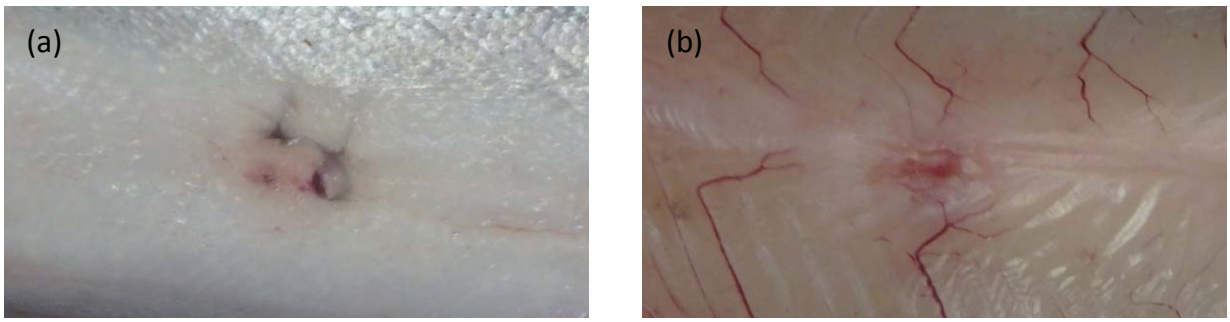


**Figure 11. The external (a) and internal (b) appearance of the surgical site on the only steelhead of 50 to lose both its anterior and posterior sutures after the 30 or 60 day retention tests. Photo credit: USFWS**

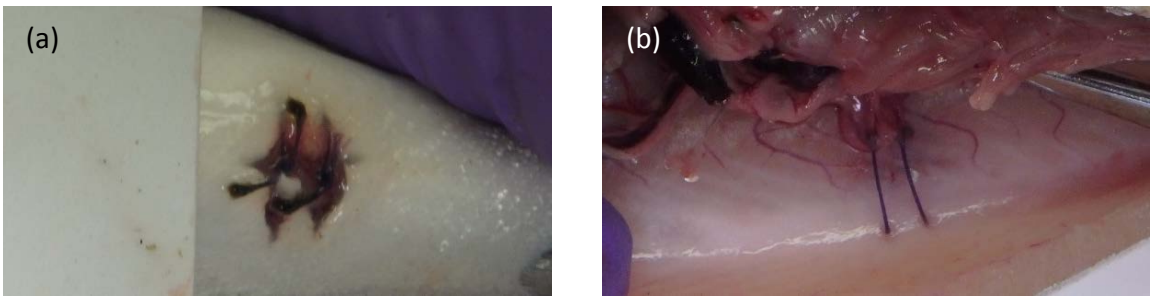


**Figure 12. The external (a) and internal (b) appearance of the surgical site on the only steelhead of 50 to display organ damage (sutured pyloric caeca) after the 30 or 60 day retention tests. Photo credit: USFWS**

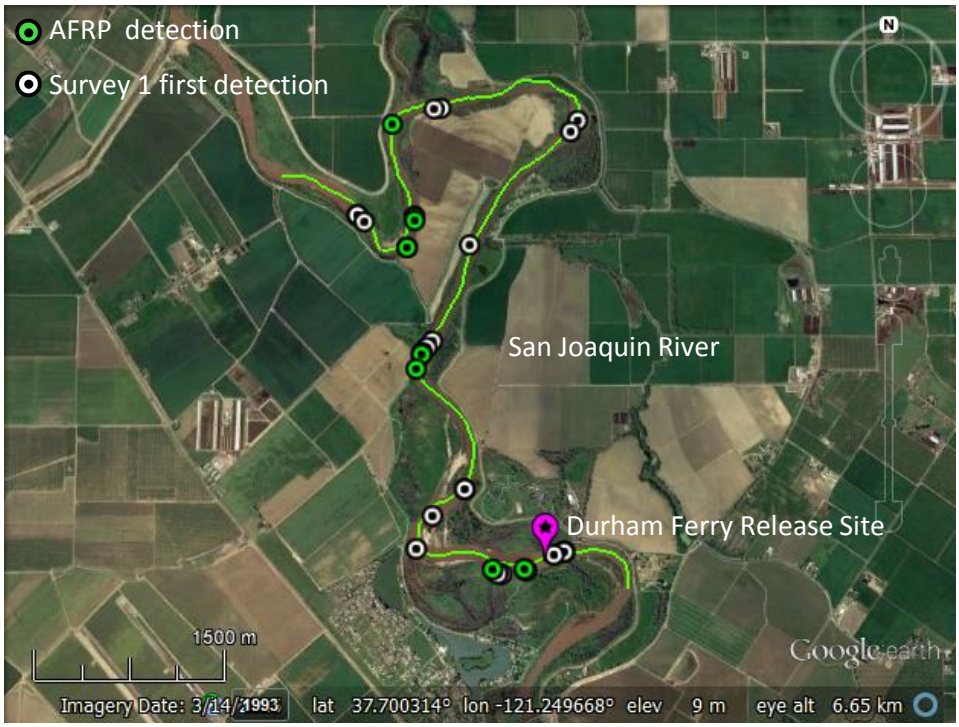


**Figure 13. Tag detections from the 2016 Steelhead Survival Study along the San Joaquin River during mobile monitoring survey 1, and detections of study tags by the AFRP mobile monitoring surveys along the same route.**

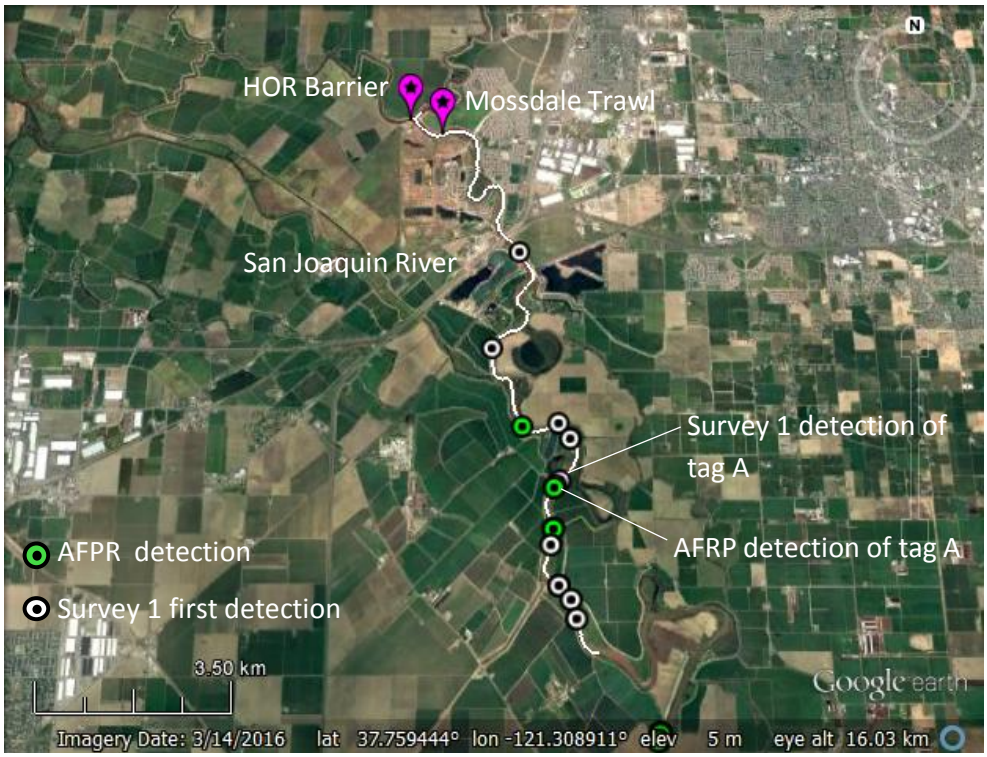


**Figure 14. Tag detections from the 2016 Steelhead Survival Study along the San Joaquin River during mobile monitoring survey 2, and detections of study tags by the AFRP mobile monitoring surveys along the same route. One tag (tag A) was detected by survey 2 approximately 180 m downstream from the AFRP detection.**

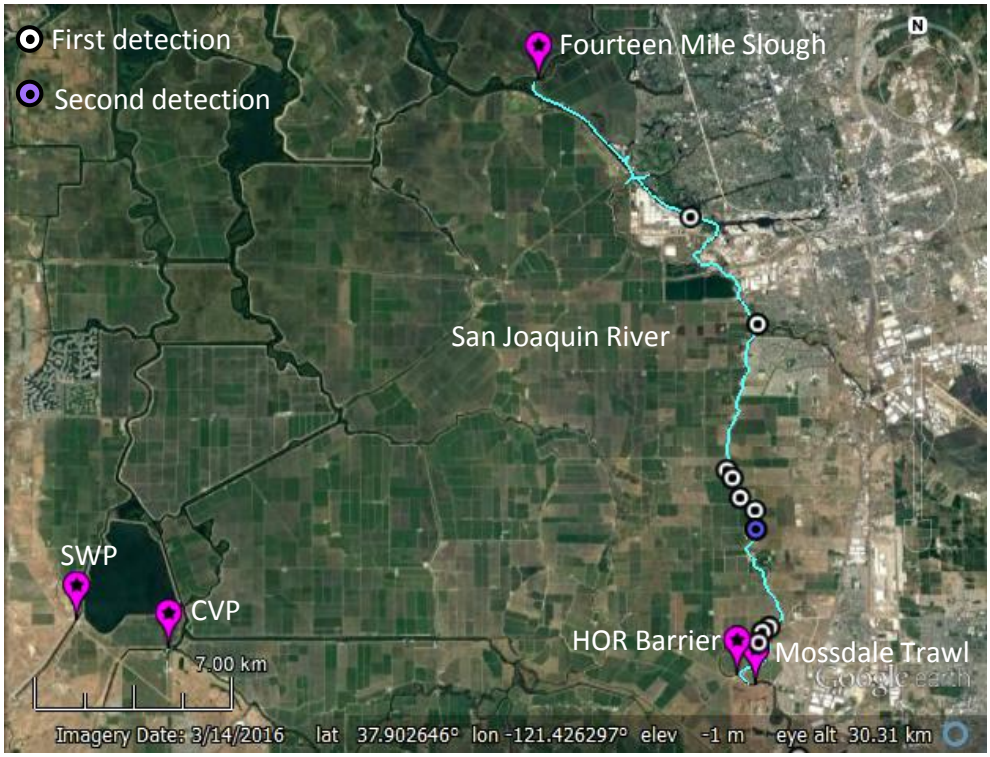


**Figure 15. Tag detections from the 2016 Steelhead Survival Study along the San Joaquin River during mobile monitoring survey 3. A second detection of one tag occurred about 19 km downstream from the tag's first detection during survey 1.**

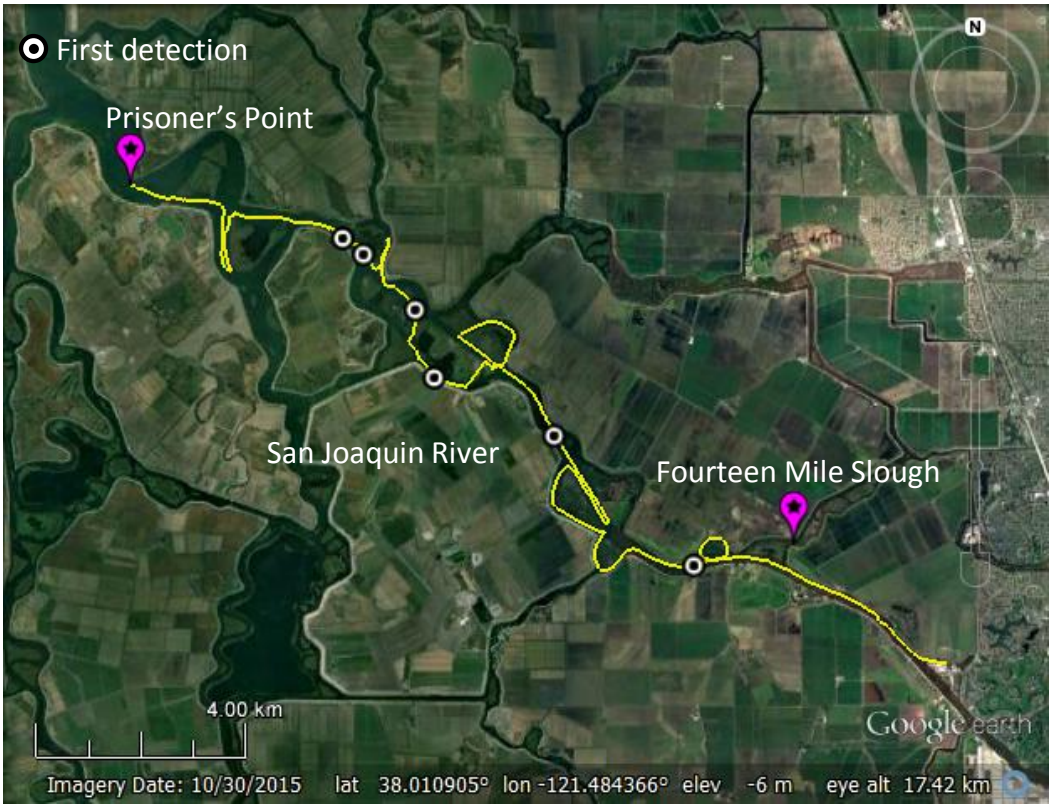


**Figure 16. Tag detections from the 2016 Steelhead Survival Study along the San Joaquin River during mobile monitoring survey 4.**

# Tables

**Table 1. The tagging, transport, holding date and times, and numbers of steelhead released during the 2016 Steelhead Survival Study. Fish were released over a 24 hour period after being held for a minimum of 24 hours.**

| *Tagging & Transport Date* | *Transport Tank #* | *Transport Start Time* | *Start Holding Time* | *Release A Date; Time* | *Release A Number Released* | *Release B Date; Time* | *Release B Number Released* | *Release C Date; Time* | *Release C Number Released* | *Release D Date; Time* | *Release D Number Released* | *Release E Date; Time* | *Release E Number Released* | *Release F Date; Time* | *Release F Number Released* | *Total Released (A+B+C+D+E+F)* | *Total Number Dummy-Tagged Fish* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *2/23/2016* | 1 | 1100 | 1400 | 2/24; 1504 | 24 | 2/24; 1858 | 30 | | | | | | | | | 54 | 3 |
| | 2 | 1325 | 1538 | | | 2/24; 1858 | 6 | 2/24; 2257 | 24 | 2/25; 300 | 24 | | | | | 54 | 3 |
| | 3 | 1632 | 1848 | | | | | | | | | 2/25; 703 | 36 | 2/25; 1102 | 18 | 54 | 6 |
| *2/24/2016* | 1 | 1015 | 1223 | 2/25; 1502 | 24 | 2/25; 1857 | 24 | 2/25; 2257 | 6 | | | | | | | 54 | 3 |
| | 2 | 1235 | 1431 | | | | | 2/25; 2257 | 30 | 2/26; 258 | 24 | | | | | 54 | 3 |
| | 3 | 1505 | 1711 | | | | | | | | | 2/26; 701 | 36 | 2/26; 1103 | 18 | 54 | 6 |
| *2/25/2016* | 1 | 1045 | 1237 | 2/26; 1456 | 24 | 2/26; 1857 | 24 | 2/26; 2256 | 4 | | | | | | | 52 | 6 |
| | 2 | 1245 | 1447 | | | | | 2/26; 2256 | 28 | 2/27; 301 | 24 | | | | | 52 | 6 |
| | 3 | 1455 | 1701 | | | | | 2/26; 2256 | 4 | | | 2/27; 703 | 24 | 2/27; 1101 | 24 | 52 | 12 |
| *3/15/2016* | 1 | 1045 | 1252 | 3/16; 1515 | 24 | 3/16; 1900 | 30 | | | | | | | | | 54 | 3 |
| | 2 | 1245 | 1503 | | | 3/16; 1900 | 6 | 3/16; 2259* | 24 | 3/17; 301* | 24 | | | | | 54 | 3 |
| | 3 | 1548 | 1730 | | | | | | | | | 3/17; 700 | 36 | 3/17; 1100 | 18 | 54 | 6 |
| *3/16/2016* | 1 | 1030 | 1236 | 3/17; 1458 | 24 | 3/17; 1857 | 24 | 3/17; 2258 | | 6 | | | | | | 54 | 3 |
| | 3** | 1250 | | 1508 | | | | | 3/17; 2258 | 30 | 3/18; 300 | 24 | | | | 54 | 3 |
| | 2** | 1440 | | 1659 | | | | | | | | 3/18; 701 | 36 | 3/18; 1102 | 18 | 54 | 6 |
| *3/17/2016* | 1 | 1015 | 1225 | 3/18; 1500 | 24 | 3/18; 1858 | 24 | 3/18; 2257 | 4 | | | | | | | 52 | 6 |
| | 2 | 1230 | 1449 | | | | | 3/18; 2257 | 28 | 3/19; 302 | 24 | | | | | 52 | 6 |

| *Tagging & Transport Date* | *Transport Tank #* | *Transport Start Time* | *Start Holding Time* | *Release A Date; Time* | *Release A Number Released* | *Release B Date; Time* | *Release B Number Released* | *Release C Date; Time* | *Release C Number Released* | *Release D Date; Time* | *Release D Number Released* | *Release E Date; Time* | *Release E Number Released* | *Release F Date; Time* | *Release F Number Released* | *Total Released (A+B+C+D+E+F)* | *Total Number Dummy-Tagged Fish* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 1455 | 1711 | | | | | 3/18; 2257 | 4 | | | 3/19; 702 | 24 | 3/19; 1059 | 24 | 52 | 12 |
| *4/26/2016* | 1 | 1115 | 1315 | 4/27; 1459 | 24 | 4/27; 1857 | 30 | | | | | | | | | 54 | 3 |
| | 2 | 1350 | 1555 | | | 4/27; 1857 | 6 | 4/27; 2255 | 24 | 4/28; 311 | 24 | | | | | 54 | 3 |
| | 3 | 1620 | 1827 | | | | | | | | | 4/28; 658 | 36 | 4/28; 1104 | 18 | 54 | 6 |
| *4/27/2016* | 1 | 1015 | 1242 | 4/28; 1459 | 24 | 4/28; 1856 | 24 | 4/28; 2257 | 6 | | | | | | | 54 | 3 |
| | 2 | 1220 | 1437 | | | | | 4/28; 2256 and 2257 | 30 | 4/29; 259 | 24 | | | | | 54 | 3 |
| | 3 | 1423 | 1647 | | | | | | | | | 4/29; 700 | 36 | 4/29; 1057 | 18 | 54 | 6 |
| *4/28/2016* | 1 | 945 | 1210 | 4/29; 1456 | 24 | 4/29; 1857 | 24 | 4/29; 2256 | 4 | | | | | | | 52 | 6 |
| | 2 | 1155 | 1431 | | | | | 4/29; 2256 | 28 | 4/30; 301 | 24 | | | | | 52 | 6 |
| | 3 | 1405 | 1617 | | | | | | | | | 4/30; 657 | 24 | 4/30; 1057 | 28 | 52 | 12 |

*The lids were switched between one tote from 3/16 2259 and one tote from 3/17 301. It is likely that the fish from each tote were assigned the correct release time based on the way in which totes were loaded at the tagging site. However, it is possible that the release times are switched for three fish from each release time.

**Transport tank #3 was used to transport the second batch of fish on 3/16.

**Table 2. Parameters and criteria assessed during the quality assurance and quality control tagging inspections of the 2016 Steelhead Survival Study. Parameters were provided by T. Liedtke, USGS.**

| Parameter Assessed | Assessment Criteria |
|---|---|
| **Anesthesia bucket temp** | Was temp in anesthesia bucket <2°C different than fish source? |
| **Gravity feed temp** | Was temp in gravity feed <2°C different than fish source? |
| **Recovery bucket DO** | Was DO in recovery buckets within target? |
| **Recovery bucket temp** | Was temp in recovery buckets <2°C different than fish source? |
| **SOP components** | 1. Were surgical instruments sterile at the start of the tagging operation? |
| | 2. Were transmitters disinfected in chlorhexidine (20 min contact time) and rinsed prior to implantation? |
| | 3. Did the taggers wear gloves during fish handling and tag implantation procedures? |
| | 4. Were disinfected transmitters handled with gloves or clean instruments? |
| | 5. Was anesthesia exposure time monitored? If fish exceeded 5 min in anesthesia were they rejected |
| | 6. Were labels applied to recovery buckets to ensure transfer to proper transport containers? |
| | 7. Was stress coat used appropriately on surfaces and in buckets? (especially important on the tagging platform and in the recovery buckets) |
| | 8. Were source fish netted carefully? Was care taken to minimize chasing? |
| | 9. Were lids used on all containers holding fish? |
| | 10. Did staff ensure that all fish in a recovery bucket were held for at least 10 min and had regained equilibrium before transferring them to the transport tote? |
| | 11. If water quality measurements were outside the acceptable range, was corrective action taken? |
| | 12. Were fish held at appropriate densities for short-term holding (i.e., no more than 3 fish per recovery bucket, no more than 3 fish per tote)? |

**Table 3. External characteristics assessed for steelhead smolt condition and short-term survival. Percent scale loss was also assessed on one side of the fish. Parameters were provided by T. Liedtke, USGS.**

| Character | Normal | Abnormal |
|---|---|---|
| Body color | High contrast dark dorsal surfaces and light sides | Low contrast dorsal surfaces and coppery colored sides |
| Fin hemorrhaging | No bleeding at base of fins | Blood present at base of fins |
| Eyes | Normally shaped | Bulging or with hemorrhaging |
| Gill color | Dark beet red to cherry red colored gill filaments | Grey to light red colored gill filaments |
| Vigor | Active swimming (prior to anesthesia) | Lethargic or motionless (prior to anesthesia) |

**Table 4. The parameters assessed during the necropsy of dummy-tagged and tag retention steelhead during the 2016 Steelhead Survival Study. The score from each of the 7 numerical parameters was added together to generate a composite score (0–12) to consider possible tagging effects on survival. The anterior and posterior sutures were scored separately and each was included in the composite score. Parameters were provided by T. Liedtke, USGS.**

| Composite Score Parameter | Score | Score Definition |
|---|---|---|
| Signs of tag expulsion | 0 | No signs of tag expulsion. I.e., no signs that the tag is being forced out through the incision or the lateral body wall. Simple encapsulation may be present |
| | 1 | Some bulging or lateral pressure. I.e., some evidence that the tag is causing pressure on the incision or the lateral body wall |
| | 2 | Expulsion process obvious or complete. I.e., the tag is obviously being forced out through the incision or the lateral body wall, or the tag is already out |
| Suture present? | 0 | Yes |
| | 1 | Yes, but untied or becoming untied |
| | 2 | No |
| Incision apposition | 0 | Completely closed, perfect apposition |
| | 1 | Incision partially open due to gape or overlap |
| | 2 | Incision completely open (>75%) |
| Peritoneal apposition | 0 | Peritoneum completely closed, perfect apposition |
| | 1 | Peritoneum partially closed |
| | 2 | Peritoneum completely open (>75%) |
| Organ damage | 0 | No organ damage present. I.e., no signs of damage either due to the surgery or the presence of the tag. Tags can be adhered to organs as part of encapsulation process, but that does not constitute damage |
| | 1 | Some organ damage present. I.e., the suture captures, punctures, or entangles the pyloric caeca, stomach, spleen, or intestine |
| Fungus present? | 0 | No fungus present |
| | 1 | Fungus present |
| Fungus location | Suture | Fungus on the suture material |
| | Incision | Fungus on skin in/around incision |
| | Tail | Fungus on skin on the tail |
| | Body | Fungus on skin on the body |

**Table 5. Water temperature and dissolved oxygen (DO) in the transport tank after loading, prior to transport, after transport, and in the river at the Durham Ferry release site, just prior to placing fish in holding containers and the number of mortalities after transport and prior to release for steelhead as part of the 2016 Steelhead Survival Study.**

| *Transport Date* | *Loading Time* | *Tank Prior to Loading Temp (˚C)* | *Tank Prior to Loading DO (mg/L)* | *Tank After Loading Temp (˚C)* | *Tank After Loading DO (mg/L)* | *Tank After Transport Temp (˚C)* | *Tank After Transport DO (mg/L)* | *Mortalities After Transport* | *River Temp (˚C)* | *River DO (mg/L)* | *Mortalities Just Prior to Release* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *2/23/2016* | 905 | 11.2 | 9.87 | 13.2 | 9.64 | 12.6 | 9.42 | 0 | 15.2 | 10.95 | 0 |
| *2/23/2016* | 1146 | 12.5 | 9.73 | 12.8 | 9.51 | 13.7 | 9.29 | 0 | 15.8 | 11.47 | 0 |
| *2/23/2016* | 1414 | 11.6 | 9.73 | 11.2 | 9.47 | 12.1 | 9.44 | 0 | 15.8 | 11.43 | 0 |
| *2/24/2016* | 900 | 10.8 | 9.69 | 13 | 9.4 | 13.7 | 9.19 | 0 | 14.7 | 10.41 | 0 |
| *2/24/2016* | 1050 | 11.7 | 9.95 | 12.3 | 9.65 | 13.5 | 10.17 | 0 | 16.2 | 11.14 | 0 |
| *2/24/2016* | 1315 | 11.6 | 9.95 | 11.5 | 9.75 | 12.8 | 10.24 | 0 | 16.5 | 11.76 | 0 |
| *2/25/2016* | 915 | 10.7 | 9.46 | 12.6 | 9.65 | 13.1 | 10.06 | 0 | 15.8 | 10.64 | 0 |
| *2/25/2016* | 1130 | 11.8 | 9.89 | 11.9 | 9.77 | 14.1 | 9.94 | 0 | 16.3 | 11.64 | 0 |
| *2/25/2016* | 1315 | 11.5 | 9.89 | 11.9 | 10.19 | 13 | 10.17 | 0 | 16.8 | 12.01 | 0 |
| *Mean* | | 11.49 | 9.8 | 12.27 | 9.67 | 13.18 | 9.77 | 0 | 15.9 | 11.27 | 0 |
| *3/15/2016* | 915 | 10.9 | 10.73 | 12.5 | 10.02 | 12.3 | 10.27 | 0 | 14.4 | 7.38 | 0 |
| *3/15/2016* | 1125 | 11.7 | 10.18 | 11.8 | 9.87 | 12.9 | 9.98 | 0 | 15.3 | 7.4 | 0 |
| *3/15/2016* | 1343 | 11.7 | 9.33 | 12.5 | 10 | 13.1 | 10.46 | 0 | 15.5 | 7.54 | 0 |
| *3/16/2016* | 850 | 10.8 | 10.76 | 11 | 10.16 | 11.7 | 10.47 | 0 | 14.7 | 7.66 | 0 |
| *3/16/2016* | 1120 | 11.4 | 10.24 | 12 | 10.01 | 12.6 | 10.72 | 0 | 16 | 7.45 | 0 |
| *3/16/2016* | 1320 | 12 | 10.06 | 12.1 | 9.68 | 14 | 9.68 | 0 | 15.9 | 7.56 | 0 |
| *3/17/2016* | 850 | 10.7 | 9.86 | 13.1 | 9.71 | 12.8 | 10.56 | 0 | 16 | 7 | 0 |
| *3/17/2016* | 1100 | 11.6 | 10 | 11.9 | 9.35 | 13.5 | 9.8 | 0 | 17 | 6.97 | 0 |
| *3/17/2016* | 1310 | 11.8 | 9.82 | 12.1 | 9.68 | 13.5 | 9.81 | 0 | 17.3 | 7.06 | 0 |
| *Mean* | | 11.4 | 10.11 | 12.11 | 9.83 | 12.93 | 10.19 | 0 | 15.8 | 7.34 | 0 |
| *4/26/2016* | 915 | 12 | 11.82 | 13.3 | 9.86 | 13.8 | 10.56 | 0 | 15.1 | 9.44 | 0 |
| *4/26/2016* | 1155 | 12.1 | 10.23 | 12.9 | 9.94 | 14.8 | 9.23 | 0 | 15.7 | 10.88 | 0 |
| *4/26/2016* | 1430 | 12 | 10.06 | 12 | 9.86 | 13.5 | 9.69 | 0 | 15.7 | 10.34 | 0 |
| *4/27/2016* | 830 | 11 | 10.48 | 12.5 | 9.92 | 12.6 | 11.04 | 0 | 14.7 | 9.39 | 0 |
| *4/27/2016* | 1050 | 12.1 | 10.16 | 12 | 10.01 | 13.8 | 9.28 | 0 | 14.8 | 9.79 | 0 |

| *Transport Date* | *Loading Time* | *Tank Prior to Loading Temp (˚C)* | *Tank Prior to Loading DO (mg/L)* | *Tank After Loading Temp (˚C)* | *Tank After Loading DO (mg/L)* | *Tank After Transport Temp (˚C)* | *Tank After Transport DO (mg/L)* | *Mortalities After Transport* | *River Temp (˚C)* | *River DO (mg/L)* | *Mortalities Just Prior to Release* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *4/27/2016* | 1253 | 11.7 | 10.03 | 11.6 | 9.9 | 12.7 | 9.88 | 0 | 15.3 | 10.31 | 0 |
| *4/28/2016* | 830 | 11 | 10.45 | 11.3 | 10.31 | 13.1 | 11.1 | 0 | 14.7 | 9.29 | 0 |
| *4/28/2016* | 1040 | 11.9 | 10.15 | 12.1 | 9.9 | 14 | 9.2 | 0 | 15.6 | 10.67 | 0 |
| *4/28/2016* | 1238 | 12.4 | 9.99 | 12 | 9.9 | 13.8 | 9.41 | 0 | 15.7 | 9.83 | 0 |
| *Mean* | | 11.8 | 10.37 | 12.19 | 9.96 | 13.57 | 9.93 | 0 | 15.3 | 9.99 | 0 |

**Table 6. Results of external criteria assessed on dummy-tagged steelhead after being held for 48 hours at the Durham Ferry release site during the 2016 Steelhead Survival Study. Criteria are defined in Table 3. All examinations occurred at 1130 hours.**

| *Tagging Week* | *Examination Date* | *Water Temperature (°C) and DO (mg/L) in Can Prior to Assessment* | *Mean (SD) Fork length (mm)* | *Mortality* | *Mean (SD) Scale Loss* | *Normal Body Color* | *No Fin Hemorrhaging* | *Normal Eye Quality* | *Normal Gill Color* |
|---|---|---|---|---|---|---|---|---|---|
| *1* | 2/25/2016 | 15.4; 9.73 | 233.1 (10.8) | 0/12 | 4.5 (2.5) | 12/12 | 12/12 | 12/12 | 12/12 |
| | | 15.7; 9.96 | 236.1 (10.3) | 0/12 | 8.9 (5.4) | 10/12 | 12/12 | 12/12 | 12/12 |
| | 2/26/2016 | | | | | | | | |
| | | | | 0/24* | | | | | |
| *2* | 3/17/2016 | 15.9; 6.86 | 243.6 (26.5) | 0/12 | 4.6 (1.4) | 12/12 | 12/12 | 12/12 | 12/12 |
| | | 16.9; 6.61 | 243.4 (13.6) | 0/12 | 5.0 (3.0) | 12/12 | 12/12 | 12/12 | 12/12 |
| | 3/18/2016 | | | | | | | | |
| | | | | 0/24* | | | | | |
| *3* | 4/28/2016 | 15.1; 9.82 | 259.3 (11.2) | 0/12 | 7.5 (4.5) | 12/12 | 12/12 | 12/12 | 12/12 |
| | | 14.8; 9.17 | 252.3 (13.2) | 0/12 | 7.9 (4.5) | 12/12 | 12/12 | 12/12 | 12/12 |
| | 4/29/2016 | | | | | | | | |
| | | | | 0/24* | | | | | |

* Fish given to CA-NV Fish Health Center for further evaluation

**Table 7. Results of characteristics assessed on dummy-tagged steelhead before necropsy during the 2016 Steelhead Survival Study. The parameters, as outlined in Table 4, included presence of the (1) anterior and (2) posterior suture (0=present, 1=untied, 2=not present), (3) incision and (4) peritoneal apposition (0=closed, good apposition, 1=partial gape or overlap, 2=completely open [>75%]), (5) organ damage (0=none, 1=yes), (6) signs of tag expulsion (0=none, 1=some signs present, 2=tag expelled or partially expelled), and (7) whether fungus was present (0=no, 1=yes). A composite score (the sum of the 7 parameters; 0–12) was calculated to consider possible compounding effects of compromised parameters on survival. All examinations occurred at 1130 hours.**

| *Tagging Week* | *Date* | *Mean (SD) of Signs Of Expulsion (0–2)* | *Mean (SD) of Anterior Suture Presence (0–2)* | *Mean (SD) of Posterior Suture Presence (0–2)* | *Mean (SD) of Incision Apposition (0–2)* | *Mean (SD) of Peritoneal Apposition (0–2)* | *Mean (SD) of Organ Damage (0–1)* | *Mean (SD) of Fungus Presence (0–1)* | *Mean (SD) of Composite Score (0–12)* |
|---|---|---|---|---|---|---|---|---|---|
| *1* | 2/25/2016 | 0 (0) | 0 (0) | 0 (0) | 0.1 (0.3) | 0.9 (0.9) | 0.2 (0.4) | 0 (0) | 1.2 (1.3) |
| | 2/26/2016 | 0 (0) | 0(0) | 0(0) | 0.1 (0.3) | 1.1 (0.7) | 0.1 (0.3) | 0 (0) | 1.3 (0.8) |
| *2* | 3/17/2016 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1.1 (0.5) | 0 (0) | 0 (0) | 1.1 (0.5) |
| | 3/18/2016 | 0 (0) | 0 (0) | 0 (0) | 0.1 (0.3) | 1.4 (0.5) | 0 (0) | 0 (0) | 1.5 (0.7) |
| *3* | 4/28/2016 | 0 (0) | 0.1 (0.3) | 0.2 (0.4) | 0 (0) | 0.9 (0.3) | 0 (0) | 0 (0) | 1.2 (0.7) |
| | 4/29/2016 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1.1 (0.5) | 0 (0) | 0 (0) | 1.1 (0.5) |

**Table 8. Results of the external criteria assessed after holding tag retention steelhead for 30 days after tagging during the 2016 Steelhead Survival Study. The weight and forklength (FL) of each fish was measured, the scales were assessed on the most compromised side of the fish as "normal" (0-5%; 0), partial (6–19%; 1), or descaled (>19%; 2), and the final position of the acoustic and PIT tags was determined (0=no, 1=yes).**

| Fish # | Weight Day 0 (g) | Weight Day 30 (g) | FL Day 0 (mm) | FL Day 30 (mm) | Scales Day 0 (0/1/2) | Scales Day 30 (0/1/2) | Acoustic Tag Position Over Incision (0/1) | Acoustic Tag Position Anterior to Incision (0/1) | Acoustic Tag Position Posterior to Incision (0/1) | PIT Tag Position Over Incision (0/1) | PIT Tag Position Anterior to Incision (0/1) | PIT Tag Position Posterior to Incision (0/1) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 129.9 | 152.9 | 233 | 248 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 |
| 2 | 136.3 | 166.8 | 251 | 265 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| 3 | 123.6 | 148.5 | 231 | 250 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| 4 | 171.7 | 203.1 | 253 | 268 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| 5 | 93 | 118.2 | 213 | 223 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| 6 | 141.8 | 165 | 242 | 258 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| 7 | 117.4 | 144.6 | 226 | 235 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| 8 | 157.6 | 175.8 | 256 | 268 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| 9 | 131.3 | 147.9 | 242 | 250 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| 10 | 158 | 185.7 | 247 | 262 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| 11 | 143.5 | 168.6 | 242 | 250 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| 12 | 147.2 | 168.8 | 250 | 263 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| 13 | 144.3 | 164.3 | 240 | 252 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| 14 | 172 | 207.9 | 259 | 275 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| 15 | 142.7 | 169.4 | 240 | 256 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| Mean | 140.69 | 165.83 | 241.67 | 254.87 | 0.33 | 0.2 | 0.93 | 0 | 0.07 | 0.47 | 0.4 | 0.13 |
| SD | 19.88 | 21.9 | 11.78 | 12.96 | 0.47 | 0.4 | 0.25 | 0 | 0.25 | 0.5 | 0.49 | 0.34 |

**Table 9. Results of seven recovery parameters assessed on tag retention steelhead held for 30 days after tagging. The parameters, as outlined in Table 4, included presence of the (1) anterior and (2) posterior suture (0=present, 1=untied, 2=not present), (3) incision and (4) peritoneal apposition (0=closed, good apposition, 1=partial gape or overlap, 2=completely open [>75%]), (5) organ damage (0=none, 1=yes), (6) signs of tag expulsion (0=none, 1=some signs present, 2=tag expelled or partially expelled), and (7) whether fungus was present (0=no, 1=yes). A composite score (the sum of the 7 parameters; 0–12) was calculated to consider possible compounding effects of compromised parameters on survival.**

| Fish # | Suture Anterior (0/1/2) | Suture Posterior (0/1/2) | Incision Apposition (0/1/2) | Peritoneal Apposition (0/1/2) | Organ Damage (0/1) | Fungus (0/1 [location]) | Signs of Tag Expulsion (0/1/2) | Composite Score (0–12) |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 1 | 0 | 0 | | 1 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 |
| 5 | 0 | 0 | 0 | 1 | 0 | 1 (suture) | 0 | 2 |
| 6 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 |
| 11 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mean | 0 | 0 | 0.27 | 0.33 | 0 | 0.07 | 0 | 0.67 |
| SD | 0 | 0 | 0.44 | 0.47 | 0 | 0.25 | 0 | 0.79 |

**Table 10. Results of the external criteria assessed after holding tag retention steelhead for 60 days after tagging. The weight and fork length (FL) of each fish was measured, the scales were assessed on the most compromised side of the fish as "normal" (0-5%; 0), partial (6–19%; 1), or descaled (>19%; 2), and the final position of the acoustic and PIT tags was determined (0=no, 1=yes).**

| *Fish #* | *Weight Day 0 (g)* | *Weight Day 30 (g)* | *FL Day 0 (mm)* | *FL Day 30 (mm)* | *Scales Day 0 (0/1/2)* | *Scales Day 30 (0/1/2)* | *Acoustic Tag Position Over Incision (0/1)* | *Acoustic Tag Position Anterior to Incision (0/1)* | *Acoustic Tag Position Posterior to Incision (0/1)* | *PIT Tag Position Over Incision (0/1)* | *PIT Tag Position Anterior to Incision (0/1)* | *PIT Tag Position Posterior to Incision (0/1)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *16* | 153.8 | 301.5 | 247 | 290 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *17* | 67.5 | 124.8 | 196 | 231 | 0 | 1 | N/A | N/A | N/A | 1 | 0 | 0 |
| *18* | 125.3 | 208.1 | 244 | 276 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| *19* | 164 | 307.1 | 252 | 298 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| *20* | 99.5 | 182.2 | 223 | 254 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| *21* | 105.5 | 197.5 | 219 | 258 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 |
| *22* | 100.6 | 212.5 | 220 | 269 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| *23* | 97.4 | 196.8 | 216 | 256 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| *24* | 145.6 | 251.8 | 248 | 287 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *25* | 154.4 | 278.5 | 252 | 290 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *26* | 102.5 | 212.4 | 219 | 269 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 |
| *27* | 91.6 | 183.4 | 214 | 251 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| *28* | 113.5 | 223.7 | 230 | 277 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *29* | 91.1 | 160.4 | 211 | 249 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| *30* | 117.5 | 225.2 | 238 | 285 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 |
| *31* | 152.6 | 285.1 | 240 | 294 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *32* | 147.7 | 227.5 | 249 | 283 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *33* | 150.3 | 243.9 | 241 | 280 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 |
| *34* | 161 | 315.5 | 253 | 301 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| *35* | 113.7 | 221.5 | 229 | 274 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *36* | 119.8 | 250.8 | 236 | 286 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |

| *Fish #* | *Weight Day 0 (g)* | *Weight Day 30 (g)* | *FL Day 0 (mm)* | *FL Day 30 (mm)* | *Scales Day 0 (0/1/2)* | *Scales Day 30 (0/1/2)* | *Acoustic Tag Position Over Incision (0/1)* | *Acoustic Tag Position Anterior to Incision (0/1)* | *Acoustic Tag Position Posterior to Incision (0/1)* | *PIT Tag Position Over Incision (0/1)* | *PIT Tag Position Anterior to Incision (0/1)* | *PIT Tag Position Posterior to Incision (0/1)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *37* | 182.4 | 344 | 257 | 305 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 |
| *38* | 96.8 | 153.9 | 219 | 252 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| *39* | 78.5 | 181.2 | 214 | 255 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 |
| *40* | 124.4 | 259.2 | 234 | 271 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 |
| *41* | 128.5 | 265.6 | 245 | 295 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| *42* | 187.1 | 351.2 | 264 | 310 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 |
| *43* | 108.2 | 224.4 | 230 | 276 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| *44* | 135.6 | 228.4 | 231 | 266 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *45* | 168 | 266.7 | 259 | 290 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *46* | 179.7 | 359.7 | 263 | 311 | 0 | 2 | 1 | 0 | 0 | 1 | 0 | 0 |
| *47* | 105.9 | 216.9 | 219 | 260 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *48* | 133.1 | 236.9 | 242 | 285 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *49* | 101.4 | 204.6 | 221 | 261 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *50* | 146.9 | 251 | 250 | 287 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *Mean:* | 127.18 | 238.68 | 235 | 276.63 | 0.37 | 0.69 | 0.65 | 0.03 | 0.32 | 0.6 | 0.06 | 0.34 |
| *SD:* | 30.39 | 54.37 | 16.61 | 18.88 | 0.48 | 0.52 | 0.48 | 0.17 | 0.47 | 0.49 | 0.23 | 0.47 |

**Table 11. Results of seven parameters assessed on tag retention steelhead held for 60 days after tagging. The parameters, as outlined in Table 4, included presence of the (1) anterior and (2) posterior suture (0=present, 1=untied, 2=not present), (3) incision and (4) peritoneal apposition (0=closed, good apposition, 1=partial gape or overlap, 2=completely open [>75%]), (5) organ damage (0=none, 1=yes), (6) signs of tag expulsion (0=none, 1=some signs present, 2=tag expelled or partially expelled), and (7) whether fungus was present (0=no, 1=yes). A composite score (the sum of the 7 parameters; 0–12) was calculated to consider possible effects of tagging parameters on survival.**

| *Fish #* | *Anterior Suture(0/1/2)* | *Posterior Suture (0/1/2)* | *Incision Apposition (0/1/2)* | *Peritoneal Apposition (0/1/2)* | *Organ Damage (0/1)* | *Fungus (0/1 [location])* | *Signs of Tag Expulsion (0/1/2)* | *Composite Score (0–12)* |
|---|---|---|---|---|---|---|---|---|
| *16* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *17* | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 4 |
| *18* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *19* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *20* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *21* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *22* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *23* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *24* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *25* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *26* | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 4 |
| *27* | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 3 |
| *28* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *29* | 0 | 0 | 0 | 0 | 0 | 1 (suture) | 0 | 1 |
| *30* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *31* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *32* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *33* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *34* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *35* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *36* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *37* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| *Fish #* | *Anterior Suture(0/1/2)* | *Posterior Suture (0/1/2)* | *Incision Apposition (0/1/2)* | *Peritoneal Apposition (0/1/2)* | *Organ Damage (0/1)* | *Fungus (0/1 [location])* | *Signs of Tag Expulsion (0/1/2)* | *Composite Score (0–12)* |
|---|---|---|---|---|---|---|---|---|
| *38* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *39* | 0 | 0 | 0 | 0 | 0 | 1 (suture) | 0 | 1 |
| *40* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *41* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *42* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *43* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *44* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *45* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *46* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *47* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *48* | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 2 |
| *49* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *50* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Mean:* | 0.06 | 0.17 | 0.26 | 0 | 0.03 | 0.06 | 0.06 | 0.63 |
| *SD:* | 0.33 | 0.56 | 0.44 | 0 | 0.17 | 0.23 | 0.33 | 1.07 |

# Appendices

## Appendix A. Standard Operating Procedure for Tagging Operations (2/18/2016)

### Materials Needed

- Dissolved oxygen (DO) meter (e.g., YSI 85)
- Acoustic tags and dummy tags (V5)
- VEMCO acoustic tag activator
- VEMCO acoustic tag verification equipment (VRHR or VR100)
- PIT tags
- PIT tag reader
- Pill boxes for tag distribution
- Distilled or de-ionized water (D-$H_2O$)
- Chlorhexidine solution (Novalsan; 30 mL/L D-$H_2O$)
- Aqui-S 20E (undiluted, directly from manufacturer)
- Stress coat - stock concentration and 25% solution (250 mL/L D-$H_2O$)
- Disinfectant solution (Virkon Aquatic or 70% ETOH)
- 19 L bucket(s) marked at 10 L and clearly labeled 'Anesthesia'
- 19 L buckets clearly labeled 'Reject' for fish not selected for tagging procedures
- 19 L buckets clearly labeled "Lethal" for fish that need to be euthanized
- 19 L buckets for post-surgical recovery of fish
- Two gravity feed containers marked at 10 L, and connected by rubber tubing with in-line shut-off valves (one labeled 'anesthesia' and one labeled 'freshwater')
- Designated syringes (1 and/or 5 mL) for measuring anesthetic and stress coat
- Oxygen delivery system (cylinder, regulator, airline, air diffusers) for recovery buckets
- Dip nets
- Nitrile gloves (in all sizes)
- Scale measuring to the nearest 0.1 g (weighing fish)
- 300 g calibration weights
- Scale measuring to the nearest 0.001 g (weighing tags)
- Large plastic weigh boats or Tupperware container to weigh fish
- Measuring board with ruler to the nearest millimeter
- Surgical platform (cradle or foam)
- Autoclave
- Trays for holding solutions used to disinfect surgical tools
- Trays to rinse disinfected tools
- Needle drivers (multiple sets)
- Forceps (multiple sets)
- Scalpel handle and 5 mm blades (multiple sets)
- Scissors (multiple sets)
- Sutures: Vicryl plus 4-0 with an RB-1 needle
- Spray bottles for disinfectant solution
- Timer(s)
- Sharps container
- Datasheets, clipboards, and writing tools
- Clip on tag labels to identify fish in recovery buckets

- Clean rags for keeping tagging areas clean and dry
- Aerators for bucket use (tagger recovery bucket, recovery at code out)

## Pre-tagging Activities

- All acoustic tags will be weighed to the nearest 0.001 g
- All acoustic tags need to be soaked a minimum of 24 hours prior to surgery in a saline solution to ensure that the tags are waterproof, and that the seals encapsulating the tags are functional (see the SOP on tag soak procedures)
- PIT tags and acoustic tags will be assigned to pairs and loaded into tag trays so that taggers remove both tags and implant them into the same fish
- Rinse, dry, and activate transmitters the day before they are to be implanted. Confirm operational status with the VEMCO tag activator and record the date and time when a tag is activated

## Equipment Set-up

- Remove transport containers from the freezer and prepare them to receive tagged fish
    - Transport containers that leave the hatchery grounds and are delivered to the release site at Durham Ferry must be frozen for at least 24 h prior to being used again for the next tagging operation. These details are outlined in the project Biosecurity Plan
    - When removing containers from the freezer, be sure to consult with the tagging coordinator to ensure that all containers undergo the minimum 24 h of exposure before they are removed and used
- Prepare the transport truck to be able to circulate water through containers
- Select and isolate steelhead for the first tag session
    - Use the raceway crowder to concentrate source fish into a small section of the raceway to facilitate netting
    - Carefully net enough fish for the tag session into perforated 32 gallon cans positioned near the raceway wall
    - Monitor the density of fish in the holding cans to ensure they are not overcrowded and add additional cans as needed
    - Keep the holding cans covered to reduce disturbance and risk of fish loss back to the main raceway area
    - Move the crowder back to allow remaining fish sufficient space to reduce stress
- Water temperatures during all aspects of the tagging operations cannot exceed 2 °C difference from the reference water source (for this study, the raceway where source fish are held)
    - Anesthesia buckets, gravity feed carboys, recovery buckets, and totes should not be filled until near the time they are needed to avoid warming
- Anesthesia bucket and gravity feed carboys should be replaced regularly to prevent increasing water temperatures over time
- Each Aqui-S dose needs to be logged on the reject and Aqui-S/Reject datasheet
- Fill disinfection trays for surgical instruments with Novalsan
- Fill rinse tray with de-ionized or distilled water
- Fill pill boxes containing study tags (acoustic & PIT) with Novalsan and allow at least 20 minutes of contact time with the disinfectant. Following disinfection, thoroughly rinse transmitters in distilled or de-ionized water prior to implantation. Transmitters should only be handled by gloved hands or clean surgical instruments such as forceps following the disinfection step

- Set up and calibrate scale. Set up the measuring board and surgical platform
- Fill gravity feed carboys with water from raceway
    - Add 1 ml Aqui-S 20E to the 10L of water in the anesthesia carboy and briefly agitate to ensure dispersal. Log the Aqui-S amount on datasheet.
    - The freshwater carboy is filled from the raceway and has no chemicals added
- Fill anesthesia bucket to 10 L line with water from source tank or raceway. Add 3.0 or 3.5 mL Aqui-S 20E (check with tagging coordinator for quantity) and briefly agitate to ensure dispersal
- Add a squirt of stress coat. Cover with a lid, and log Aqui-S use on the datasheet
- Adding Aqui-S 20E to any container should be done carefully, with communication between the tagger and the assistant to avoid double dose or no dose outcomes
- Retrieve a 5 gallon fish recovery bucket filled with water from the raceway that has been supersaturated with 130% to 150% oxygen and has stress coat
- Reference the tote summary at the bottom of the tagging datasheet and retrieve clip-on tag ID labels for recovery buckets to be used during tag operations
- Check that a reject bucket has been filled with water from the source tank or raceway and is outfitted with an air bubbler
- Check that a clearly labeled lethal bucket is ready for fish that need to be euthanized. This bucket should be positioned well away from the tagging stations to ensure that it is not confused with an anesthesia bucket
- Start a tag data sheet and a daily fish reject tally and Aqui-S use datasheet for each tagging station to account for fish that are handled but not tagged and for the amount of Aqui-S used
- The tagger should wear clean medical grade exam gloves during all procedures that involve handling fish
- If the assistant will handle disinfected acoustic or PIT tags, then they should also wear gloves or be prepared to use an instrument for handling tags

## Surgical Implantation of the Transmitter

- Food should be withheld from fish for ~24 h prior to surgical implantation of the transmitter

### *Anesthetize Fish and Collect Morphometric Data*

- Net one fish from holding containers in the raceway and place directly into an anesthesia bucket:
    - Work from one holding container until it is empty to avoid stressing fish in all containers
    - Start a stopwatch immediately after the fish has been placed into the anesthesia bucket in order to track how long the fish is exposed to anesthesia
    - Place a lid on the bucket and make available to a tagging station
- Remove the lid after about 1 minute to observe the fish for loss of equilibrium. Keep the fish in the water for an additional 30–60 seconds after it has lost equilibrium
    - Time of sedation should normally be 2–4 minutes, with an average of about 3 minutes
    - If loss of equilibrium takes less than 1 minute or if a fish is in the anesthesia bucket for more than 5 minutes, reject that fish
    - If after sedating a few fish, they are consistently losing equilibrium in more or less time than typical, the anesthesia concentration may need to be adjusted. This should only be done after consultation with the tag coordinator, and should be done in 0.5 ml increments. Concentration changes should be executed for all taggers simultaneously and recorded on the tagging datasheet
- If a fish is unacceptable for tagging, place the fish in the reject bucket, inform the data recorder, and record it on the daily reject tally sheet

- Record fish length, weight, and scale condition:
    - Start “air time” timer when a fish is removed from the anesthesia bucket
    - Transfer the fish to the scale and weigh to the nearest 0.1 g.
        - A fish is acceptable for tagging if it weighs at least 13 g, so that the tag burden does not exceed 5% of the weight of the fish. The transmitters used for this study are VEMCO brand, model V5, which weigh about 0.65 g in air
        - In order to keep study fish in a reasonable size range, representing the average fish reared at the hatchery, fish will not be tagged if they weigh 200 g or more (i.e., fish that weigh 199 g can be tagged, fish at 200 g should be rejected)
    - Transfer the fish to the measuring board. Measure fork length (FL) to the nearest mm
    - Check for any abnormalities and descaling
        - A fish is acceptable for tagging when it lacks deformations such as: non-normal color, gross anatomical deformations, damaged opercula with exposed gill filaments, gross scarring, bleeding scratches, any bulging eyes, gross signs of disease, any fungal infection, or any fin hemorrhaging
        - Scale condition is noted as Normal (N), Partial (P), or Descaled (D) and is assessed on the most compromised side of each fish. The normal scale condition is defined as loss of 5% or less of scales on one side of the fish. Partial descaling is defined as loss of 6–19% of scales on one side of the fish. Fish are classified as descaled if they have lost 20% or more of the scales on one side of the fish, and should not be tagged due to potentially compromised osmoregulatory ability
- Data should be vocally relayed to the recorder and the recorder should repeat the information back to the tagger to avoid miscommunication
- Any fish dropped on the floor should be rejected. Fish dropped from the surgical platform to the table or working surface may be advanced through the tagging process or rejected based on the tagger’s evaluation of the fish.
- The anesthesia containers should be emptied and remixed at regular intervals throughout the tagging operation to ensure the appropriate concentration and to avoid warming
- Always record new doses on the reject and Aqui-S use datasheet
- The gravity feed containers should be monitored for volume and temperature and changed as needed to avoid inadequate volume to complete a surgery and significant warming (difference in water temperature from the raceway cannot exceed 2 °C)

## *Transmitter Implantation*

- Place the fish into the surgical platform ventral side up
- Anesthesia should be administered through the gravity feed tube as soon as the fish is on the surgery platform. Using the valve system, adjust the flow as needed so that the gilling rate of the fish is steady
- Using a scalpel, make an incision approximately 5 mm in length beginning a few mm in front of the pelvic girdle. The incision should be just deep enough to penetrate the peritoneum, avoiding the internal organs. The spleen is generally near the incision point so pay close attention to the depth of the incision
- Use forceps to open the incision to check that you did not damage any internal organs or cause excessive bleeding. If you observe damage or think you damaged an organ, do not implant the tag – reject that fish
- One scalpel blade can be used on about 5–7 fish. If the scalpel is pulling rough or making jagged incisions, it needs to be changed prior to tagging the next fish

- Remove two disinfected tags from the pill box bin for a given fish:
    - Each fish will receive both a PIT tag and an acoustic tag
    - Disinfected tags will only be handled with clean, gloved hands or disinfected surgical instruments
- Gently insert the PIT tag into the body cavity, followed by the acoustic tag. Position the acoustic tag so that it lies directly beneath the incision and the ceramic head is facing forward. This positioning will provide a barrier between the suture needle and internal organs
- Suture the incision with two to three interrupted stitches. Make note on the datasheet when three stitches are used, as two stiches is assumed to be the typical condition
- Transfer the fish from the surgical platform to the appropriate recovery bucket with minimal handling by moving the platform as close as possible to the bucket or using a liner material to lift the fish for transfer
    - Immediately following surgery fish will be held in recovery containers that provide 130% to 150% DO for a minimum of 10 minutes
    - Holding time in recovery containers begins when the last fish is added to the container and will be monitored using a timer
- A recovery bucket should include all fish for each clip-on tag ID for each tagger. Coordinate/communicate the number of fish in the recovery buckets to the tagging assistant/recorder for confirmation. Put the lids back on the buckets and ensure that each bucket has the proper label attached to the handle. Once the correct number of fish are in a single or pair of recovery bucket corresponding to one clip-on tag ID ("tote ID"), have the tagging assistant move the buckets to the tag verification staging area
    - Two recovery buckets are used for each group of 3 fish that will be transferred into one tote for transport to the release site
    - You will only be assigned 1 or 2 fish for "shared" tote IDs. These will require only 1 recovery bucket. Consult the tote summary on the Surgical Tagging datasheet to determine whether totes are shared between taggers and how many fish are assigned to each tote for each tagger. All shared totes will occur as one group, either at the beginning or the end of the tagging session. If the shared totes occur at the end of the tagging session, taggers may be required to wait for other taggers to catch up so all shared totes are started at the same time. This will ensure that no fish endure excessive wait times before being combined with other fish in the tote and placed into the transport truck
- Between surgeries the tagger should replace the instruments that were just used into the disinfectant bath. Each tagger will have at least 3 sets of surgical instruments to rotate to ensure that tools get a thorough soaking in disinfectant between uses. Once disinfected, instruments should be rinsed in distilled or deionized water. Organic debris in the disinfectant bath reduces effectiveness so be sure to change the bath regularly

## Transmitter Verification

- Obtain buckets from tagging crew and start a timer for the 10 minute surgical recovery period
- If the buckets correspond to dummy fish on the Tag and Tote Verification datasheet, replace the bucket tags with the appropriate dummy tote tag
- Gently place a hydrophone into each bucket containing active tags (i.e., not dummy tags)
- Watch the display for tag codes that appear on the monitoring screen. As tag codes are verified check off the tag code on the copy of the Tag and Tote Designation & Verification datasheet provided to the tag verifier

- Once all tags in a bucket have been verified, remove the hydrophone and secure the lid until the recovery period is complete
- Once the 10 minute recovery period is complete, transfer the 2 buckets (un-shared) or 2–3 buckets (shared) to the corresponding 18 gallon tote and confirm that all fish have recovered from anesthesia and are swimming normally. Move the tote to the truck loading area. If after the 10 minute recovery period, tag codes are not verified, continue to attempt verification by separating fish to one per bucket.
- If a tag does not code out, notify the tag coordinator and return the fish to the tagger who performed the surgery for tag extraction. Once the tag is removed, return to tag coordinator for a replacement tag to complete tag implantation

## Loading for Transport

- Begin completion of fish loading, transport, and release data sheets
- Fill hauling tank with water at same temperature as the raceway and make sure the flow through system is established before notifying the tag coordinator that tagging can commence
- Record temperature and DO in the transport tank
- Bring totes to the truck and check each for general fish condition and dead fish before placing into the tank. If a dead fish is found, notify the tag coordinator and return the fish to the tagger who performed the surgery for tag extraction. Once the tag is removed, return it to the tag coordinator so the tag code can be verified and a plan for reuse of the tag can be determined. The original entry should be crossed out in the data sheet with a comment of "mort at loading"
- Call out the number of the bucket to the recorder and the number of fish in the bucket
- Once all buckets have been loaded, confirm that the number of buckets matches the number that should be loaded and that there are no buckets remaining in the tagging area
- In order to ensure that the taggers do not know when they are tagging dummy fish, dummy fish totes have been randomly assigned tote numbers, which will be replaced with dummy tote numbers by the verifier (SX-). This means that that any tote numbers observed by the tagger that contained dummy fish will be skipped in the final tote sequence loaded onto the transport truck
- Secure the tank and tank lid for transport
- Send copies of the tagging session's datasheets with transport crew for data entry

## End of Session Activities

- Validation of tag data and datasheet accuracy
    - Working together, each tagger and assistant team will review the tagging datasheet and the tote summary on the datasheet to verify that all of the transmitters provided for the session were implanted into study fish
    - The steps of the verification process should include completing all fields on the datasheet and a count of the tags provided (as shown on the tote summary) vs. the tag tubes and data rows on the datasheets. In addition, the tagger and assistant should confirm that no tags (either acoustic tags or PIT tags) remain in the pill boxes that were provided them for the tagging session
    - The tubes in the pill box bins should remain in the bins, as assigned. They do not need to be reviewed by the tagger/assistant, just counted
    - Once the validation steps have been completed, both the tagger and the assistant initial the datasheet to document the process
- Collect all datasheets, pill boxes, and tag tubes and hand them in to the tagging coordinator

- Organize tagging solutions, supplies, and surgical instruments to be ready for the next tagging session

## End of Day Clean Up

- At the end of each tagging day, wipe down or spray all surfaces with Virkon or 70% ETOH to disinfect
- Use a toothbrush to remove all large organic debris from instruments, rinse them and dry them to prevent rust
- Return all surgical instruments to the office for autoclaving
- Make surgical tagging solutions as needed to be ready for the next tagging session
- Inventory chemical solutions and tagging supplies (blades and suture)
- Return any soiled rags to the office and have them washed
- Rinse buckets and place upside down to dry
- Turn off oxygen cylinder

## General Fish Handling Reminders

- Anesthesia and freshwater carboys and buckets should be filled just prior to tagging to avoid temperature changes and should be changed often. Check levels of carboys before each surgery to be certain that you will not run out of water during a surgery
- **Use caution and communication** when adding Aqui-S 20E to any container to avoid adding two doses or no doses to the container
- Keep a lid on any bucket or tote that contains fish
- Any fish dropped on the floor should be rejected. If a fish is dropped on the floor after it has been tagged, euthanize the fish, remove the tag, and place it into another fish. Note the event on the datasheet and coordinate with the tagging coordinator
- **Carefully handle buckets**. Try not to bang them around, slam the handles, or otherwise handle in a rough manner as this can stress fish

## Appendix B. Quality Assurance and Quality Control Tagging Inspection

**Table A.1. Results of the quality assurance and quality control tagging inspections. Six measurements were found to be out of compliance of standard operating procedures (highlighted in gray).**

### a. Was the temperature in the anesthesia bucket <2°C different than the raceway?

| *Week* | *Date* | *Temp in Anesthesia Bucket 1 (°C)* | *Temp in Anesthesia Bucket 2 (°C)* | *Temp in Anesthesia Bucket 3 (°C)* | *Temp in Anesthesia Bucket 4 (°C)* | *Raceway Temp 1 (°C)* | *Raceway Temp 2 (°C)* |
|---|---|---|---|---|---|---|---|
| *Tag Retention* | 2/22/2016 | 10.7 | 10.7 | 10.7 | 10.6 | 10.7 | 10.7 |
| *1* | 2/23/2016 | 12.3 | 12.5 | 12.5 | 11.7 | 11.2 | 11.2 |
| | 2/24/2016 | 11.7 | 11.3 | 11.8 | 12.2 | 11.6 | 11.5 |
| | 2/25/2016 | 11.6 | 11.7 | 11.9 | 12 | 11.1 | 11.2 |
| *2* | 3/15/2016 | 11.8 | 11.6 | 11.8 | 12.4 | 11.3 | 11.2 |
| | 3/16/2016 | 11.7 | 11.7 | 12.2 | 12 | 11.1 | 11.2 |
| | 3/17/2016 | 12.1 | 12 | 12.8 | 12.7 | 11.3 | |
| *3* | 4/26/2016 | 13.1 | 12.9 | 12.8 | | 11.3 | 11.3 |
| | 4/27/2016 | 12.2 | 12.3 | 12.3 | | 11.2 | 11.4 |
| | 4/28/2016 | 13.2 | 13.2 | 13.3 | | 11.9 | |

### b. Was the temperature in recovery buckets <2°C different than the raceway?

| *Week* | *Date* | *Pre-surgery Temp in Recovery Bucket 1 (°C)* | *Pre-surgery Temp in Recovery Bucket 2 (°C)* | *Pre-surgery Temp in Recovery Bucket 3 (°C)* | *Post-surgery Temp in Recovery Bucket 1 (°C)* | *Post-surgery Temp in Recovery Bucket 2 (°C)* | *Post-surgery Temp in Recovery Bucket 3 (°C)* |
|---|---|---|---|---|---|---|---|
| *Tag Retention* | 2/22/2016 | 10.5 | | | 10.4 | 10.6 | |
| *1* | 2/23/2016 | 11.5 | | | 12 | 12.1 | **15.0*** |
| | 2/24/2016 | 11.9 | | | 12.3 | 12.7 | |
| | 2/25/2016 | 11.6 | | | 11.9 | 11.9 | |
| *2* | 3/15/2016 | 11.5 | | | 12.1 | 12.2 | |
| | 3/16/2016 | 11.4 | | | 12.1 | 12.2 | |
| | 3/17/2016 | 11.6 | | | 13.2 | 13.1 | |
| *3* | 4/26/2016 | 11.9 | 12 | 11.6 | 12.9 | 13.1 | |
| | 4/27/2016 | 11.7 | | | 12.2 | 12.5 | |
| | 4/28/2016 | 12.1 | | | 12.9 | | |

### c. Was temperature in the gravity feed <2°C different than the raceway?

| *Week* | *Date* | *Temp in Gravity Feed 1 (°C)* | *Temp in Gravity Feed 2 (°C)* | *Temp in Gravity Feed 3 (°C)* | *Temp in Gravity Feed 4 (°C)* |
|---|---|---|---|---|---|
| *Tag Retention* | 2/22/2016 | 10.7 | 11 | 10.8 | |
| *1* | 2/23/2016 | 12.2 | 12.3 | 12.3 | |
| | 2/24/2016 | 12.4 | 12.4 | 12.7 | |
| | 2/25/2016 | 11.7 | 12.2 | 11.9 | |
| *2* | 3/15/2016 | 12.1 | 12.3 | 11.7 | |
| | 3/16/2016 | 12.2 | 12.2 | 12 | |
| | 3/17/2016 | 12.2 | 12.9 | 12.5 | |
| *3* | 4/26/2016 | 13.1 | 13 | 13.2 | 13.2 |
| | 4/27/2016 | 12.2 | 12.2 | 12.8 | 12.4 |
| | 4/28/2016 | 13 | 12.8 | 13.1 | |

### d. Was the DO in pre-surgery recovery buckets within target?

| *Week* | *Date* | *Pre-surgery DO in Recovery Bucket 1 (%)* | *Pre-surgery DO in Recovery Bucket 2 (%)* | *Pre-surgery DO in Recovery Bucket 3 (%)* | *Pre-surgery DO in Recovery Bucket 4 (%)* |
|---|---|---|---|---|---|
| *Tag Retention* | 2/22/2016 | 141.8 | | | |
| *1* | 2/23/2016 | 146 | | | |
| | 2/24/2016 | **152.8** | **152** | 148.1 | 146 |
| | 2/25/2016 | **152.2** | | | |
| *2* | 3/15/2016 | **155.2** | 147 | 148 | |
| | 3/16/2016 | **161** | 136 | 140 | |
| | 3/17/2016 | 137.8 | | | |
| *3* | 4/26/2016 | 141.3 | 146.5 | 149 | |
| | 4/27/2016 | 142.4 | | | |
| | 4/28/2016 | 144 | | | |

### e. Was the DO in post-surgery recovery buckets within target?

| *Week* | *Date* | *Post-surgery DO in Recovery Bucket 1 (%)* | *Post-surgery DO in Recovery Bucket 2 (%)* | *Post-surgery DO in Recovery Bucket 3 (%)* |
|---|---|---|---|---|
| *Tag Retention* | 2/22/2016 | 124 | 105 | |
| *1* | 2/23/2016 | 132.4 | 145 | |
| | 2/24/2016 | 120.4 | 122 | |
| | 2/25/2016 | 110 | 111 | |
| *2* | 3/15/2016 | 129 | 112 | |
| | 3/16/2016 | 129.6 | 127 | |
| | 3/17/2016 | 104.1 | 126.1 | 140.1 |
| *3* | 4/26/2016 | 140 | 115.3 | |
| | 4/27/2016 | 136.1 | 126 | |
| | 4/28/2016 | 144.2 | | |

*Measurement was obtained after QA/QC inspection was complete, when it was noticed that a bucket felt hot to the touch and its temperature was checked.

# Appendix C. Transport Tank Temperatures

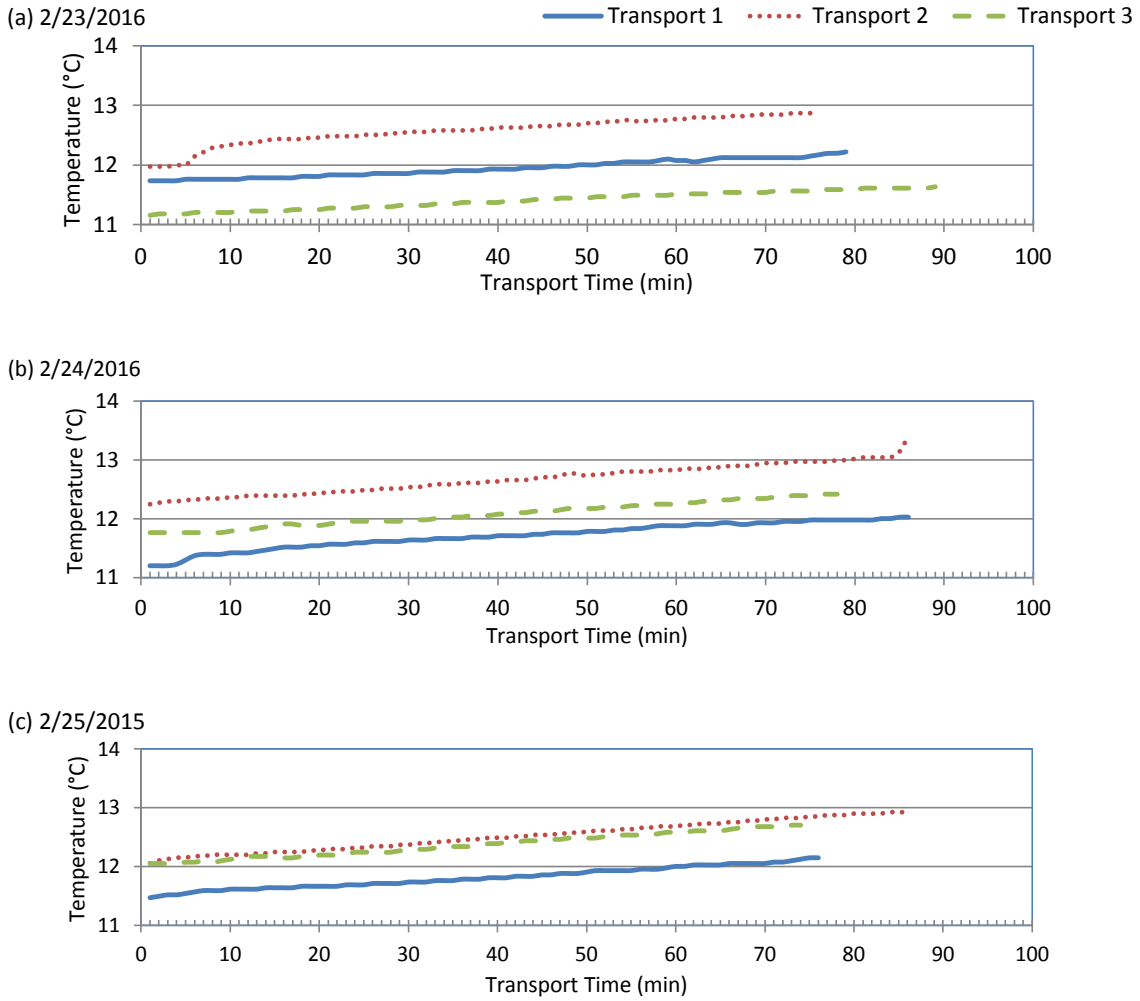


**Figure A.1. Temperature in each of three transport tanks (transport 1, 2, and 3) during the transport of steelhead during week 1 of transport to the Durham Ferry release site.**

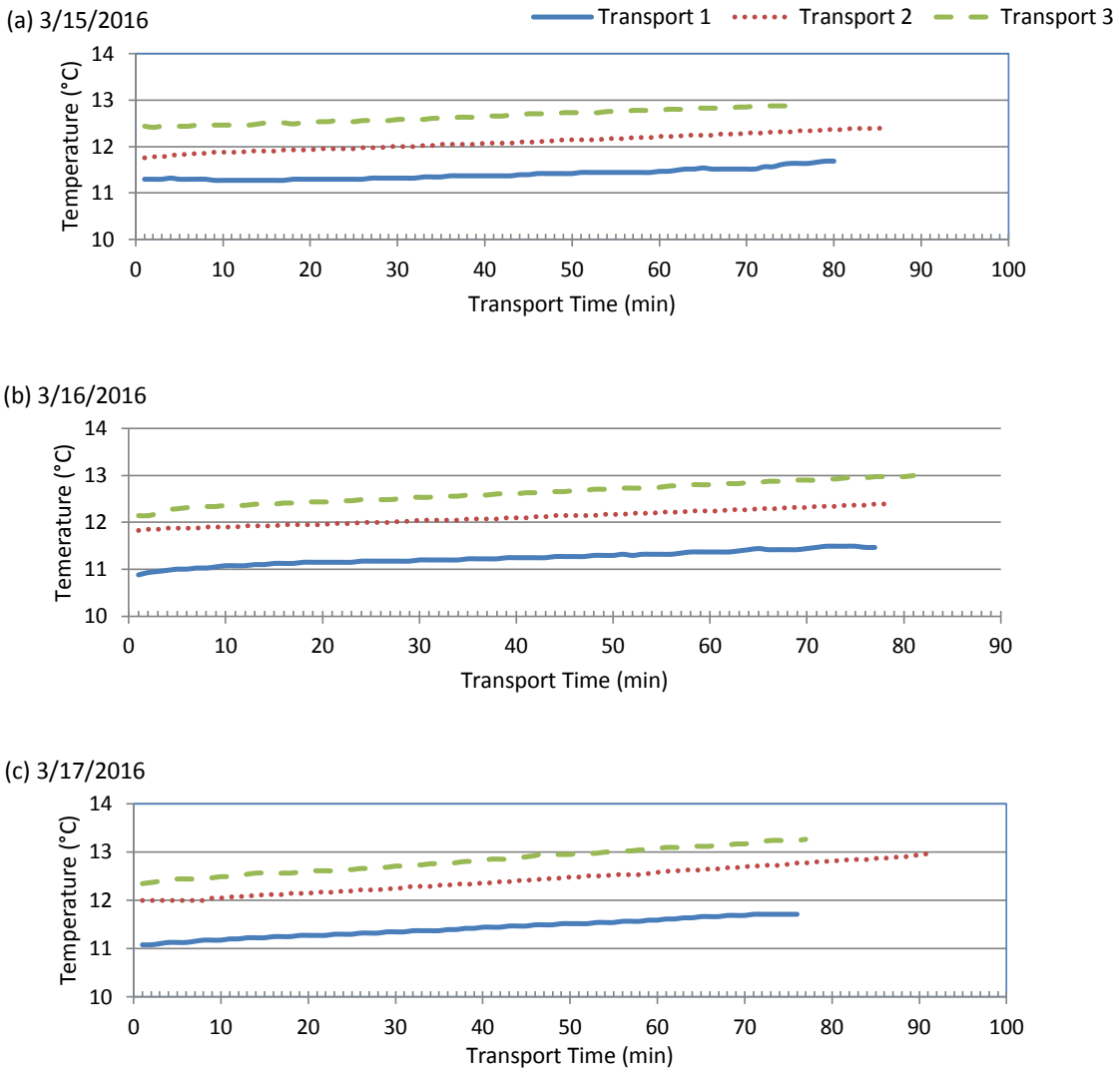


**Figure A.2. Temperature in each of three transport tanks (transport 1, 2, and 3) during the transport of steelhead during week 2 of transport to the Durham Ferry release site.**

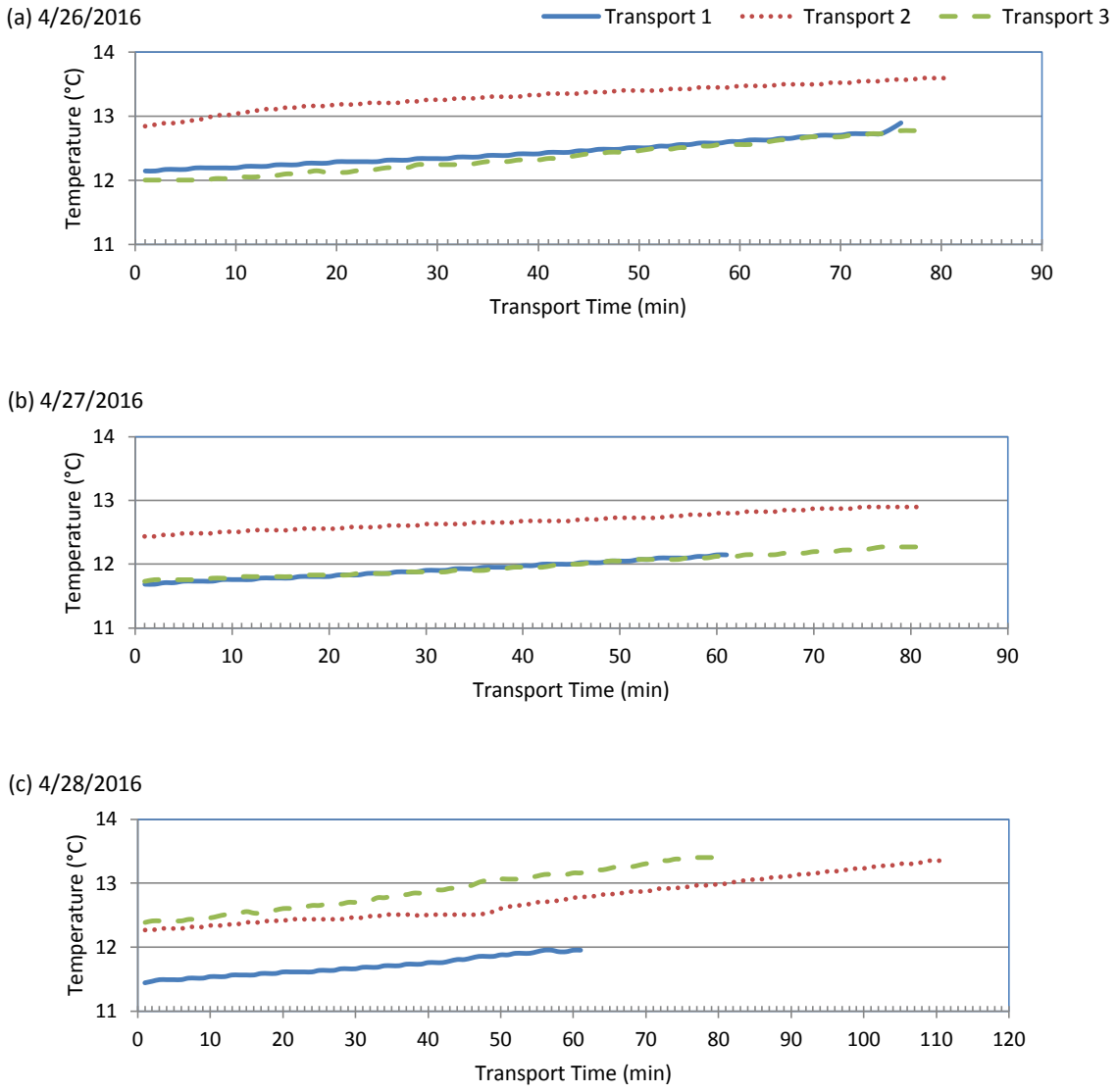


**Figure A.3. Temperature in each of three transport tanks (transport 1, 2, and 3) during the transport of steelhead during week 3 of transport to the Durham Ferry release site.**