Tagging, Releases and Fish Health components of the 2015 Steelhead Survival Study

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Introduction

The Lodi U.S. Fish and Wildlife Service (USFWS) office was responsible for the tagging and release of steelhead, *Oncorhynchus mykiss,* during the 2015 Steelhead Survival Study, the fifth year of the six-year study. The following report includes the methods and implementation results for the tagging, release, tag retention tests, and mobile monitoring components of the project. This report also contains the fish health component of the study conducted by the USFWS California/Nevada Fish Health Center. The complete report from the fish health component of the study is attached as Appendix A. This report is intended to act as a preliminary supplement to a final, comprehensive report combining all aspects of the study, including the fish tagging, release, health and mobile monitoring components of the study as well as the receiver deployment, survival models and statistical analysis components of the study.

Study Fish

Steelhead were obtained and tagged at the Mokelumne River Hatchery (MKRH). Tagging operation equipment was set-up in early February and tag training took place at the hatchery from February 23–27, 2015. The fish weight criteria for steelhead used in the study was a minimum of 13 g and a maximum of 200 g.

Tags (Transmitters) and Activation

Steelhead were tagged with VEMCO V-5 180 kHz transmitters that weighed on average 0.674 g in air (SD = 0.006 g); range (0.655 g to 0.690 g). Tags were 12.7 mm long, 4.3 mm in height, and 5.6 mm wide (http://vemco.com/products/v4-v5-180khz/; accessed June 15, 2015). The percentage of tag weight to body weight averaged 0.5% (SD = 0.2%) for the 954 fish where tag weights were measured, well below the recommended 5%. This average only includes tag weight to body weight ratios of fish from Weeks 2 and 3, since Week 1 tags did not arrive in time to allow for weighing prior to tagging. Consequently, the actual average tag weight to body weight ratio is slightly higher than this estimate since the study fish in Week 1 had lower weights (mean = 126. 4 g, SD = 27.5 g) than Week 2 (mean = 143.4 g; SD = 29.9 g) or Week 3 (mean = 151.4 g; SD = 28.8 g).

Tags were custom programmed with two separate codes; a traditional Pulse Position Modulation (PPM) style coding along with a hybrid PPM/High Residence (HR) coding. The HR component of the coding allows for improved detection in areas where collisions (many tags emitting signals at the same time to the same receiver) are anticipated. 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 8 pings

approximately every 1.2 to 1.5 seconds. The PPM/HR code consisted of 1 PPM code and 8 HR codes (all the same for each individual fish) with 8 pings approximately every 1.2–1.5 seconds.

Tags were soaked in saline water for at least 24 hours prior to tag activation. Tags were activated approximately 24 hours prior to tag implantation using a VEMCO tag activator. Once activated, each tag was placed into a designated pillbox cell, and each pill box was assigned to a surgeon. The time of activation for each tag was recorded to the nearest minute.

Surgeon Training

Steelhead surgeon training was conducted between February 23 and February 27, 2015 at the MKRH. The training was conducted by staff from the U.S. Geological Survey (USGS)'s Columbia River Research Laboratory (CRRL). 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 train or refresh surgeons on acoustic transmitter implantation methods (based on Liedtke 2012; Liedtke et al. 2012). One experienced surgeon (from USFWS) received refresher training and was required to tag a minimum of 35 fish. The two new surgeons (one from USFWS and one from the California Department of Water Resources (CDWR) received more extensive training on surgical techniques and were required to tag a minimum of 75 fish. Training included sessions on knot tying, mock surgery on bananas, tagging dead fish, and finally tagging live fish. Live fish were held overnight and necropsied the next day to evaluate techniques and recovery. A mock tagging session was held on February 27 to practice logistical procedures, establish a flow to operations, and to identify and discuss solutions to potential problems that could occur during tagging (Figure 1).

Tagging

A total of 1584 juvenile steelhead were tagged at MKRH over the course of the 2015 study, including 1,440 study fish and 144 dummy-tagged fish. Fish were tagged over three tagging weeks: March 3–5, March 24–26, and April 21–23, 2015. Each day was further divided into three sessions, each corresponding to one transport truck.

Prior to tagging fish for each transport truck, 17 fish (68 total) were netted from the raceway into each of four 166 L (44 gallon) perforated cans that were located in the raceway near the tagging trailer (Figure 1). Each can contained a minimum of 151 L (40 gallons) of water, which translated into an initial pre-tagging holding density of 15.8 g/L, close to the density recommendation for pre-tagging holding (<15 g/L); Peven et al., 2005). Once the tagging operation began, a few fish were removed from the perforated cans and space recommendations were met for the duration of each tagging session.

During each week of tagging, 480 study fish were tagged and 48 fish were dummy-tagged (Table 1). 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. Dummy-tagged fish differed from study fish in that they were tagged with inactive transmitters and Passive Integrated Transponder (PIT) tags to allow for individual identification. Dummy-tagged fish were held at the release site for 48 hours, after which they were assessed for mortality and euthanized prior to examination for condition or given to the California/Nevada Fish Health Center for fish health and pathogen screening.

During each tagging session, steelhead were surgically implanted with V-5 tags following procedures based on a standard operating procedure (SOP) developed by the Columbia River Research Lab (CRRL) of the United States Geological Survey (USGS). The SOP (Appendix B) 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. The SOP directed all aspects of the tagging operation, and at least one quality assurance check (QA/QC) was made during each tagging day to ensure compliance with the SOP guidance (Table 2).

Fish were taken off feed 24 hours prior to the beginning of each tagging day. Fish were held in the MKRH raceway (Figure 1), and were put into perforated garbage cans immediately before the start of each tagging session in order to reduce stress during capture. To begin the tagging procedure, a fish was carefully netted from the raceway perforated garbage can and placed into a 19 L (5 gallon) anesthesia bucket containing a solution of 34 mg/L AQUI-S 20E until they lost equilibrium. As each fish was removed from the anesthetic solution, fish were examined for criteria that sometimes led to a fish being rejected for use in the study. Rejection criteria included fin, eye, and operculum damage, disease, descaling, size, and injury. Fish were then measured (FL) to the nearest mm and weighed to the nearest 0.1 g. The average weight of the steelhead used in the study was 140.4 g (SD = 30.6 g), range (18.1 g to 199.9 g). The average length of steelhead tagged during the study was 235.5 mm fork length (FL; SD = 22.4 mm; range 97 mm to 287 mm). Average surgery times were 2:33 (m:ss; range: 1:17 to 5:36).

Once the tag was inserted into the fish's body cavity, the fish was placed into a 19 L bucket filled with 10 L of water with high dissolved oxygen concentrations (130–150%) and held for 10 minutes to recover from anesthesia (Figure 2). 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.

Results of the QA/QC check during tagging indicated that water temperatures were once found to be more than 2^{o}C above the raceway temperature in the anesthesia bucket (Table 3). Dissolved oxygen concentration was found to be outside the range established in the SOP (130-150%) three times pre-surgery (two above and one below the range) and four times post-surgery (four below the range) (Table 3). Corrective action was taken immediately in the event of a failed QA/QC inspection to remediate the failed component.

Transmitter Validation

Tagged fish were monitored by hydrophones placed in the recovery buckets to confirm the operational status of each transmitter prior to transportation to the release sites (Figure 3). In 2015, VEMCO provided the study with a VRHR prototype receiver that was capable of detecting and confirming both the PPM and HR code of each tag. Fish containing tags that were unable to be verified were replaced with a new fish and a new tag.

Transport to Release Site

After transmitter validation and a 10 minute recovery period, two buckets of steelhead with water were combined into one 68 L (18 gal) perforated tote held in a same-sized non-perforated tote until it was subsequently loaded into the tank on the transport truck. Generally, three tagged steelhead were in each tote. Totes were perforated starting 15 cm from the bottom to allow water exchange

within the transport truck holding tank. Each tote was covered with a labeled lid that facilitated the placement of each tote into the transport truck, as well as transfer to holding cans at the release site. 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 moving fish and to track smaller groups of individually tagged fish, three specially designed transport tanks were used to move steelhead from the hatchery, where the tagging occurred, to the holding site at Durham Ferry. Each transport tank for steelhead was designed to securely hold 24, 68-L perforated totes. Tanks had an internal frame that held totes in individual compartments to minimize contact between containers and to prevent tipping. Totes were also covered in the transport tanks with stretched cargo nets to assure they did not tip over and 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 each of the tanks during transport (Figure 4). One trip to the holding site for each truck 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 5). The trip from the MKRH to Durham Ferry took approximately 75 minutes.

Water temperature and dissolved oxygen (DO) in the transport tanks was measured using a YSI 85 or ProODO meter prior to loading totes, and after loading totes into transport tanks but before leaving the MKRH (Appendix C). No ice was added to the transport tanks prior to leaving the hatchery as the water in the tanks needed to warm during transport to better match the water temperature at the holding site. As expected during transport, water temperatures in the transport tanks generally increased (Table 4; Appendix D).

The higher water temperatures in the river during the last week of April (Table 4) increased the need to temper the fish due to the water temperature difference between the transport tank and the river. In the earlier transports, we opened the transport tank lids and let the water warm for an hour, but it was determined that for the third transport on April 22, 2015, that process would not be sufficient to raise the water temperature in the transport tank enough to achieve less than a 5^{o} C difference between water in the transport tank and in the river. Thus the protocol for tempering fish for that one group was modified (Appendix C), such that totes were taken out of the transport tank and put into sleeves holding river water and allowing the water to warm outside of the transport tank. A bubbler was added to supply oxygen in each tote, and totes were allowed to sit for 30 minutes to 1 hour prior to putting them into the holding cans in the river. Water temperature was monitored during the 1 hour and rose substantially (to 19.9^{o} C) prior to moving the fish into the holding cans in the river (Table 4). Dummy tagged fish for that transport were treated the same as the study fish.

Transfer to Holding Containers

Once the transport truck arrived at the release site, fish were moved from the transport tank to the river. Soon after the transport truck arrived, the river's water temperature and DO levels were measured (Table 4) 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. Two crew members then unloaded each perforated tote from the transport truck and handed

them to two crew members on the pick-up truck, who placed the perforated totes into the partially filled sleeves in the bed of the pickup truck. Placing the perforated totes in sleeves allowed the water level of the totes to rise above the tote perforations, and provided fish with a mix of river water and hatchery water during the transition between the transport truck and being placed in the river. 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 68-L perforated totes into 166-L (44-gal) perforated 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 steelhead each were emptied into each perforated holding can. Each holding can was covered by a lid and labeled to ensure that fish in each labeled tote were loaded into the correct holding can for later release at the correct time.

There were no mortalities observed after transport to the holding site (Table 4). Water temperature in the river at the release site ranged from 14.9°C to 22.5°C, with an average during the first week of 16.6°C, an average during second week of 19.4°C, and an average during the third week of 20.7°C (Table 4). DO levels ranged between 8.22 and 15.98 mg/L for all measurements in the transport tanks or in the river (Table 4).

Fish Releases

Tagged study fish were held in the perforated holding cans for approximately 24 hours, and were then transported downstream by boat to the release location (Figure 6a). 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 (Figure 6b). 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 originating 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, time, and GPS location of the release were recorded. Mortalities just prior to releases were noted, and their tags were removed. The tags were returned to the tagging location or office to have the individual tag identified. Carcasses were disposed of in the trash.

There were 12 steelhead mortalities that occurred just prior to release in the 2015 6 Year study; (Table 4). Four of them were from the third transport on April 22, 2015 that used the modified tempering protocol (Table 4). However, all the mortalities occurred during the last four days of tagging, which also had the highest average transport and river temperatures and the lowest average DO concentrations (Table 4). These factors likely contributed to the increase in mortalities at the end of the 2015 tagging season.

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. 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 differed from study fish only in that they were also given PIT tags for individual identification. Transporting them to the release site and holding them at the release site was 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 (parameters are described in Table 5). 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.

The dummy-tagged fish from each week (tagged on Day 1 and Day 2; Table 1) were observed to determine if any fish had died or were compromised (vigor; Table 5) 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 5). One group of 24 dummy tagged 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).

After the mortality and condition examinations, 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 six parameters to consider possible tagging effects that could affect survival of study fish (T. Liedtke, personal communication Table 6).

Two of the 72 dummy-tagged steelhead were found dead after being held for 48 hours (Table 7). These mortalities occurred at the end of the tagging season and can likely be attributed to high water temperatures. Of the remaining 70 dummy-tagged fish assessed at the holding site for healing and recovery, ten fish had light body color and five had bulging eyes (Table 7). All had normal gill color (Table 7). All remaining fish were found swimming vigorously with no fin hemorrhaging. The mean scale loss for each group of fish assessed ranged from 7.1 to 26.7%. The mean FL of the three groups of dummy tagged fish ranged from 229.8 to 252.2 mm ().

The mean composite score (0–12) of the six groups of dummy-tagged steelhead ranged between 1.0 and 1.8 (Table 8), indicating that these fish had only a few instances of inferior tagging and healing characteristics. There were minimal signs of tag expulsion (found in one fish in Week 3) or fungus (found in two fish in Week 1). Eleven of the 70 fish assessed had anterior or posterior sutures loose or untied, none of which occurred in both anterior and posterior sutures in a single fish. Nineteen fish displayed poor incision apposition, and 16 fish displayed signs of organ damage. Forty-four of the 72 dummy-tagged fish had poor peritoneal apposition, with the peritoneal cavity only partially closed or open (Table 8). 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 results discussion below). These data indicate that the steelhead used in 2015 study likely did not die from the tagging and transport processes.

Fish Health

The CNFHC performed an assessment of the health and smolt development on dummy tagged fish each week (tagged on Day 3). A suite of pathogen assays and gill Na^+/K^+-Adenosine Triphosphatasease (gill ATPase) activity measurements were performed on cohorts of dummy tagged release groups to help explain any performance and survival differences during the study. Groups of 24 yearling steelhead were sampled on March 7, March 28 and April 25, 2015. These groups were held in the San Joaquin River at the Durham Ferry release site for 48 hours prior to sampling. Fish were euthanized, fork length was recorded, any abnormalities were noted, and tissues were sampled for lab assays. A sample of kidney tissue was aseptically collected and inoculated onto brain-heart infusion agar for bacterial culture (USFWS and AFS-FHS 2014 in Appendix A). A kidney tissue imprint was collected to screen for *Renibacterium salmoninarum* (the bacteria that causes bacterial kidney disease) by fluorescent antibody test (USFWS and AFS-FHS 2014 in Appendix A). Kidney and spleen tissue were collected in 3 fish pooled samples for viral tissue culture (USFWS and AFS-FHS 2014 in Appendix A). Gill tissue was collected to assess smolt development by gill Na^+/K^+- ATPase assay (McCormick 1993 in Appendix A). Samples of gill tissue were collected from all live fish for histopathological examination.

In steelhead health assessment groups, mortality over the 48 hour holding period was low (0-4%) and no significant pathogen infections were detected. Differences in gill Na^+/K^+-ATPase activity were observed between groups; however the differences were small and likely would not affect migration or survival. Only one of the 72 steelhead that were examined for fish health died over the 48 hour holding period. The mortality occurred in the March 28 group in a fish with moderate hemorrhaging at the suture site (Figure A.1 in Appendix A). It was likely this fish died due to complications following tagging. Pale gills were observed on one fish from the March 28 group, and significant scale loss (>50% of body) was observed in two fish from the April 25 sample group. Overall condition of the steelhead groups appeared good with no evidence to suspect survival differences. No obligate bacterial or viral pathogens were detected in the 71 trout sampled. Other bacteria isolates (presumptive environmental contaminates due to field sampling conditions) were observed in 11% (8/71) of fish sampled. Minor parasitic infections were observed in 29% (20/70) of gill tissues examined

by histopathology, with no associated lesion or other signs of impairment. Gill infections included: *Capriniana piscium* (presumptive ID, formerly known as *Trichophrya*) 23% (16/70); cyst-like xenoma due to an unidentified microsporidian 4% (3/70); and an infection of *Ichthyophthirius multifiliis* 1% (1/70). None of these infections were likely to cause differences in survival between steelhead release groups. (See Appendix A for further results on fish health).

Tag Retention Study

On March 2, 2015, 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 B), and held in a raceway at the MKRH. Each of the three surgeons tagged 16 or 17 fish, so all surgeons were equally represented (approximately) in the group of fifty. The average weight of the tag retention fish at the time of tagging was 129.6 g (SD = 27.4 g) and ranged between 68.1 and 195.2 g. The average FL was 226.3 mm (SD = 23.9 mm) and ranged between 137 and 258 mm. A control sample of 15 steelhead (without surgery) was added to the group, which were generally of comparable size to the tagged retention fish. The control fish were not weighed or measured, and were only evaluated for mortality.

On April 7, 2015, after a 36-day hold, each surgeon identified five of his or her tagged fish using PIT tag codes. After a fish was identified, it was euthanized with a lethal dose of Aqui-S 20E and assessed for growth, appearance, and internal and external recovery at the surgery site by the surgeon who tagged the fish. The parameters assessed on the tag retention fish included some of the same initial and composite parameters assessed on the dummy-tagged fish (Table 5 and Table 6). Scale loss was assessed categorically as "normal" (0–5% scale loss; 0), "partial" (6–19% scale loss; 1), or "descaled" (>19% scale loss; 2) instead of estimating the specific numerical percent scale loss as was done for the dummy tag fish assessed at the release site. Tag retention was also assessed. No mortalities occurred among the 50 dummy-tagged or control retention fish held for 36 or 71 days. All dummy-tagged and control fish appeared to be swimming vigorously before the retention assessments.

36 Day Retention

The 15 dummy-tagged fish assessed after 36 days showed an average increase in growth of 25.00 mm (SD = 2.73 mm; Table 9). The amount of descaling increased between Day 0 (mean = 0.13; SD = 0.35) and Day 36 (mean = 0.67; SD = 0.72). All fish showed normal eyes, body and gill color. One fish displayed fin hemorrhaging, while the remaining 14 fish displayed normal fins. Three fish were calculated to have a composite score (0–12) of 0, six fish had a score of 1, four fish had a score of 2, one fish had a score of 3, and one fish had a score of 4 (Table 10). One fish showed an untied anterior suture, two fish showed untied posterior sutures, and two fish showed missing posterior sutures. All remaining sutures were present and normal. Six fish showed a partially open incision (score = 1), and the remaining fish showed good incision apposition. No fish displayed organ damage. Four of the 15 fish displayed fungus, which was all found on the incision. One fish displayed beginning signs of tag expulsion (score = 1). This fish was one of the fish that displayed a partially open incision and peritoneum. This fish was also the smallest fish tagged during the tag retention study, which may have

contributed to the bulging or lateral pressure observed (fish #5 in Table 9). The remaining 14 fish did not display signs of tag expulsion.

71 Day Retention

On May 12, 2015, after a 71-day hold, the remaining 35 tagged fish were euthanized, necropsied and evaluated using some of the same condition factors and surgical parameters. However, peritoneal apposition was not assessed during the 71-day hold assessments. Consequently, the composite score only included six parameters in common with the 36 day hold, and ranged from 0–10 for the 71-day hold assessments. Scale loss was also assessed categorically as "normal" (0–5% scale loss; 0), "partial" (6–19% scale loss; 1), or "descaled" (>19% scale loss; 2) instead of estimating the specific numerical percent scale loss. The 35 dummy-tagged steelhead assessed after 71 days showed an average increase in growth of 55.06 mm (SD = 17.18; Table 11). The descaling scores increased between Day 0 (mean = 0.17, SD = 0.38) and Day 71 (mean = 0.80, SD = 0.72), indicating that the longer holding period resulted in more scale loss. All fish showed normal body and gill color, eyes, and fins. Two fish were calculated to have a composite score (0–10) of 0, 3 fish had a score of 1, 10 fish had a composite score of 2, 16 fish had a composite score of 3, and 4 fish had a score of 4 (Table 12). Twenty-nine fish showed untied but present anterior sutures, and 32 fish showed untied posterior sutures. Twenty-eight of these fish displayed both anterior and posterior sutures untied. The remaining fish showed present and normal sutures, no sutures were missing on any of the 71-day hold fish. Five fish showed a partially open incision, and the remaining 30 fish displayed incisions with good apposition. Fungus was present on 20 of the 35 fish, but the location of the fungus was not specified. None of the fish showed any signs of tag expulsion after the 71 day holding period.

Mobile Telemetry Monitoring

During and after completion of all three weeks of fish releases, we conducted a pilot mobile telemetry monitoring study to locate some of the acoustic transmitters that were still active in the study area. We conducted two mobile monitoring surveys during releases around the Durham Ferry release site, two surveys downstream from the Durham Ferry release site, and one survey around Medford Island (Figure 7). Medford Island was surveyed in order to detect tags from a corresponding juvenile Chinook salmon tagging study that used Medford Island as a release site. The first survey was conducted on April 17, 2015, between the Durham Ferry release site and approximately 1 km downstream of the release site. The second survey was conducted on April 21, between approximately 1 km upstream and 1 km downstream from the Durham Ferry release site. The third survey was conducted on May 8, began at the mouth of the Stanislaus River (at the Two Rivers Marina), and followed the San Joaquin River approximately 17.5 km downstream. The fourth survey was conducted on May 15 between the end of the third survey and just past the head of Old River, for about 13.5 km downstream. The fifth survey was conducted on May 21, started approximately 1 km upstream from Medford Island, went 10.6 km downstream, and ended midway into the eastern side of Frank's Tract. We did not conduct any mobile monitoring in Old River, near the trash racks of the Central Valley Project (CVP) or outside or inside of Clifton Court Forebay.

Surveys were conducted using a VR100 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 during surveys 1 and 2, the time of detection was recorded. For surveys 4 and 5, GPS coordinates were also linked to each code and time of detection. During survey 3, GPS coordinates were only linked to detections observed during the first half of the survey due to equipment failure (the remaining detections are only linked to times). The equipment failure occurred at about the time the boat passed the Durham Ferry release site (Figure 7). After completion of the surveys, the data from the VRHR was downloaded and recorded to ensure that all codes detected were documented.

There were a total of 250 individual tag codes detected by the 2015 Steelhead Survival Study mobile monitoring surveys. Survey 1 detected 7 codes (see Durham Ferry Detail in Figure 7; Table 13). Survey 2 detected a total of 40 codes (Table 13). One of the codes detected during survey 2 was also detected on survey 1 (Table 13). Survey 3 detected a total of 156 tag codes (Figure 8). Twenty-two of the detections during survey 3 occurred between the mouth of the Stanislaus River and the Durham Ferry release site and have corresponding GPS coordinates. The remaining 134 detections occurred after the equipment failure and passing the Durham Ferry release site, and do not have corresponding GPS coordinates. During survey 3 there were also 5 second detections: one code that had been previously detected during survey 1 and four codes that were detected during survey 2. Only one of these detections had corresponding GPS codes, and was detected approximately 0.8 km upstream of the release site. Survey 4 detected 54 codes, one of which was also detected in survey 3 (Figure 9). The second detection during survey 4 was 11.3 km downstream from the Durham Ferry release site. This code's first detection during survey 3 did not have corresponding GPS coordinates. There were no steelhead study fish detected during survey 5.

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 river bottom and not be detected by the hydrophone.

Additionally, mobile monitoring can only provide information regarding the specific areas where surveys were conducted. For example, the CVP trash racks 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. 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. More detections across multiple surveys 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 stationary 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.

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Tables

Table 1. The tagging, transport, holding date and times, and numbers of steelhead released during the 2015 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 Time	Start Holding Time	Total Releaseda (A+B+C+D+E+F)	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 Releaseda	Release E Date, time	Release E Number Releaseda	Release F Date, Time	Release F Number Releaseda	Total Number Dummy Taggeda
3/3/2015	1	1100–1213	1328	162	3/4, 1458	24	3/4, 1857, 1858	28	3/4 2255	2							3
3/3/2015	2	1340–1510	1650	162			3/4, 1858	8	3/4, 2255	22	3/5, 259	24					3
3/3/2015	3	1608–1709	1805	162									3/5, 704	36	3/5, 1101	18	6
3/4/2015	1	1031–1142	1310	162	3/5, 1459	24	3/5, 1859	24	3/5, 2258	6							3
3/4/2015	2	1310–1425	1534	162					3/5, 2258	30	3/6, 259	24					3
3/4/2015	3	1527–1629	1721	162									3/6, 704	36	3/6, 1105	18	6
3/5/2015	1	1033–1152	1250	156	3/6, 1300c	24	3/6, 1858, 1859d	24	3/6, 2257	4							6b
3/5/2015	2	1235–1350	1440	156					3/6, 2257	28	3/7, 255	24					6b
3/5/2015	3	1506–1611	1705	156					3/6, 2257	4			3/7, 703	24	3/7, 1106	24	11b
3/24/2015	1	1055–1220	1315	162	3/25, 1500	24	3/25, 1857, 1858	30									3
3/24/2015	2	1330–1500	1620	162			3/25, 1858	6	3/25, 2257	24	3/26, 255	24					3
3/24/2015	3	1630–1733	1915	162									3/26, 700, 701	36	3/26, 1101	18	6
3/25/2015	1	1055–1212	1345	162	3/26, 1502	24	3/26, 1856	24	3/26, 2257	6							3
3/25/2015	2	1310–1420	1610	162					3/26, 2257	30	3/27, 259	24					3
3/25/2015	3	1545–1647	1840	162									3/27, 659, 700	36	3/27, 1056	18	6
3/26/2015	1	1030–1150	1250	154 (2)	3/27, 1459	24	3/27, 1859	24	3/27, 2300	4							6b
3/26/2015	2	1257–1410	1510	154 (2)					3/27, 2300, 2301	28	3/28, 259	24					6b
3/26/2015	3	1515–1622	1710	154 (2)					3/27, 2300	4			3/28, 700	24	3/28, 1102	22 (2)	12b
4/21/2015	1	1055–1212	1258	160 (2)	4/22, 1458	24	4/22, 1900	30									3e
4/21/2015	2	1330–1445	1527	160 (2)			4/22, 1900	6	4/22, 2257	24	4/23, 259	23 (1)					3e
4/21/2015	3	1637–1739	1920	160 (2)									4/23, 658	35 (1)	4/23, 1100	18	6e
4/22/2015	1	1027–1145	1239	156 (6)	4/23, 1457	24	4/23, 1855	24	4/23, 2259	6							3

Tagging & Transport Date	Transport Tank	Transport Time	Start Holding Time	Total Releaseda (A+B+C+D+E+F)	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 Releaseda	Release E Date, time	Release E Number Releaseda	Release F Date, Time	Release F Number Releaseda	Total Number Dummy Taggeda
4/22/2015	2	1220–1345	1439	156 (6)					4/23, 2259	30	4/24, 255	22 (2)					3
4/22/2015	3	1500–1602	1805	156 (6)									4/24, 657	32 (4)	4/24, 1102	18	6
4/23/2015	1	1136–1251	1430	154 (2)	4/24, 1503	24	4/24, 1858	24	4/24, 2258	4							6b
4/23/2015	2	1400–1530	1630	154 (2)					4/24, 2258	28	4/25, 305	22 (2)					6b
4/23/2015	3	1644–1745	1840	154 (2)					4/24, 2258	4			4/25, 700	24	4/25, 1100	24	12b

[a] The total planned number of fish were not released due to study fish mortalities before release. The total numbers of mortalities encountered after transport or pre-release are entered in parentheses.

[b] Fish given to Ken Nichols (CNFH) for fish health assessment

[c] Fish were inadvertently released 2 hours early. Planned release time was at 1500

[d] Fish were released from shore due to dead boat battery

[e] Two dummy tagged fish were found dead in the holding can with 12 fish in it, just prior to assessment. It was not clear which transport tank the mortalities were in, thus all groups from that can are footnoted.

Table 2. Parameters and criteria assessed during the quality assurance and quality control tagging inspections of the 2015 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? (130-150%)
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. Quality Assurance/Quality Control Checks for steelhead during the 2015Steelhead Survival Study. Highlighted values are those falling outside accepted criteria (Table 2). No QA/QC was completed on March 3 2015. Prior to each days tagging, oxygen tank settings were adjusted to reflect dissolved oxygen levels between 130 and 150 percent in recovery buckets entering the tagging area. Values under the criteria are highlighted in yellow, and those over the criteria are highlighted in pink.

DATE	TIME	RACEWAY TEMP (^{O}C)	ANESTHESIA BUCKET TEMP (^{O}C)	DIFF. FROM RACEWAY (^{O}C)	GRAVITY FEED TEMP (^{O}C)	DIFF. FROM RACEWAY (^{O}C)	PRE-RECOVERY BUCKET DO (%)	POST-RECOVERY BUCKET DO (%)	PRE-RECOVERY BUCKET TEMP (^{O}C)	POST-RECOVERY BUCKET TEMP (^{O}C)	PRE-DIFF. FROM RACEWAY (^{O}C)	POST-DIFF. FROM RACEWAY (^{O}C)
3/3/2015	1450	12.3	12.5	0.2	12.5	0.2	133.5		12.4		0.1	
3/4/2015	1149	12.2	12.6	0.4	12.4	0.2	171	125.5	12.4	12.4	0.2	0.2
3/24/2015	1223	12.8	13.2	0.4	13.4	0.6	144.5	140.1	13.1	13.3	0.3	0.5
3/25/2014	1145	12.7	13.3	0.6	13	0.3	126	132.2	13.1	13.2	0.4	0.5
3/26/2015	945	12.5	12.7	0.2	12.7	0.2	153.8	138.4	12.8	12.9	0.3	0.4
4/21/2015	1139	13.7	14.4	0.7	15	1.3	139.6	123.9	14.2	14.3	0.5	0.6
4/22/2015	1050	13.7	15.4	1.7	15.7	2	133.2	129.4	14.2	15.1	0.5	1.4
4/23/2015	1045	13.9	16.1	2.2	15.9	2	141.9	128.2	14.2	15.2	0.3	1.3
AVERAGE		13	13.8	0.8	13.8	0.9	142.9	131.1	13.3	13.8	0.3	0.7
SD		0.7	1.4	0.7	1.5	0.8	14.1	6.2	0.8	1.1	0.1	0.5

Table 4: Water temperature and dissolved oxygen (DO) in the transport tank after loading, 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 2015 Steelhead Survival Study.

TRANSPORT DATE	TANK #	LOADING TIME	TEMP (˚C) TANK PRIOR TO LOADING	DO (MG/L) TANK PRIOR TO LOADING	TEMP (˚C) TANK AFTER LOADING	DO (MG/L) TANK AFTER LOADING	TEMP (˚C) TANK AFTER TRANSPORT (TAT) JUST PRIOR TO UNLOADING	DO (MG/L) TAT JUST PRIOR TO UNLOADING	MORTALITIES AFTER TRANSPORT	RIVER TEMP (˚C)	RIVER DO (MG/L)	MORTALITIES JUST PRIOR TO RELEASE	WATER TEMP DIFFERENCE BETWEEN TAT AND RIVER (˚C)
3/3/2015	1	907	12.1	11.47	12.3	11.2	13.4	15.3	0	14.9	11.88	0	1.5
3/3/2015	2	1140	12.7	11.19	12.7	11.54	13.8	11.21	0	16.2	13.47	0	2.4
3/3/2015	3	1417	13.2	10.88	12.9	11.33	13.3	11.69	0	16.2	12.94	0	2.9
3/4/2015	1	859	11.8	13.34	12.2	13.22	12.9	12.71	0	16.5	13.88	0	3.6
3/4/2015	2	1110	13	11.29	12.7	11.85	14.9	11.65	0	17.6	15.55	0	2.7
3/4/2015	3	1347	13.2	11	13.5	11.21	13.3	11.88	0	18.1	15.98	0	4.8
3/5/2015	1	857	12.3	13.42	12.4	13.33	13	12.5	0	15.7	13.94	0	2.7
3/5/2015	2	1105	13.4	10.97	13.3	11.69	14.2	11.73	0	17.1	13.9	0	2.9
3/5/2015	3	1316	13.2	11.16	13.4	11.38	14.3	11.41	0	17.5	14.05	0	3.2
AVG			12.8	11.64	12.8	11.86	13.7	12.23		16.6	13.95		3
3/24/2015	1	900	12.3	11.74	12.5	11.6	13.5	11.15	0	18.2	10.43	0	4.7
3/24/2015	2	1200	13.1	11.76	13.5	11.79	14.7*	12.72*	0	19.7	11.25	0	5
3/24/2015	3	1422	13.8	11.05	14.2	11.25	15.3*	10.55*	0	20.1	11.08	0	4.8
3/25/2015	1	910	12.3	11.65	12.7	11.49	13.5	11.16	0	17.8	11.49	0	4.3
3/25/2015	2	1140	13.4	11.5	14	12.97	15.3	10.92	0	20	11.78	0	4.7
3/25/2015	3	1416	13.8	11.77	14.8	11.62	16.1*	11.05*	0	21.2	12.2	0	5.1
3/26/2015	1	900	12.4	11.7	12.8	11.75	14	11.11	0	18	10.61	0	4
3/26/2015	2	1120	13.8	11.17	14.8	12.56	16.2	12.31	0	19.7	10.79	0	3.5
3/26/2015	3	1343	14	10.61	15.2	10.88	16.4	11.22	0	20	11.34	2	3.6
AVG			13.2	11.44	13.8	11.77	14.9	11.5		19.4	11.22		4.5
4/21/2015	1	920	13.3	11.5	13.7	11.38	14.5	10.89	0	19.1	8.23	0	4.6
4/21/2015	2	1145	14.2	11.67	14.7	11.4	16.1	10.54	0	20.5	8.45	1	4.4
4/21/2015	3	1505	14.4	10.69	14.5	10.76	15.3*	9.58*	0	20.8	8.62	1	5.5

TRANSPORT DATE	TANK #	LOADING TIME	TEMP (˚C) TANK PRIOR TO LOADING	DO (MG/L) TANK PRIOR TO LOADING	TEMP (˚C) TANK AFTER LOADING	DO (MG/L) TANK AFTER LOADING	TEMP (˚C) TANK AFTER TRANSPORT (TAT) JUST PRIOR TO UNLOADING	DO (MG/L) TAT JUST PRIOR TO UNLOADING	MORTALITIES AFTER TRANSPORT	RIVER TEMP (˚C)	RIVER DO (MG/L)	MORTALITIES JUST PRIOR TO RELEASE	WATER TEMP DIFFERENCE BETWEEN TAT AND RIVER (˚C)
4/22/2015	1	900	13.3	11.43	13.7	11.16	14.2	10.67	0	18.5	8.22	0	4.3
4/22/2015	2	1100	14.1	11.74	14.7	11.78	17.1	10.37	0	20.5	8.34	2	3.4
4/22/2015	3**	1339	14.6	10.87	15.3	10.88	16.4/19.9**	10.85/NA	0	21.7	8.37	4	1.8
4/23/2015	1	1010	13.3	11.53	14.3	11.33	15.7	10.45	0	20.7	8.45	0	5
4/23/2015	2	1230	15.1	11.66	16.9	11.47	18.7	9.92	0	21.9	8.64	2	3.2
4/23/2015	3	1507	16.4	10.44	16.9	10.6	17.7	10.86	0	22.5	8.73	0	4.8
AVG			14.3	11.28	15	11.2	16.2	10.56		20.7	8.45		4.5

* Fish were held in transport tank for up to 1 hour to let water temperature increase before transferring them to the river. Reported values are water temperature and dissolved oxygen levels just prior to transfer

**Fish in this tank were tempered outside of transport tank for one hour prior to transfer to the river. At arrival transport water temperature was 16.4 ^{0}C but was 19.9 ^{0}C just prior to loading fish into holding cans in the river. All fish were alive when placed into holding cans.

Table 5: External characteristics assessed for steelhead smolt condition and short-term survival during the 2015 Steelhead Survival Study. Scale loss was also assessed as the percent of the total scale loss on both sides of the fish.

Character	Normal (0)	Abnormal (1)
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, motionless (prior to anesthesia) or mortality

Table 6. The parameters assessed during the necropsy of dummy-tagged and 36 day tag retention steelhead during the 2015 Steelhead Survival Study. The score from each of the seven 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 7: Results of external criteria assessed on dummy-tagged steelhead after being held for 48 hours at the Durham Ferry release site during the 2015 Steelhead Survival Study. Criteria are defined in Table 5. All examinations occurred at 1130 hours. Fish that died did not have condition characteristics assessed.

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 Hemorr-haging*	*Normal Eye Quality*	*Normal Gill Color*
1	3/5/2015	N/A	237.6 (15.7)	0/12	26.7 (18.1)	10/12	12/12	12/12	12/12
	3/6/2015	16.7; 10.83	229.8 (11.9)	0/12	27.1 (17.1)	10/12	12/12	11/12	12/12
				0/24*					
2	3/26/2015	17.6; 10.15	252.2 (12.2)	0/12	22.1 (7.5)	9/12	12/12	10/12	12/12
	3/27/2015	16.2; 10.81	238.4 (13.9)	0/12	7.1 (3.3)	12/12	12/12	12/12	12/12
				0/24*					
3	4/23/2015	19.9; 8.44	239.2 (16.7)	2/12	12.5 (11.1)	9/10	10/10	9/10	10/10
	4/24/2015	20.2; 7.77	252.0 (11.5)	0/12	16.7 (9.4)	10/12	12/12	11/12	12/12
				0/24*					

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

Table 8: Results of characteristics assessed on dummy-tagged steelhead after being held at the release site for 48 hours before necropsy during the 2015 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 seven parameters; 0–12) was calculated to consider possible compounding effects of compromised parameters on survival. All examinations occurred at 1130 hours.

TAGGING WEEK (N)	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 (12)	3/5/2015	0 (0)	0 (0)	0.2 (0.6)	0.4 (0.8)	0.8 (0.6)	0.4 (0.5)	0.1 (0.3)	1.8 (1.3)
1 (12)	3/6/2015	0 (0)	0 (0)	0.3 (0.5)	0.6 (0.7)	0.5 (0.7)	0.3 (0.5)	0.1 (0.3)	1.8 (1.2)
2 (12)	3/26/2015*	0 (0)	0.1 (0.3)	0 (0)	0.2 (0.4)	0.7 (0.5)	0.2 (0.4)	0 (0)	1.2(0.9)
2 (12)	3/27/2015	0 (0)	0 (0)	0.2 (0.4)	0.4 (0.7)	0.8 (0.7)	0.3 (0.5)	0 (0)	1.8 (1.1)
3 (12)	4/23/2015**	0.1 (0.3)	0.0 (0)	0.2 (0.4)	0.2 (0.4)	1.0 (0.8)	0.0 (0.0)	0 (0)	1.5 (1.0)
3 (12)	4/24/2015	0 (0)	0 (0)	0.2 (0.4)	0.2 (0.4)	0.6 (0.5)	0.1 (0.3)	0 (0)	1.0 (0.7)

* Only 11 fish were assessed for surgical parameters.
** Only 10 fish were assessed for surgical parameters.

Table 9: Results of the external criteria assessed after holding tag retention steelhead for 36 days after tagging during the 2015 Steelhead Survival Study. The weight and fork length (FL) of each fish was measured, and 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). See Table 5 for explanation of criteria. A ranking of 0 is "normal" and a ranking of 1 is "abnormal". Weight was not retaken on day 36.

FISH #	WEIGHT DAY 0 (G)	FL DAY 0 (MM)	FL DAY 36 (MM)	SCALES DAY 0 (0/1/2)	SCALES DAY 36 (0/1/2)	COLOR	FIN HEMORRHAGE	EYES	GILL COLOR
1	151.8	246	271	0	2	0	0	0	0
2	180.0	257	281	0	0	0	0	0	0
3	128.5	233	264	1	0	0	0	0	0
4	135.2	239	264	0	0	0	0	0	0
5	74.7	194	225	0	1	0	0	0	0
6	161.9	250	271	0	1	0	0	0	0
7	155.4	248	273	1	1	0	1	0	0
8	135.5	243	268	0	0	0	0	0	0
9	155.0	254	278	0	1	0	0	0	0
10	110.2	225	252	0	1	0	1	0	0
11	182.0	234	257	0	0	0	0	0	0
12	107.9	199	223	0	2	0	0	0	0
13	109.7	208	230	0	0	0	0	0	0
14	131.1	214	237	0	1	0	0	0	0
15	100.2	200	225	0	0	0	0	0	0
MEAN:	134.61	229.60	254.60	0.13	0.67	0.00	0.13	0.00	0.00
SD:	30.41	21.51	20.97	0.35	0.72	0.00	0.35	0.00	0.00

Table 10. Results of seven recovery parameters assessed on tag retention steelhead held for 36 days after tagging. The parameters, as outlined in Table 6, 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 seven parameters; 0–12) was calculated to consider possible compounding effects of compromised parameters on survival.

FISH #	ANTERIOR SUTURE (0-2)	POSTERIOR SUTURE (1-2)	INCISION APPOSITION (0/1/2)	PERITONEAL APPOSITION (0/1/2)	ORGAN DAMAGE (0/1)	FUNGUS PRESENT? (0/1 [LOCATION])	SIGNS OF TAG EXPULSION (0/1/2)	COMPOSITE SCORE (0–12)
1	0	0	1	0	0	0	0	1
2	0	0	0	1	0	0	0	1
3	0	0	1	0	0	0	0	1
4	0	0	1	0	0	0	0	1
5	0	0	1	1	0	0	1	3
6	0	0	1	0	0	1 (Incision)	0	2
7	0	0	0	1	0	1 (Incision)	0	2
8	1	0	0	0	0	0	0	1
9	0	1	0	0	0	0	0	1
10	0	1	0	0	0	1 (Incision)	0	2
11	0	2	1	0	0	1 (Incision)	0	4
12	0	0	0	0	0	0	0	0
13	0	2	0	0	0	0	0	2
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0
MEAN	0.07	0.4	0.4	0.2	0	0	0.07	1.4
SD	0.26	0.74	0.51	0.41	0	0	0.26	1.12

Table 11. Results of the external criteria assessed after holding tag retention steelhead for 71 days after tagging. The weight and fork length (FL) of each fish was measured, and 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). See Table 5 for explanation of criteria. A ranking of 0 is "normal" and a ranking of 1 is "abnormal". Weight was not retaken on day 71.

FISH #	WEIGHT DAY 0 (G)	FL DAY 0 (MM)	FL DAY 71 (MM)	SCALES DAY 0 (0/1/2)	SCALES DAY 71 (0/1/2)	COLOR	FIN HEMORRHAGE	EYES	GILL COLOR
16	115.4	232	275	0	1	0	0	0	0
17	107.7	206	274	0	1	0	0	0	0
18	129.8	246	302	0	1	0	0	0	0
19	121.6	237	290	1	0	0	0	0	0
20	144.6	247	296	0	0	0	0	0	0
21	114.5	222	276	0	1	0	0	0	0
22	161.1	252	301	0	1	0	0	0	0
23	126.3	137	270	1	1	0	0	0	0
24	145.6	248	286	0	1	0	0	0	0
25	80.6	203	251	0	0	0	0	0	0
26	68.1	177	215	0	0	0	0	0	0
27	120.5	206	269	0	1	0	0	0	0
28	119.7	208	266	0	1	0	0	0	0
29	141.0	242	286	0	1	0	0	0	0
30	132.2	243	288	1	0	0	0	0	0
31	195.2	258	318	0	0	0	0	0	0
32	122.4	218	284	0	2	0	0	0	0
33	157.2	253	310	0	2	0	0	0	0
34	114.3	221	259	0	0	0	0	0	0
35	111.6	200	276	0	1	0	0	0	0
36	152.2	253	295	0	1	0	0	0	0
37	100.6	197	249	0	0	0	0	0	0
38	167.7	255	303	0	1	0	0	0	0
39	117.9	228	274	0	0	0	0	0	0
40	79.2	203	255	1	2	0	0	0	0
41	118.3	228	269	0	0	0	0	0	0
42	123.9	216	290	0	1	0	0	0	0
43	150.5	224	290	1	2	0	0	0	0
44	148.5	246	285	0	2	0	0	0	0
45	120.2	238	292	0	2	0	0	0	0

FISH #	WEIGHT DAY 0 (G)	FL DAY 0 (MM)	FL DAY 71 (MM)	SCALES DAY 0 (0/1/2)	SCALES DAY 71 (0/1/2)	COLOR	FIN HEMORRHAGE	EYES	GILL COLOR
46	131.9	215	280	0	0	0	0	0	0
47	160.8	243	302	1	1	0	0	0	0
48	144.6	223	289	0	1	0	0	0	0
49	104.3	221	264	0	0	0	0	0	0
50	110.1	225	269	0	0	0	0	0	0
MEAN	127.43	224.89	279.94	0.17	0.80	0.00	0.00	0.00	0.00
SD	26.13	24.98	20.00	0.38	0.72	0.00	0.00	0.00	0.00

Table 12. Results of six parameters assessed on tag retention steelhead held for 71 days after tagging (peritoneal apposition was not assessed for this necropsy). The parameters, as outlined in Table 6, included presence of the (1) anterior and (2) posterior suture (0 = present, 1 = untied, 2 = not present), (3) incision apposition (0 = closed, good apposition, 1 = partial gape or overlap, 2 = completely open [>75%]), (4) organ damage (0 = none, 1 = yes), (5) signs of tag expulsion (0 = none, 1 = some signs present, 2 = tag expelled or partially expelled), and (6) whether fungus was present (0 = no, 1 = yes). A composite score (the sum of the six parameters; 0–10) was calculated to consider possible effects of tagging parameters on survival. The location of fungus was not provided.

FISH #	ANTERIOR SUTURE (0-2)	POSTERIOR SUTURE (0-2)	INCISION APPOSITION (0/1/2)	ORGAN DAMAGE (0/1)	FUNGUS PRESENCE (0-1)	SIGNS OF TAG EXPULSION (0-2)	COMPOSITE SCORE (0–10)
16	0	0	0	0	0	0	0
17	1	1	1	0	0	0	3
18	1	1	0	0	0	0	2
19	1	1	0	0	1	0	3
20	1	1	0	0	0	0	2
21	1	1	0	0	0	0	2
22	1	1	0	0	0	0	2
23	1	0	0	0	1	0	2
24	1	1	0	0	1	0	3
25	1	1	0	0	1	0	3
26	0	0	0	0	0	0	0
27	0	1	0	0	0	0	1
28	1	1	1	0	0	0	3
29	1	1	0	0	1	0	3
30	1	1	0	0	0	0	2
31	1	1	0	0	0	0	2
32	1	1	1	0	1	0	4
33	1	1	0	0	0	0	2
34	1	1	0	0	1	0	3
35	0	1	0	0	0	0	1
36	1	1	0	0	1	0	3
37	1	1	1	0	1	0	4
38	1	1	0	0	0	0	2
39	1	1	0	0	1	0	3
40	1	1	0	0	1	0	3
41	1	1	0	1	1	0	4
42	1	1	0	0	1	0	3
43	1	1	0	0	1	0	3
44	0	1	0	0	0	0	1
45	0	1	0	0	1	0	2
46	1	1	1	0	1	0	4
47	1	1	0	0	1	0	3
48	1	1	0	0	1	0	3

FISH #	ANTERIOR SUTURE (0-2)	POSTERIOR SUTURE (0-2)	INCISION APPOSITION (0/1/2)	ORGAN DAMAGE (0/1)	FUNGUS PRESENCE (0-1)	SIGNS OF TAG EXPULSION (0-2)	COMPOSITE SCORE (0–10)
49	1	1	0	0	1	0	3
50	1	1	0	0	1	0	3
MEAN	0.83	0.91	0.14	0.03	0.57	0	2.49
SD	0.38	0.28	0.36	0.17	0.5	0	1.01

Table 13: Tag detections in mobile monitoring for the first and second surveys, conducted just downstream and upstream of the release site on April 17 and April 21, respectively. Some tags were detected multiple times (second detection date).

FIRST MOBILE DETECTION DATE	SECOND DETECTION DATE	TAG CODE	DATE FISH TAGGED	RELEASE DATE AND TIME	LOCATION OF DETECTION
4/17/2015		21801	3/3/2015	3/4/2015 18:58	Downstream of Release site
4/17/2015		22649	3/3/2015	3/5/2015 11:01	Downstream of Release site
4/17/2015	4/21/2015	25031	3/4/2015	3/5/2015 22:58	Downstream of Release site
4/17/2015		25244	3/4/2015	3/6/2015 11:05	Downstream of Release site
4/17/2015		33387	3/24/2015	3/26/2015 2:55	Downstream of Release site
4/17/2015		33430	3/24/2015	3/26/2015 7:00	Downstream of Release site
4/17/2015		33626	3/25/2015	3/27/2015 2:59	Downstream of Release site
4/21/2015		21029	3/3/2015	3/4/2015 14:58	Upstream of Release Site
4/21/2015		21278	3/3/2015	3/4/2015 18:57	Upstream of Release Site
4/21/2015		22792	3/3/2015	3/5/2015 7:04	Upstream of Release Site
4/21/2015		22984	3/3/2015	3/5/2015 7:04	Upstream of Release Site
4/21/2015		23991	3/4/2015	3/5/2015 22:58	Upstream of Release Site
4/21/2015	5/8/15, 11:53	26556	3/5/2015	3/6/2015 18:59	Upstream of Release Site
4/21/2015		26964	3/5/2015	3/6/2015 22:57	Upstream of Release Site
4/21/2015		27097	3/5/2015	3/6/2015 22:57	Upstream of Release Site
4/21/2015		27195	3/5/2015	3/7/2015 2:55	Upstream of Release Site
4/21/2015		27546	3/5/2015	3/7/2015 2:55	Upstream of Release Site
4/21/2015		27702	3/5/2015	3/7/2015 2:55	Upstream of Release Site
4/21/2015		33334	3/24/2015	3/25/2015 18:57	Upstream of Release Site
4/21/2015	5/8/2015	33361	3/24/2015	3/25/2015 18:58	Upstream of Release Site
4/21/2015		33428	3/24/2015	3/26/2015 7:00	Upstream of Release Site
4/21/2015		33755	3/26/2015	3/27/2015 18:59	Upstream of Release Site
4/21/2015		33776	3/26/2015	3/28/2015 2:59	Upstream of Release Site
4/21/2015		33912	3/26/2015	3/28/2015 11:02	Upstream of Release Site
4/21/2015		22270	3/3/2015	3/5/2015 2:59	Downstream of Release Site

FIRST MOBILE DETECTION DATE	SECOND DETECTION DATE	TAG CODE	DATE FISH TAGGED	RELEASE DATE AND TIME	LOCATION OF DETECTION
4/21/2015		22334	3/3/2015	3/5/2015 2:59	Downstream of Release Site
4/21/2015		22448	3/3/2015	3/5/2015 7:04	Downstream of Release Site
4/21/2015		23445	3/4/2015	3/5/2015 14:59	Downstream of Release Site
4/21/2015		25031	3/4/2015	3/5/2015 22:58	Downstream of Release Site
4/21/2015		25058	3/4/2015	3/5/2015 22:58	Downstream of Release Site
4/21/2015	5/8/15, 12:54	25174	3/4/2015	3/6/2015 11:05	Downstream of Release Site
4/21/2015		25474	3/4/2015	3/6/2015 7:04	Downstream of Release Site
4/21/2015		25969	3/4/2015	3/6/2015 11:05	Downstream of Release Site
4/21/2015		27731	3/5/2015	3/7/2015 2:55	Downstream of Release Site
4/21/2015		27742	3/5/2015	3/7/2015 7:03	Downstream of Release Site
4/21/2015		27769	3/5/2015	3/7/2015 7:03	Downstream of Release Site
4/21/2015		33167	3/24/2015	3/25/2015 18:58	Downstream of Release Site
4/21/2015		33194	3/24/2015	3/25/2015 15:00	Downstream of Release Site
4/21/2015		33420	3/24/2015	3/26/2015 2:55	Downstream of Release Site
4/21/2015		33510	3/24/2015	3/26/2015 11:01	Downstream of Release Site
4/21/2015		33536	3/25/2015	3/26/2015 15:02	Downstream of Release Site
4/21/2015		33616	3/25/2015	3/27/2015 2:59	Downstream of Release Site
4/21/2015		33633	3/25/2015	3/27/2015 2:59	Downstream of Release Site
4/21/2015		33695	3/25/2015	3/27/2015 10:56	Downstream of Release Site
4/21/2015	5/8/2015	33737	3/26/2015	3/27/2015 14:59	Downstream of Release Site
4/21/2015		33767	3/26/2015	3/27/2015 23:00	Downstream of Release Site
4/21/2015		33901	3/26/2015	3/28/2015 7:00	Downstream of Release Site

Figures

Figure 1. Steelhead were held in perforated garbage cans in the raceway before surgery during the 2015 Steelhead Survival Study. The surgical procedure began by netting a fish from a holding can into an anesthesia bucket. Colored balls were used as mock fish during simulated tagging during the training week. Photo credit: USFWS.

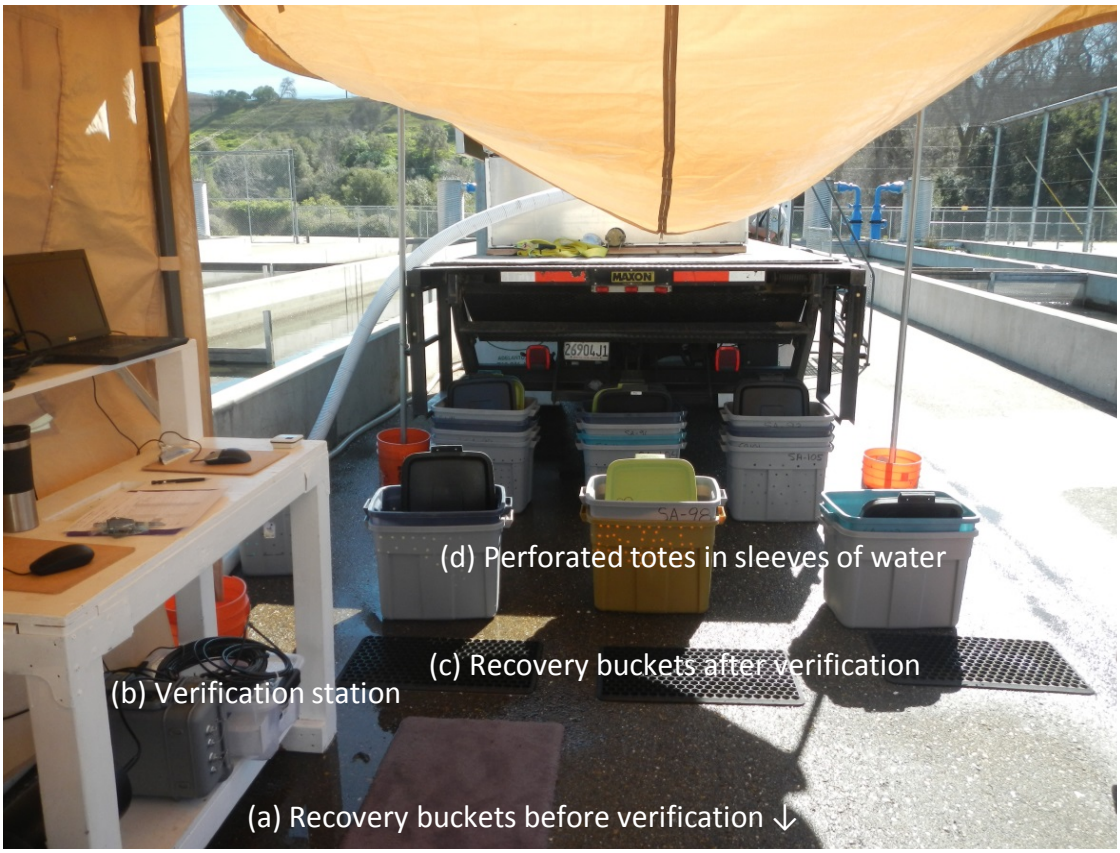

Figure 2. The recovery bucket staging area at the Mokelumne River Hatchery during the 2015 Steelhead Survival Study. Recovery buckets, which each contained 1–2 tagged fish, were placed to the left of the staging area immediately after surgery (a). Their tags were validated (b), and the recovery buckets were moved to the right side of the staging area to await completion of 10 minutes in the oxygenated recovery buckets (c). After recovery, 2–3 buckets were combined into a perforated tote in a sleeve of water (d). Photo credit: USFWS.

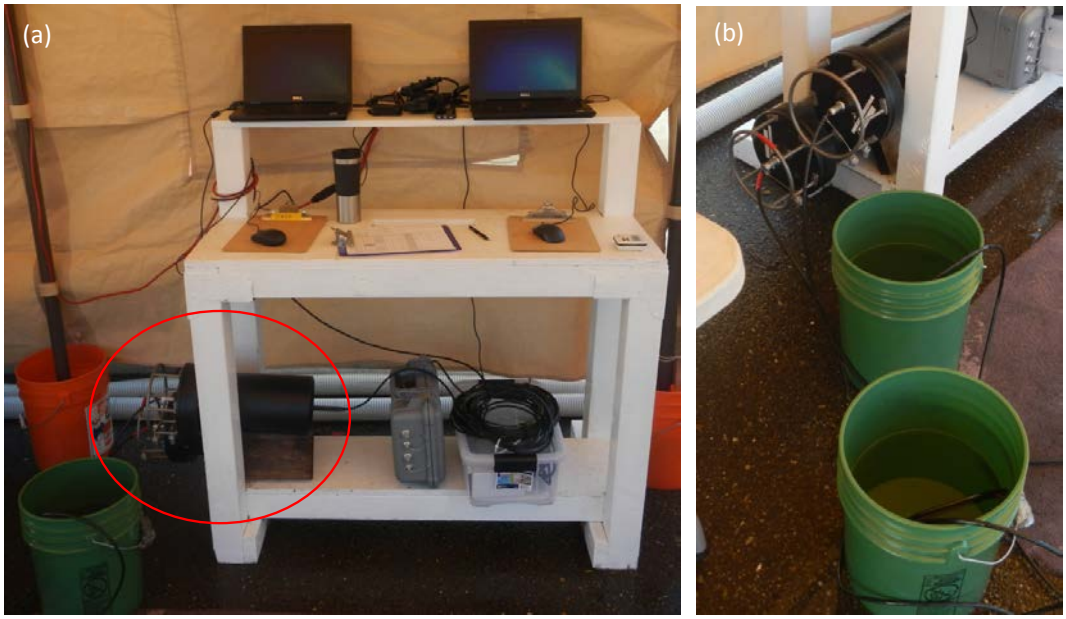

Figure 3. The transmitter validation station (a) at the Mokelumne River Hatchery during the 2015 Steelhead Survival Study consisted of two VEMCO High Residency Receiver prototypes (circled), each attached to a 180 kHz hydrophone. The hydrophones were placed into recovery buckets to verify the tag codes emitted by tagged steelhead while fish recovered from surgery (b). Photo credits: USFWS.

Figure 4. The prepared transport truck used to transport tagged steelhead during tagging operations of the 2015 Steelhead Survival Study (a). Perforated totes, each containing three tagged and verified fish, were added to the transport tank after 10 minutes of recovery. Intake and outtake hoses provided constant flow-through of raceway water. The oxygen level of the water was monitored and adjusted as necessary during tagging and immediately before transport (b). Photo credits: USFWS.

Figure 5. The Durham Ferry holding site of the 2015 Steelhead Survival Study. Tagged steelhead were held in perforated 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 6. A fish release during the 2015 Steelhead Survival Study. Tagged fish were held in perforated holding cans in the river for approximately 24 hours after tagging. The cans were then transported (a) to the middle of the channel approximately 200 m downstream of the holding cans, where the fish were released. Immediately before being transported downstream for release, the perforated cans were placed into non-perforated sleeves (b) to reduce the chance of predators smelling fish in the cans and following them to the release site. Photo credit: Pat Brandes/USFWS.

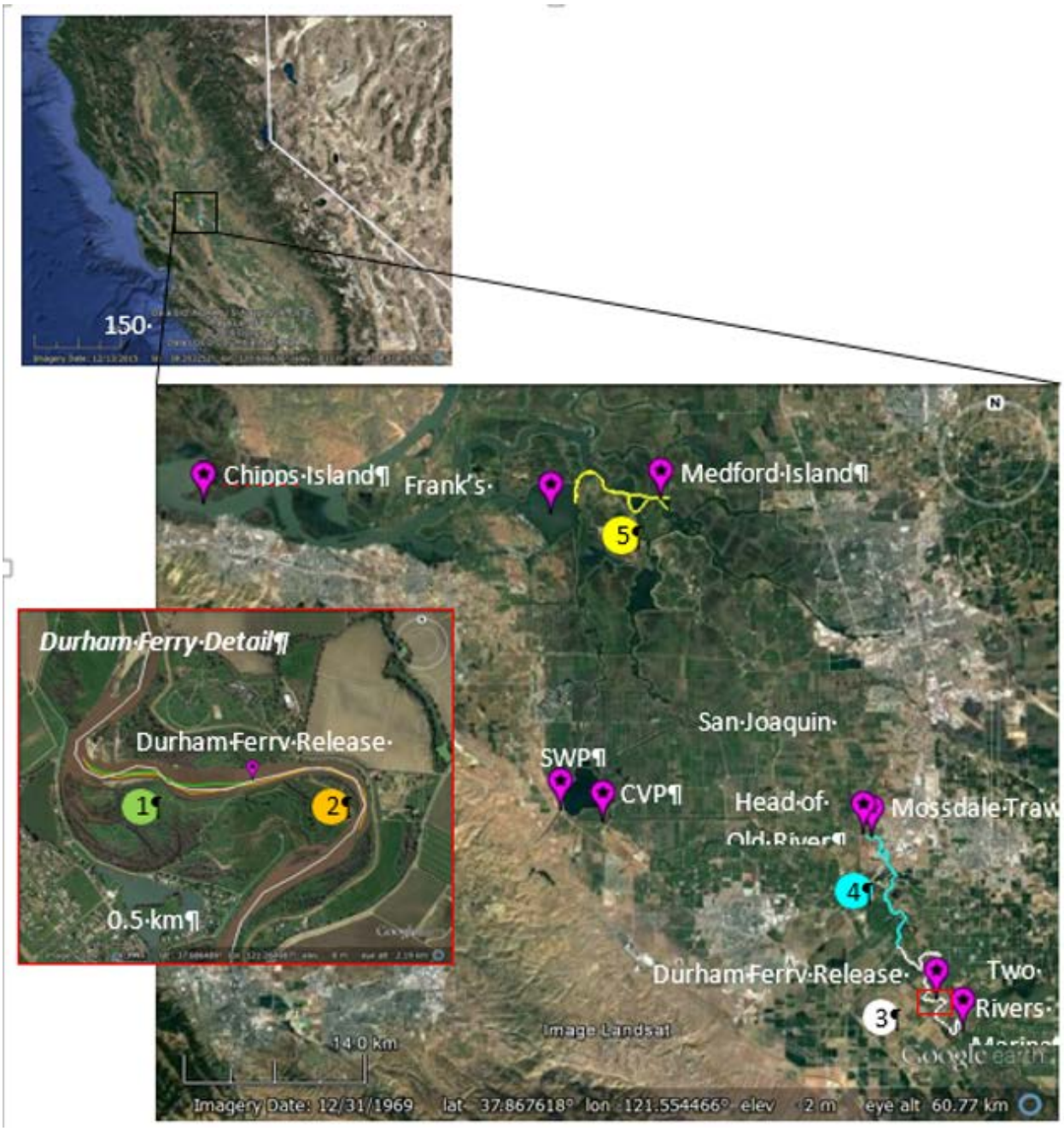

Figure 7. The area along the San Joaquin River covered by five mobile monitoring surveys during the 2015 Steelhead Survival Study. Survey 1 (green) was conducted on April 17, 2015, between the Durham Ferry release site and approximately 1 km downstream of the release site. Survey 2 (orange) was conducted on April 21, between approximately 1 km upstream and 1 km downstream from the Durham Ferry release site. Survey 3 (white) was conducted on May 8, began at the mouth of the Stanislaus River (at the Two Rivers Marina), and followed the San Joaquin River approximately 17.5 km downstream. Survey 4 (blue) was conducted on May 15 between the end of the third survey and the head of Old River, for about 13.5 km downstream. Survey 5 (yellow) was conducted on May 21, started approximately 1 km upstream from Medford Island, went 10.6 km downstream, and ended midway into the eastern side of Frank's Tract.

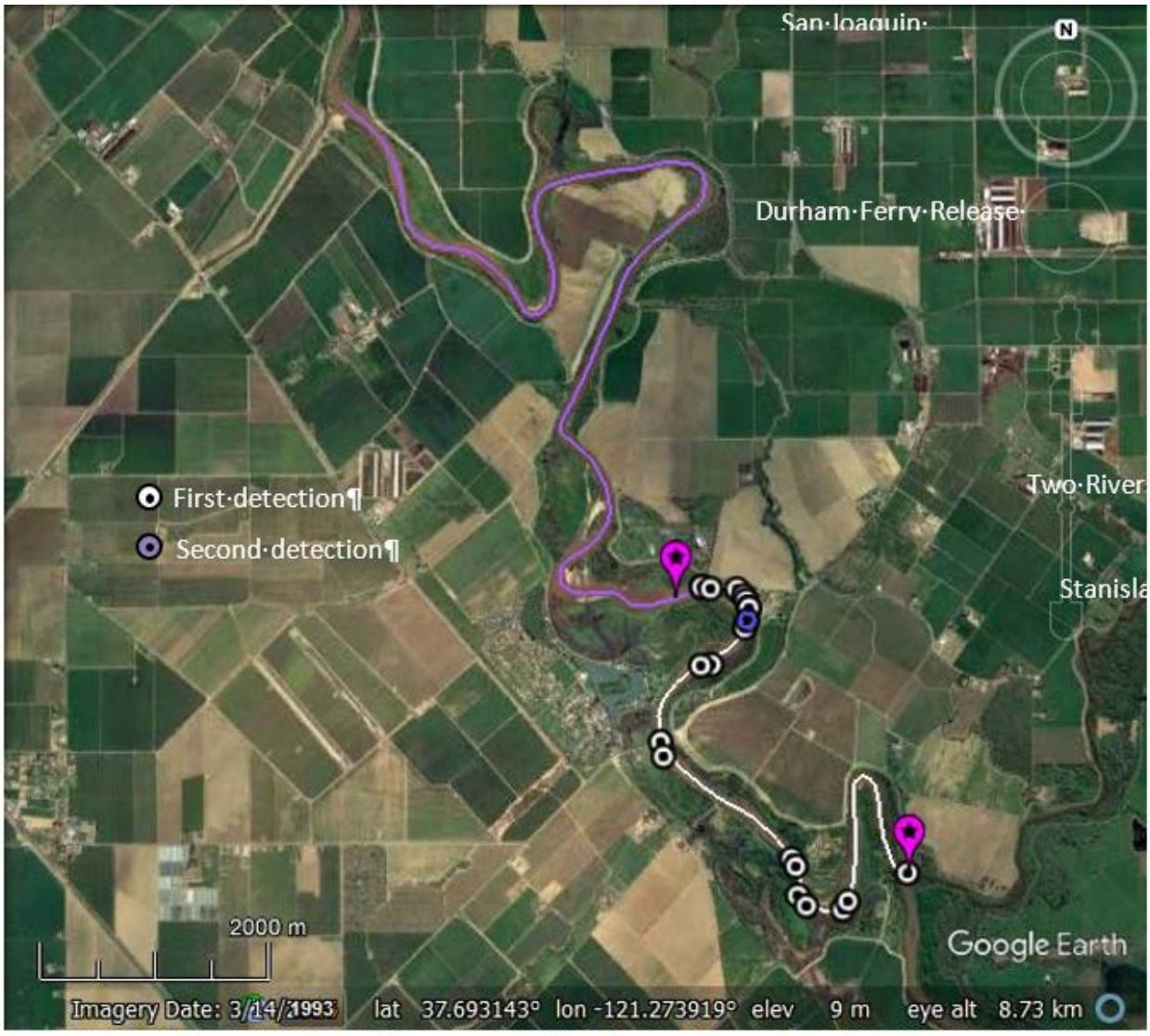

Figure 8. Tag detections from the 2015 Steelhead Survival Study mobile monitoring survey 3. One hundred and thirty-four detections were recorded past the Durham Ferry release site, but no GPS coordinates were obtained (purple track). Of the detections with no coordinates, four were second detections.

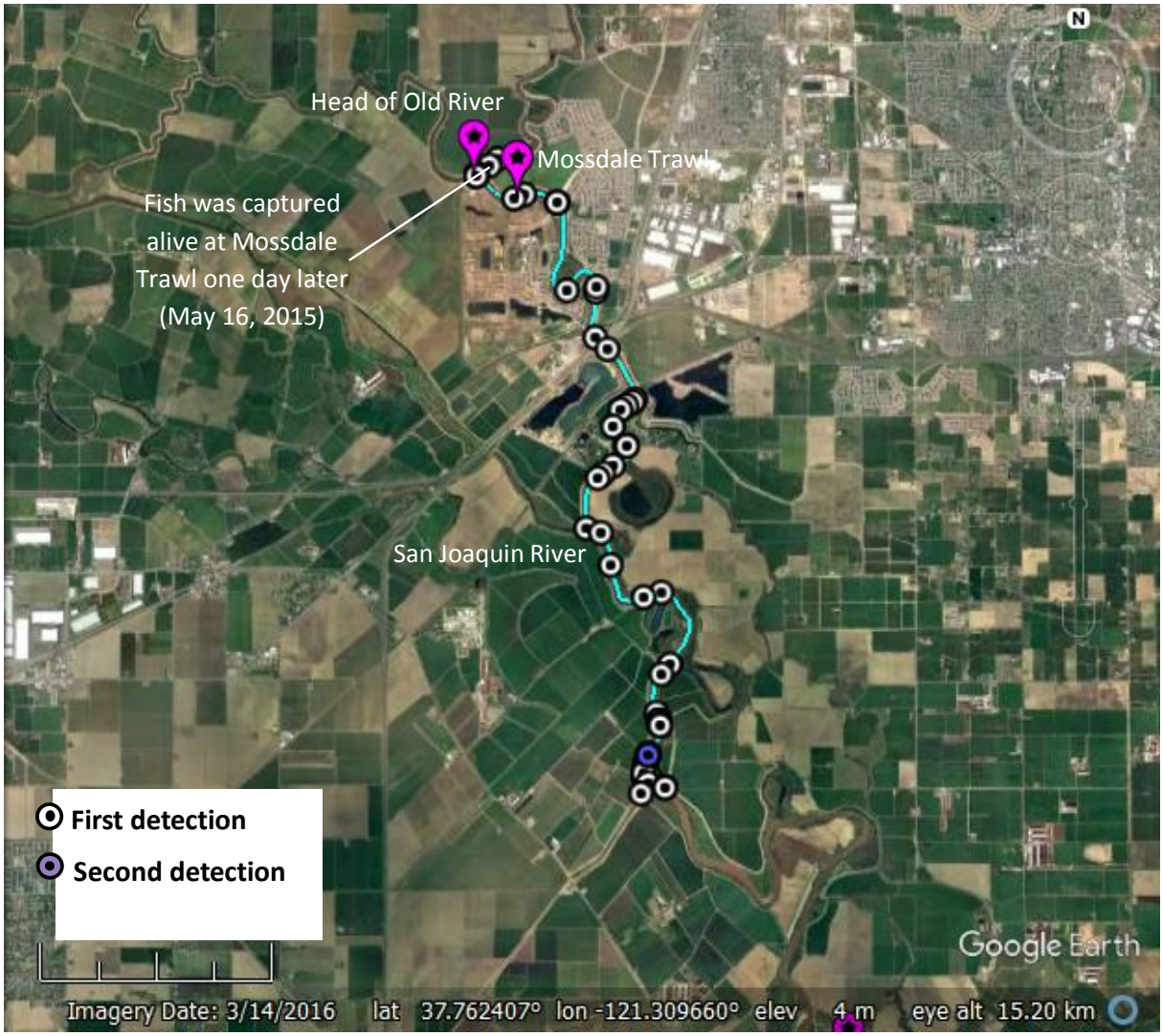

Figure 9. Tag detections from the 2015 Steelhead Survival Study mobile monitoring survey 4.

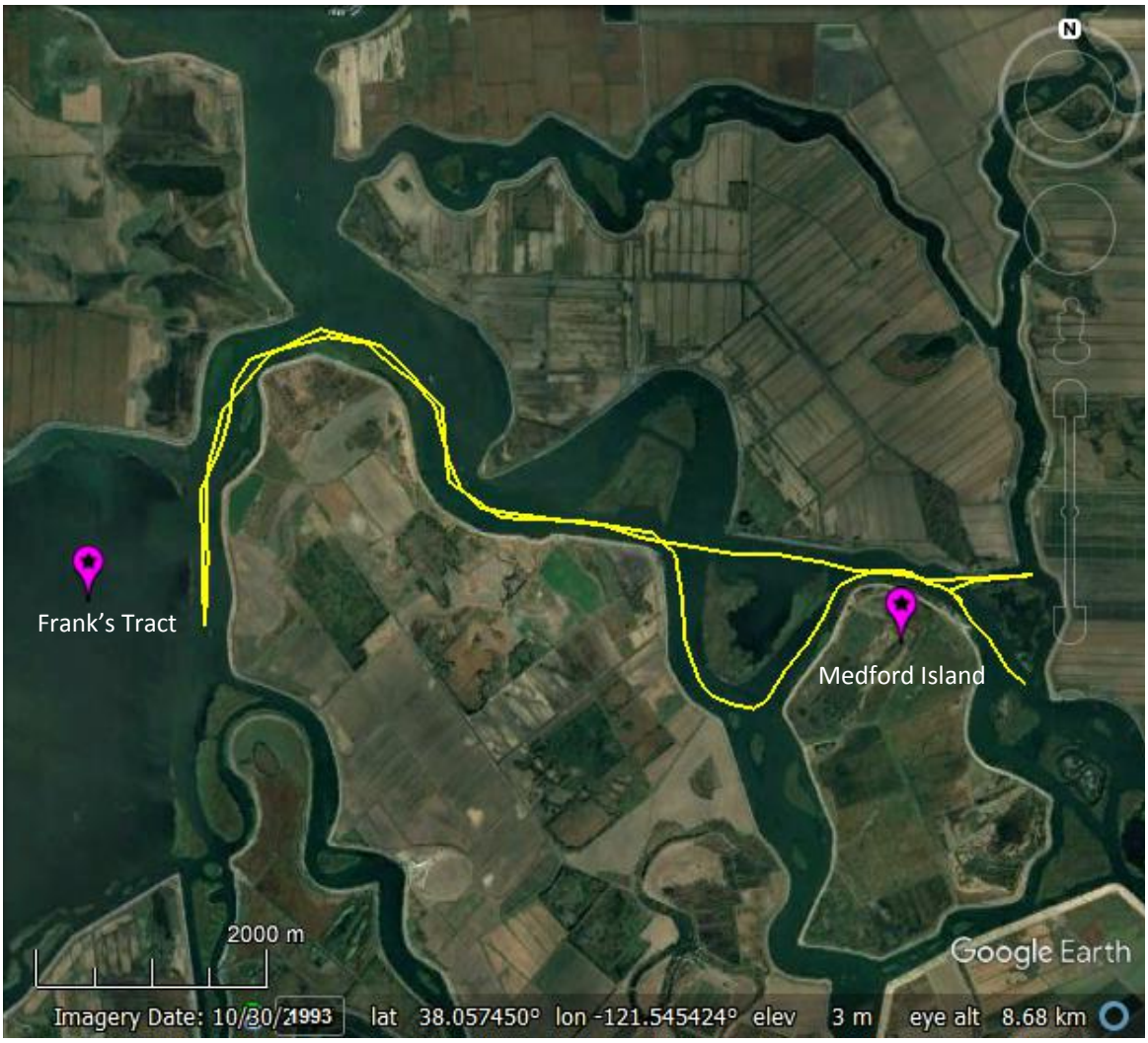

Figure 10. The track followed during the 2015 Steelhead Survival Study mobile monitoring survey 5. No study fish were detected during the mobile survey.

Appendices

Appendix A.

U.S. Fish & Wildlife Service

PATHOGEN SCREENING AND GILL NA^+/K^+- ATPASE ASSESSMENT OF SOUTH DELTA CHINOOK AND STEELHEAD 2015 RELEASE GROUPS

Ken Nichols

October 2015

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Summary

The California-Nevada Fish Health Center performed an assessment of the health and smolt development of steelhead trout and Chinook salmon utilized in south Delta salmonid survival studies in March-May, 2015. A suite of pathogen assays and gill Na^+/K^+-ATPase activity measurements were performed on cohorts of acoustic tagged release groups to help explain any performance and survival differences during the studies. In both steelhead and Chinook health assessment groups, mortality over the holding period was low (0-4%) and no significant pathogen infections were detected. Differences in gill Na^+/K^+-ATPase activity were observed in both steelhead and Chinook sample groups; however the differences were small and likely would not affect migration or survival.

Recommended citation for this report is:
Nichols, K. 2015. Pathogen screening and gill Na+/K+- ATPase assessment of south Delta Chinook and steelhead 2015 release groups. U.S. Fish & Wildlife Service, California-Nevada Fish Health Center, Anderson, CA. Accessible at: http://www.fws.gov/canvfhc.

Background

As a component of the south Delta salmonid survival studies examining reach-specific survival and distribution of migrating juvenile Chinook salmon and steelhead trout in the San Joaquin River and Delta, the California-Nevada Fish Health Center conducted a general pathogen screening and smolt physiological assessment. Steelhead trout were sampled in support of the 6-year Study required by the 2009 Biological Opinion on the Central Valley Project and State Water Project operations (RPA IV.2.2). The health and smolt development of the study fish can help explain their survival and migration performance during the studies. Similar pathogen screening and physiological assessments have been conducted on Chinook used in various studies in the south Delta since 1996. In the majority of these past studies, juvenile Merced River Hatchery Chinook were utilized, and occasionally significant infections with the myxozoan parasite *Tetracapsuloides bryosalmonae*, the causative agent of Proliferative Kidney Disease (PKD), were observed (Foott, Stone and Nichols 2007; Foott 2012). In 2014, the source for the juvenile Chinook changed to Mokelumne River Hatchery due to health concerns. No significant health issues were observed in the Mokelumne River fish in 2014 (Nichols 2014). Steelhead trout from Mokelumne River Hatchery have been assessed for these studies since 2010 and no significant health issues have been observed in these fish to date.

Methods

Study Fish

Both Chinook salmon and steelhead trout were obtained from the California Department of Fish and Wildlife Mokelumne River Hatchery. Health assessment groups were cohorts of acoustic tagged release groups and shadowed their tagged cohorts through handling, tagging (dummy tagged), transport, and in-river holding.

Steelhead – Groups of 24 yearling steelhead were sampled on 7 March, 28 March and 25 April, 2015. These groups were held in the San Joaquin River at the Durham Ferry release site for 48 hours prior to sampling.

Chinook – Groups of 30 juvenile Chinook salmon were sampled on 19 April and 4 May, 2015. The 19 April group was held in the San Joaquin River at the Durham Ferry release site for 48 hours before sampling. The 4 May group was held at the Mokelumne River Hatchery instead of the river due to elevated water temperatures at the release site. The 4 May Chinook group was held for 72 hours instead of 48 hours prior to sampling due to a schedule conflict.

Sample Collection

Fish were euthanized, fork length was recorded, any abnormalities were noted and tissues were sampled for lab assays. A sample of kidney tissue was aseptically collected and inoculated onto brain-heart infusion agar for bacterial culture (USFWS and AFS-FHS 2014). A kidney tissue imprint was collected to screen for *Renibacterium salmoninarum* (the bacteria that causes bacterial kidney disease) by fluorescent antibody test (USFWS and AFS-FHS 2014). Kidney and spleen tissue were collected in 3 fish pooled samples for viral tissue culture (USFWS and AFS-FHS 2014). Gill tissue was collected to assess smolt development by gill Na+/K+-Adenosine Triphosphatase (gill ATPase) assay (McCormick 1993). For Chinook, samples of gill, liver, kidney and intestine tissues were collected from 12 fish from each group for histopathological examination (Humason 1979). In steelhead, samples of gill tissue were collected from all live fish for histopathological examination.

Results and Discussion

Fish Condition

Steelhead – A total of 72 steelhead were examined (Table A.1) and only one fish died over the 48 hour holding period. The mortality occurred in the 28 March group in a fish with moderate hemorrhaging at the suture site (Figure A.1A), and it was likely this fish died due to complications following tagging. Pale gills were observed on one fish from the 28 March group, and significant scale loss (>50% of body) was observed in two fish from the 25 April sample group. Overall condition of the steelhead groups appeared good with no evidence to suspect survival differences.

Table A.1. Fork length (FL), mortality, external abnormalities (Ext Abn), or internal abnormalities (Int Abn) in Steelhead health assessment groups. Observed external abnormalities included pale gills and scale loss.

Sample Date	FL ±SE (mm)	Mortality	Ext Abn	Int Abn
7 March	255 ±5	0/24	0/24	0/24
28 March	239 ±3	1/24 (4%)	1/23 (4%)	0/23
25 April	252 ±3	0/24	2/24 (8%)	0/24

Chinook – A total of 60 Chinook salmon were examined (Table A.2) and there was no mortality in the health assessment groups over the holding period. Minor hemorrhaging (Figure A.1B) or slightly pale gills (Figure A.1C) were observed in 3 fish from the 19 April sample group. In the 4 May group, one fish had a small amount the liver extruding between the sutures with no hemorrhaging, and two other fish had minor hemorrhaging at the suture site. No evidence to suspect survival differences between Chinook groups due to fish condition was observed.

Table A.2. Fork Length (FL), mortality, external abnormalities (Ext Abn), and internal abnormalities (Int Abn) in Chinook salmon health assessment groups. Observed external abnormalities included: pale gills, minor hemorrhaging and partly open sutures.

Sample Date	FL ±SE (mm)	Mortality	Ext Abn	Int Abn
19 April	102 ±0.9	0/30	3/30 (10%)	0/30
4 May	104 ±1.0	0/30	3/30 (10%)	0/30

Pathogen Screening

Steelhead – No obligate bacterial or viral pathogens were detected in the 71 trout sampled. Other bacteria isolates (presumptive environmental contaminates due to field sampling conditions) were observed in 11% (8/71) of fish sampled. Minor parasitic infections were observed in 29% (20/70) of gill tissues examined by histopathology, with no associated lesion or other signs of impairment. Gill infections included: *Capriniana piscium* (presumptive ID, formerly known as Trichophrya) 23% (16/70); cyst-like xenoma due to an unidentified microsporidian 4% (3/70); and an infection of *Ichthyophthirius multifiliis* 1% (1/70). None of these infections were likely to cause differences in survival between steelhead release groups.

Chinook – No obligate bacterial or viral pathogens were detected in 60 salmon sampled. Other bacterial isolates (presumptive environmental contaminates) were observed in 20% (12/60) of samples. No infections or signs of impairment were observed by histopathology of gill, liver, kidney or intestine tissues from 25 Chinook. No differences in survival due to infections would be expected in the Chinook groups,

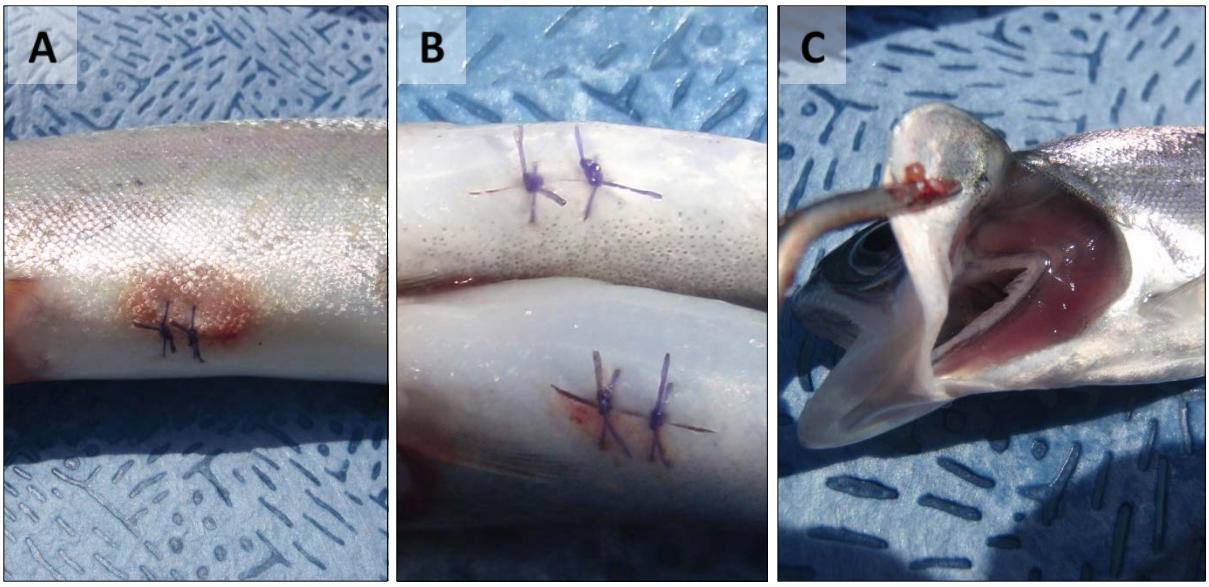

Figure A.1. Examples of external abnormalities including: (A) Moderate hemorrhaging at suture site of a steelhead; (B) minor hemorrhaging (lower) and normal (upper) sutures in Chinook; and (C) slightly pale gills in Chinook.

Gill Na^+/K^+-ATPase Activity

Steelhead – Gill ATPase activity levels (µmol ADP*mg $protein^{-1}$*hr^{-1}) ranged from 0.3 to 6.9 in all groups (Figure A.2). Median activity levels in the 7 March group were significantly lower than the later groups (Kruskal-Wallis test, $P<0.001$). Higher gill ATPase activity levels are associated with migrating smolts relative to their residual cohorts (Hanson et. al. 2011). In our experience, steelhead ATPase activity levels may increase over a short time period. While high levels may indicate salt-water readiness, lower values may have little biological significance due to this ability to rapidly change. Overall, the gill ATPase activity levels were low in all groups and the would not point to differences in migration or survival of the steelhead release groups.

Chinook – Gill ATPase activity levels ranged from 4.5 to 16.8 in all groups (Figure A-3). Median activity levels in the 19 April group were higher than in the 4 May group. The modifications in holding conditions of the 4 May Chinook health assessment groups (mentioned above) and variability due to lab assay conditions likely contributed to the observed difference, and a direct comparison may not be useful. The majority of fish from both Chinook health assessment groups had activity levels consistent with smolts (>6.7, CA-NV Fish Health Center unpublished data). Active migration in both release groups would be consistent with the observed activity levels.

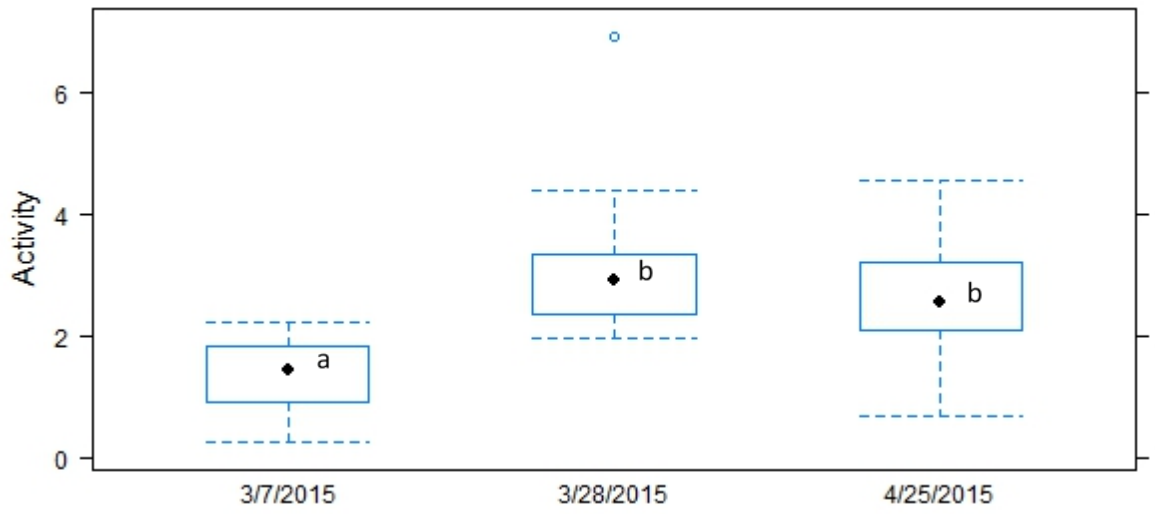

Figure A.2. Boxplot of median gill ATPase activity in 2015 steelhead health assessment groups. Medians with the same letter are not significantly different (Kruskal-Wallis, P<0.001). Number of fish sampled for each group was 16.

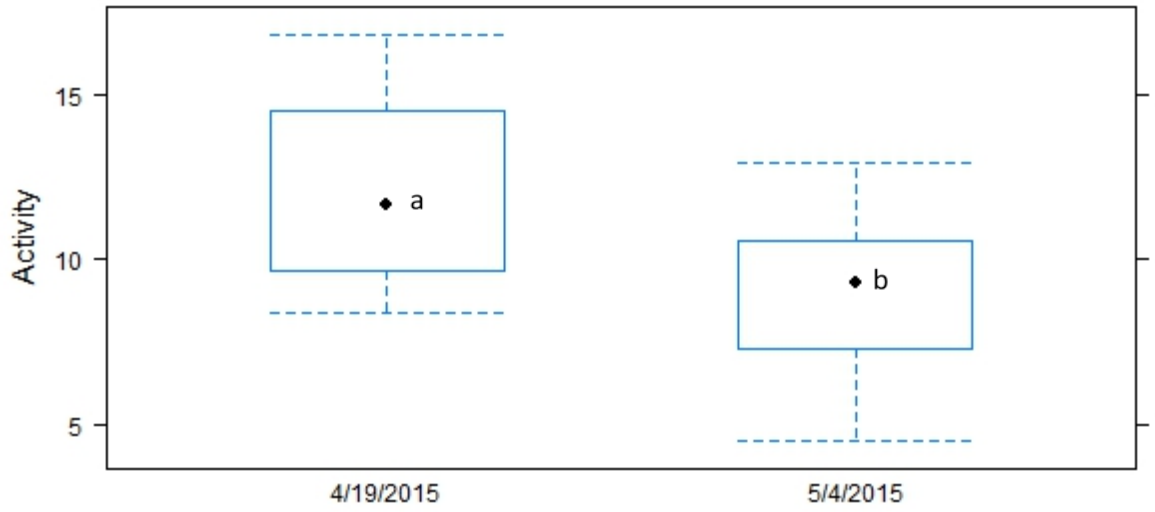

Figure A.3. Boxplot of median gill ATPase activity in 2015 Chinook health assessment groups. Medians with the same letter are not significantly different (Kruskal-Wallis, P=0.005). Number of fish sampled from each group was 16.

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Standard Operating Procedure

Acoustic Tagging for Steelhead 2015 South Delta Studies

MATERIALS NEEDED:

- Dissolved oxygen (DO) meter (e.g., YSI 85)
- Acoustic tags (V-5)
- VEMCO acoustic tag activator
- VEMCO acoustic tag verification equipment (VR-100)
- 14 day pill boxes for tag distribution
- Chlorhexidine solution (Novalsan ; 30mL/L D-H_2O)
- Distilled or de-ionized water (D-H_2O)
- Aqui-S 20E (undiluted, directly from manufacturer)
- Stress coat - stock concentration and 25% solution (250mL/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 (5 mL) for measuring anesthetic and stress coat
- Oxygen delivery system (cylinder, regulator, airline, air diffusers) for recovery buckets
- Dip nets
- Sanctuary nets
- Nitrile gloves (in all sizes)
- Scale measuring to the nearest 0.1 g (weighing fish)
- 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)
- 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 blades (multiple sets)
- Scissors (multiple sets)
- Tissue collection supplies: scissors, blotter paper, labeled coin envelopes
- 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).
- 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 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
- 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

- Fill disinfection trays for surgical instruments with Novalsan
- Fill rinse tray with de-ionized or distilled water
- Fill pill boxes containing study tags 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, 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
    - The freshwater carboy is filled from the raceway and has no anesthesia added
- Fill anesthesia bucket to 10 L line with water from source tank or raceway. Add 3 ml Aqui-S 20E and briefly agitate to ensure dispersal. Cover with a lid
- 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. Add stress coat
- Reference Tag and Tote inventory sheet 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 datasheet for each tagging stations to account for fish that are handled but not tagged
- The tagger should wear clean medical grade exam gloves during all procedures that involve handling fish

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 raceway using a sanctuary net and place directly into an anesthesia bucket.
    - Use a standard net inside the sanctuary net to avoid adding water to the anesthesia bucket and diluting the working concentration of the Aqui-S 20E.
    - 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 deliver the bucket 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, if 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 0.1g.
    - 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 fish 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 less than 5% 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 compromised osmoregulatory ability.
- Data must 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
- 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 in-line valve, adjust the flow as needed so that the gilling rate of the fish is steady
  - Remove a 2 mm by 2 mm section of the ventral portion of the caudal fin and place on filter paper. Put filter paper in pre-labeled coin envelopes that indicate the individual identification of the fish.

    - Recorders should mark off on the datasheet that tissue sample was collected.
    - In the event of fish that are tagged and later rejected, discard the tissue sample and envelope and use a marker to record the serial number of the new/alternate tag.
    - Once tagging is completed, during QA/QC, confirm number of envelopes. The coin envelopes will be presented to the tagging coordinator at the completion of each tagging session.
    - The coin envelopes will be returned to the FWS office daily, and should stay dry and be at room temperature. Putting envelopes into a sealed plastic bag should be avoided. Back at the office the tissues may be put into a desiccator and then mailed to NMFS.
- 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 a disinfected transmitter from the pill box
- Confirm the tube ID with the recorder and place the empty vial into the lid of the tray which holds the tags
- Gently insert the tag into the body cavity and position it 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

- Two recovery buckets are used for each group of three fish that will be transferred into one tote for transport to the release site. Call out the count of fish in the recovery buckets to the tagging assistant/recorder for confirmation. Put the lids back on the buckets. Once 3 fish are in the 2 buckets that make up a respective tote, attach the clipboard with tag datasheet to one of the two buckets and have the tagging assistant move the buckets to the tag verification staging area
- 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 through 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 reduced effectiveness so be sure to change the bath regularly

Transmitter Verification:

- Obtain buckets and datasheet from tagging crew and start a timer for the 10 minute surgical recovery period
- Gently place hydrophones attached to a VEMCO VR-100 into each bucket
- Watch the display on the VR-100 for tag codes that appear on the monitoring screen. As tag codes are verified circle the tag code that is read on the VR-100 on the copy of the Tag and Tote 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 to an 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. One the tag is removed, return to tag coordinator for a replacement tag to complete tag implantation
- Return the datasheet to the tagging crew

Loading for Transport:

- Begin completion of fish loading, transport, and release data sheets
- Fill hauling tank with water at same temperature as source tank 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 buckets 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
- Secure the tank and tank lid for transport
- Send previous days datasheets with transport crew (first transport truck)

End of session activities:

- Validation of tag data and datasheet accuracy
    - Working together, each tagger and assistant team will review the transmitter tubes/serial numbers against the tag and tote inventory and the datasheets to verify that all of the transmitters provided for the session were implanted into study fish
    - The steps of the verification process should include reading the serial number on each tag tube, finding that serial number on the datasheet to confirm that it was

implanted, and a simple count of the tags provided (as shown on the tag-tote inventory) vs. the tag tubes and data rows on the datasheets
    - Once the validation steps have been completed, both the tagger and the assistant initial the datasheet to confirm that the validation step has been completed
- Validation of genetic sample accuracy
    - Following a similar process to what was done for tag data, the tagger and assistant should work together to confirm that they have a complete and accurate collection of coin envelopes containing genetic samples
    - The steps of the verification process should include reading the serial number on each envelope and comparing it to the tags listed on the tag and tote inventory to ensure that all appropriate genetic samples were collected
    - Once the validation steps have been completed, both the tagger and the assistant initial the datasheet to confirm that this validation step has been completed
- Review all datasheets and complete any missing information (e.g., tag end time, page numbers, validation initials)
- Collect all datasheets, pill boxes, coin envelopes, and tag tubes and hand them in to the tagging coordinator
- Organize tagging solutions 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 with hose 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
- **CAREFULLY HANDLE BUCKETS**. Try not to bang them around, slam the handles, or otherwise handle in a rough manner as this can stress fish
- **USE A SANCTUARY NET** to capture source fish and place them into an anesthesia bucket. A recommended approach is to use a non-sanctuary net in the container of source

fish in order to be able to capture the fish without them detecting the pressure wave in front of the sanctuary net. Once a fish is in the traditional net, place the sanctuary net immediately below the fish so that the handles of the two nets are aligned and can be handled together.

Standard Operating Procedure and Checklist

Holding and Releasing Acoustically Tagged Fish 2015 South Delta Steelhead Studies

Steelhead Transport

_____Before loading, totes will be checked for any dead or impaired fish and if any are found they will be returned to the tag coordinator. In addition, the number of fish in each tote will be noted on the Transport and Release datasheet.

_____ Use the appropriate loading plan, so the first tote numbers to come off the truck at the release site are loaded on the passenger side of the truck.

Totes will be loaded into the transport tank

_____After all the fish are loaded into the transport tank, the driver will record the water temperature and dissolved oxygen (DO) in the tank on the Transport and Release datasheet. Also include the tank number on the data sheet.

_____The driver should call the unload/set-up/release crew and let them know he is leaving the hatchery and let the crews at the release site know the temperature in the transport truck after loading so they can assess the need for tempering the fish at the release site.
_____The driver will also take a copied set of the Surgical Tagging datasheets to the release site crew.

_____The driver will record the time he leaves the hatchery on the release datasheet

The driver will drive the truck from Mokelumne River Hatchery to Durham Ferry release site

____The driver will record the time he arrives at the release site.

_____The driver will take the water temperature and DO of the transport tank and record it on the datasheet.

____One unloader puts on clean, bio-hazard free waders, to assist the truck driver in moving totes to personnel in the pick-up truck.

_____The release/set-up/unload crew will take a water temperature and dissolved oxygen reading from the river.

Holding site tempering

If the water temperature in the transport tank is lower than 5˚C than the water in the river, the lids of the transport tank will be opened to allow the water to warm until it is within the 5ºC of that in the river.
If after 1 hour, there is still more than a 5ºC difference between the water temperature in the river versus that in the transport truck, we will start moving perforated totes to the river.

Addendum added to SOP on 4/22/15: Fill 18 gallon tote sleeves with 1 bucket (5 gallons) of river water. Add totes with hatchery water from transport truck and add bubbler. Wait 1 hour and then put them into garbage cans in the river. Monitor temperature and DO during the 1 hour.

Preparing the pick-up truck for the totes

The crew will place 8 to 10 tote sleeves into the back of the pick-up truck. The crew will then drive the pick-up truck down to the river and use buckets to fill up the tote sleeves 2/3 full of clean river water, with a minimum of sediment.

The crew will then drive the pick-up truck up the levee and park next to the transport truck.

Transfer from the transport tank to the pick-up truck

The perforated totes will be transferred, one at a time, by the truck driver and one unloader to two other unloaders in the pick-up truck. The unloaders in the pick-up truck will place the perforated totes into the half-filled tote sleeves into the back of the pick-up truck. The totes will be transferred starting with the totes that are on the passenger side of the truck in descending order. After transfer there should be approximately 12 gallons of water in each perforated tote, held within a tote sleeve. Repeat for 8-9 totes prior to delivering to the river holding site.

Once the first load of study fish has been transferred to the containers at the river's edge, the tote sleeves should be emptied and refilled 2/3 full with clean, fresh river water for transferring the second load of totes to the river's edge.

Transfer of tagged fish to the River

If water temperature difference between the river and transport tank is greater than 5°C, we will re-take water temperature in totes at the river's edge. If the difference is still greater than a 5°C, we will add a bubbler and ½ bucket full of water from the river to raise the temperature ½ degree every 15 minutes until we reach a water temperature in the totes within 2° Cof the river temperature.

The second load will be retrieved from the transport truck while we are waiting for the first group to acclimate, if acclimation is necessary.

Before fish are transferred into their in-river holding containers, fish must be observed to check if the number of fish in each tote agrees with what is written on the datasheet. If there are any mortalities or any fish is in an impaired condition, they should be removed from the tote and noted on the datasheet and euthanized if not already dead. The tag should then be retrieved/dissected from the carcass and placed into a zip-lock bag. Place the tag inside of the bag with all information required on the pre-made label (transport date and number/letter of tote that it was collected from). If there are multiple mortalities from the same transport, but different totes, *make sure that tags from each tote go into a separate zip-lock bag* and note mortalities on the datasheet. Bring bags of tag(s), each containing the appropriate label, back to office at the end of the shift and put on Jack's desk.

Once transfers of study fish to the perforated garbage cans in the river have been completed, lay clean (rinsed if necessary) perforated totes and lids on the blue tarp for drying. Once dry, stack and load them into a pick-up truck for return to Lodi. Tagging

personell will pick them up the following day, drive them to Mokelumne River Hatchery, and place them into the freezers for use on subsequent days.

Biosecurity Control Point: Emptied totes and their lids will NOT be put onto the transport truck, but returned to the Mokelumne River Hatchery after drying at the release site. Buckets/totes and lids should be clean and dried on the tarp at the release site prior to being transported to the Lodi office and then to Mokelumne River Hatchery, so that they go into the freezer at the hatchery in the cleanest possible condition.

Releasing Tagged Fish

Release crews will release fish at times provided on the schedule posted in the field crew trailer – once every 4 hours at 1500, 1900, 2300, 0300, 0700, and 1100.

Release crews should all wear appropriate field gear. This includes: waders with boots, safety belt, appropriate outerwear, and PFD with safety strobes when on the boat; head lamps should be worn at night.

When release crews arrive at the release site, they should make sure that all of the cans are in place and all are upright. Also, check to see if there is enough clearance between the bottom of the cans and the substrate. If the cans are sitting on the substrate, they need to be moved out into deeper water. This can be accomplished by either pulling the fence stakes and anchors into deeper water or, if this is not feasible, contact appropriate personnel and they can come out and assist with this process. This may be difficult in 2015 due to low San Joaquin River flows, but all attempts should be made to keep cans off the river bottom.

Identify which cans are to be released. Steelhead containers will be marked with a number (1-32). Each container is equipped with two tethers with two quick-links attached to the main anchor line (fixed between two fence stakes).

Detach the quick-links from the main anchor line and attach to the transport line located near the starboard side gunnel of the release boat. Either two or three cans will be released at a time. Put perforated cans into garbage can “sleeves” for transport down the river. Make sure perforated cans inside sleeves are full of water. If the depth of the water in the river is too shallow to allow movement downstream without hitting bottom, pull “sleeve” containing release can up higher on boat cleat using the rope ties, so cans do not hit the bottom of the river.

Prior to leaving shore during night time hours, confirm a security guard or other crew member is on site observing the release. He/she is on site to call for help, or assist if boat capsizes or other emergency-type event occurs. If the security guard is not at the site at the correct time, please notify the project manager. Don't use the boat at night unless there is someone on land observing the release.

Once you have attached the transport containers to the vessel, board the vessel and start outboard engine. The outboard is equipped with a key start; make sure that the outboard is in neutral with the throttle set at start. Once the outboard is running, safely engage the shifter into forward or reverse, depending on the orientation of the vessel and move away from the holding area.

Maintain a slow and steady speed, making sure that the cans are not tipping or submerging. Slow down if cans appear to be tipping or submerging. If cans are hitting the bottom because the river is too shallow, pull the cans up further in the water column using a rope looped around the can and the cleat on the boat.

Once the release location has been reached, remove the wing-nuts from the release can holding the lid in position. Pull the lid off and place into boat. Once the lid is removed, pull the perforated container slowly up out of the sleeve; allowing some of the water to drain. DO NOT COMPLETELY DEWATER THE CAN!!

Observe the fish inside of the container; making sure there are no mortalities. If you observe a dead fish, remove it as gently as possible using an aquarium net and place it into a zip-lock bag. Record the number of mortalities for each can on the data sheet. Once you have retrieved any mortalities from the can, slowly invert and push the can down so that one end of the opening is just under the surface of the water and allow the fish to swim out of the can. If necessary, turn the can upside down to empty the contents of the container into the river, making sure that all fish have left the container prior to bringing the container on board the vessel. Once the container is empty, place it inside the vessel. **Record the date and actual time of release (to the nearest minute in 24 hour time) on the data sheet for each can/container.** Do not write down the time from the schedule if this is not the actual time of release. Remember to change the date if the release is after midnight. Also take a GPS reading of the release location.

Repeat the procedure for the remaining containers, making sure that you record the actual release date and time for each group of fish. Return to shore and remove the

empty containers from the vessel and place on shore. Make sure that the containers are placed on their side so that the containers do not get damaged.

If you have any mortalities, retrieve the acoustic tag from within the carcass and place into a zip-lock bag. Place the tag inside of the bag with all information required on the pre-made tag (date and time of release and number or letter of can that it was collected from). If there are multiple mortalities from the different cans, *make sure that tags from separate cans go into a separate bag* and document the mortality on the datasheets.

Perform necropsies on the mortalities and fill out a necropsy data sheet. Please make sure it is clear on the datasheet what fish you are necropsying (can be obtained from bag number if there is more than one mortality in a can). Bring tag(s) back to office at the end of the shift and put it/them on Jack's desk. Bring the carcass back to the office for disposal in the office dumpster.

Continue to release fish throughout the shift at the scheduled times according to the schedule posted in the field crew trailer. At the end of your shift, make sure that the next shift of personnel or security guard arrives prior to leaving. The crew handling the last release will bring all supplies and equipment remaining at the release site and trailer back to the office.

Processing Dummy Tagged Fish:

Fish Condition Evaluations

After the last releases from each transport day, complete the following steps (refer to release schedule for time and can numbers for fish assessment):

1. Take off lid of dummy can and take water temperature and DO measurements in dummy tag can. Write it down on the Fish Condition Assessment sheet.
2. Put can containing dummy tagged fish into a "sleeve" and take it halfway to release site by boat and return to shore, duplicating the release process for the tagged fish.
3. Determine if there are any mortalities in the dummy tagged cans by putting fish into a bucket at the time fish condition assessment is to be done (see release schedule). Note mortalities on Fish Condition Assessment data sheet.
4. If the live fish will not be given to Ken Nichols for the Fish Health component of the study, euthanize the 12 dummy tagged steelhead.

5. Note PIT tag using the PIT tag reader for each fish, and the date and time of the assessment on the Fish Condition Assessment data sheet.
6. Measure each fish and check the 5 characteristics of condition (scale loss, fin hemorrhaging, body color, gill color, and eye condition) and complete datasheet entries for each fish.
7. Take picture of each fish showing sutures (turn camera date and time stamp on). Record picture number on Fish Condition Assessment data sheet
8. Do necropsy per training protocol and score tagging for each fish. Take another picture of the inside of the fish.
9. Put PIT tags and dummy tags in a zip-lock bag marked "dummy tag" and bring them back to the office and leave on Jack's desk

Fish Health Evaluations

After the last release of each week, repeat steps 1-3 above (1. take temp and DO in can, 2. take can halfway to release location and back, and 3. check the 24 dummy tagged steelhead for mortalities). **Keep fish alive** in the river until Ken Nichols of CA/NV Fish Health Center arrives and assesses them for fish health/disease. Obtain dummy tags back from Ken and return to Jack at the office. There will not be any PIT tags in these dummy tagged fish.

Disposal of MS-222:

DO NOT dispose of MS-222 into the river or within 100 feet of any water source. Dump MS-222 containers onto dry ground on the other side of the levee, on the pavement.

Disposal of carcasses:

Once the tag has been removed from study or dummy tagged fish, place the carcasses into a zip-lock bag and bring back to the office. Once you arrive at the office, discard all carcasses into the large trash bin that is located in the parking lot.

Data Entry:

Truck drivers will bring a copy of tagging sheets from the hatchery to the release site. During hours that release crews are not unloading or releasing fish, data are to be entered into the database. Crews should both enter data and QA/QC it by checking datasheets to entered data. Please initial the datasheets so it is clear who entered the data and who QA/QC'd the data. Once release sheets are complete they should also be entered into the database.

Follow guidelines for entering data. Please enter pill boxes in the A-01 to A-10 format, and the totes in the SA-01 to SA-10 format.

Each study fish will have a serial number and two tag codes. We will provide a look-up worksheet within the database where you should cut and paste the serial number, and the two codes for each tag as you enter data on that particular fish/tag.

Appendix D.

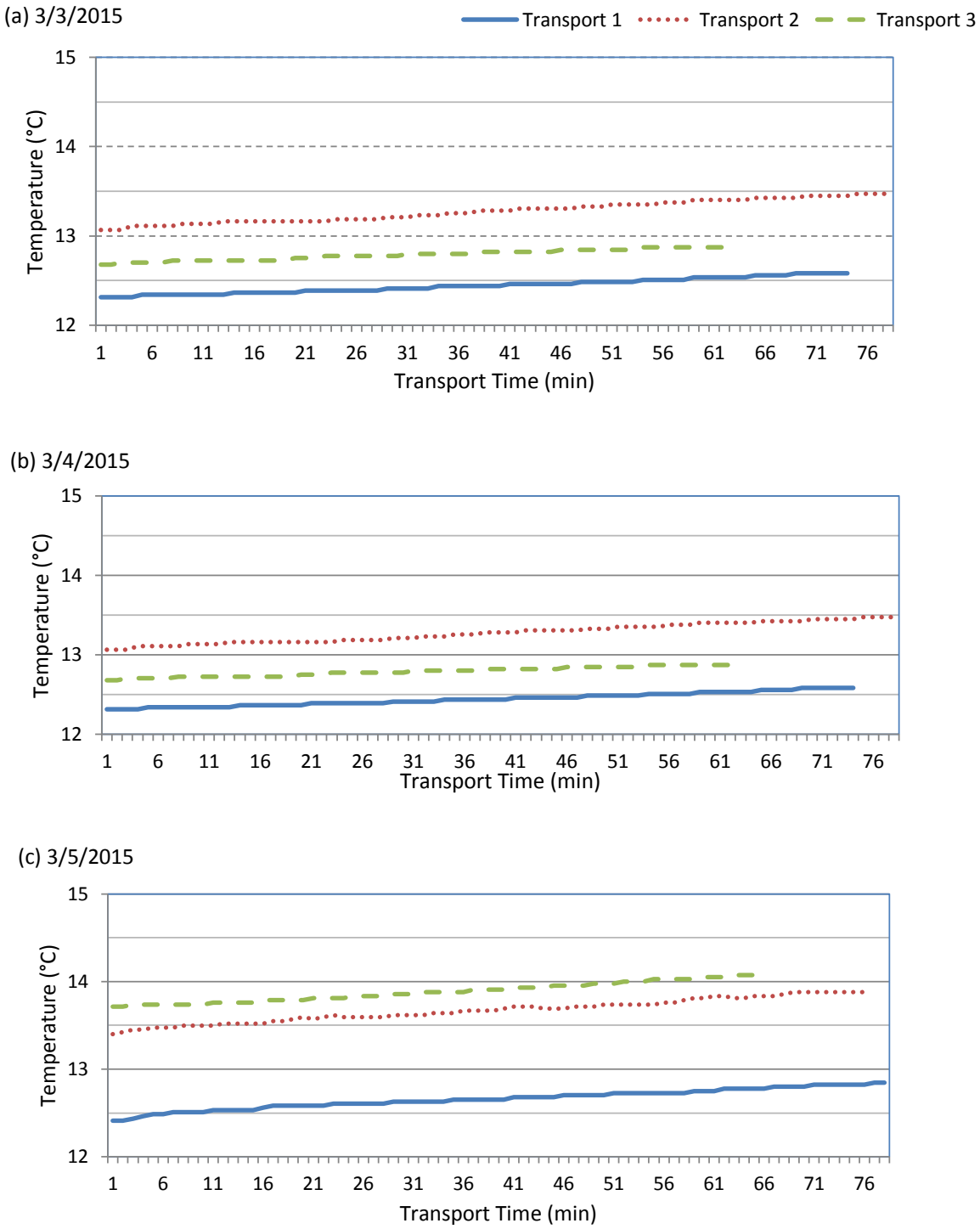


Figure D.1. Temperature in each of three transport tanks (transport 1, 2, and 3) during the transport of steelhead during week 1 of transport to the Durham Ferry release site.

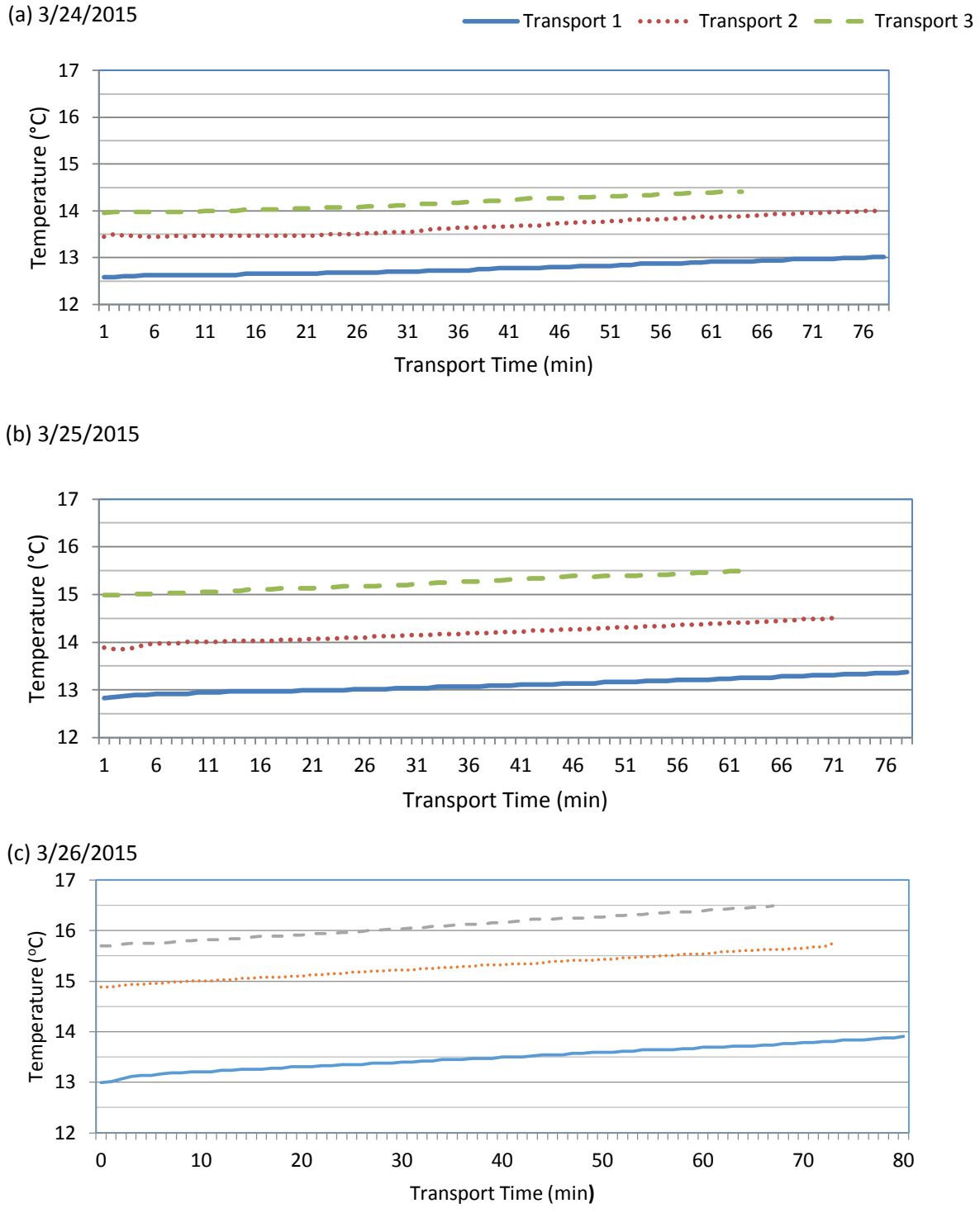
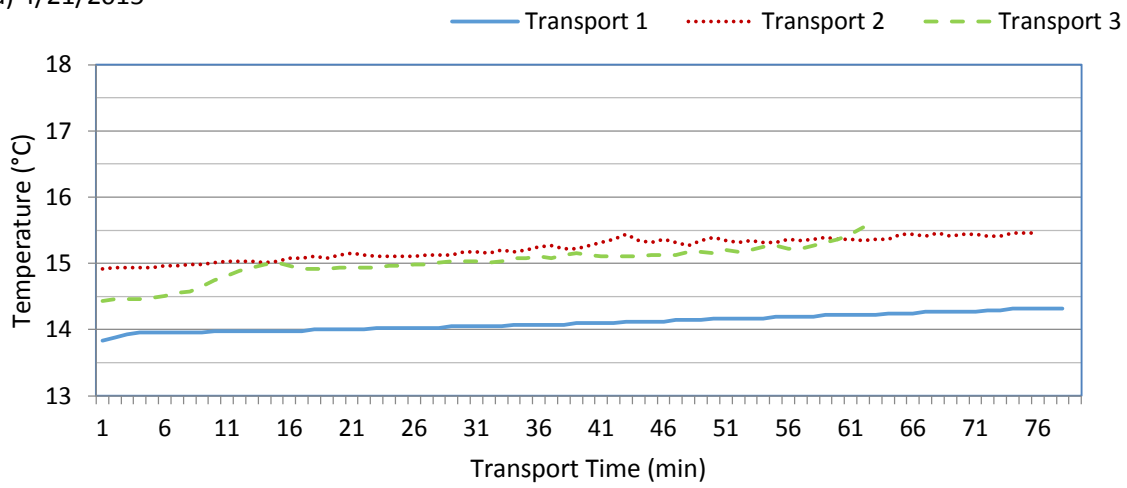
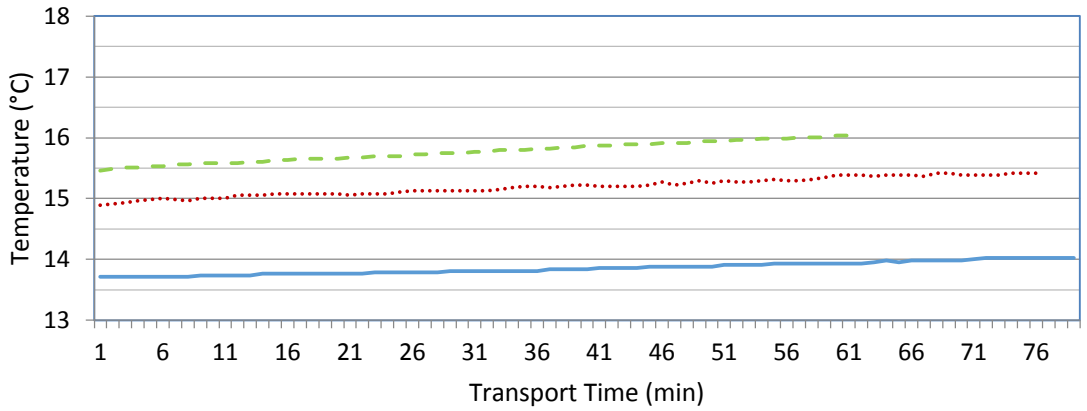


Figure D.2. Temperature in each of three transport tanks (transport 1, 2, and 3) during the transport of steelhead during week 2 of transport to the Durham Ferry release site.

(a) 4/21/2015



(b) 4/22/2015



(c) 4/23/2015

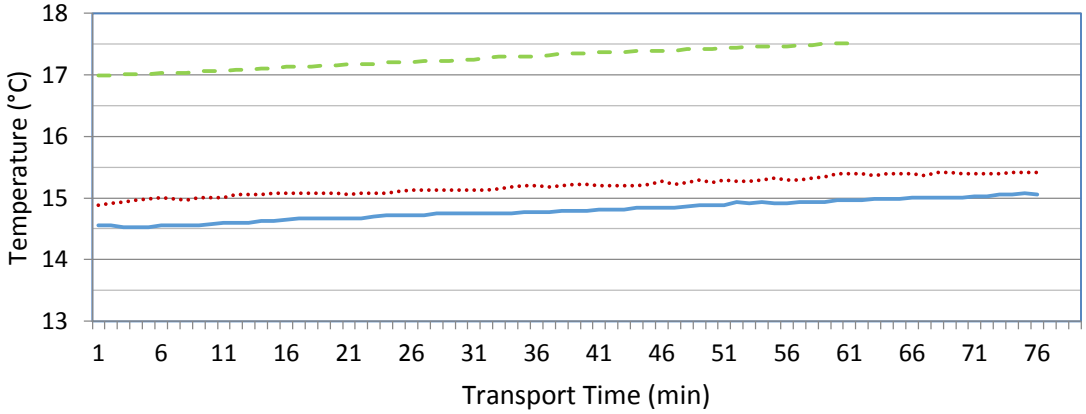


Figure D.3. Temperature in each of three transport tanks (transport 1, 2, and 3) during the transport of steelhead during week 3 of transport to the Durham Ferry release site.