Methods and Implementation of 2014 Steelhead Tagging and Releases

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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 2014 Steelhead Survival Study, the fourth year of the six-year study. The following report includes the methods and implementation results for the tagging, release, dummy tag assessments, tag retention tests, and disease monitoring components of the project. This report is intended to act as a preliminary supplement to the final, comprehensive report combining all aspects of the study, including the fish tagging, release, and health examination, receiver deployment and the survival models and statistical analysis.

Acoustic tags were implanted into juvenile steelhead at the Mokelumne River Fish Hatchery (MKRH). The fish were transported to and released at Durham Ferry, about 20 km upstream of Mossdale (the south entrance to the Sacramento-San Joaquin Delta). A total of 1,440 steelhead were tagged for the release; other steelhead from MKRH were used for assessing fish condition after tagging, transport and holding for 48 hours, tag retention or fish health. Study fish were tagged over three, 3-day periods between March 25 and May 22. Once the steelhead were tagged, they were loaded into transport tanks and transported to the holding location on the San Joaquin River near Durham Ferry (RK 109). Fish were held in-river for at least 24 h prior to release.

During the 2014 Steelhead Study, a manufacturing bug in the new VEMCO V5 tags resulted in premature failure of tags used for the first release of steelhead for this study. VEMCO personnel reprogrammed tags used in the second and third releases of steelhead, so those tags did work properly.

A tag life and a tag retention study were done to evaluate tag life and survival and tag retention in hatchery steelhead with surgically implanted acoustic V5 tags. Lastly, tissue was taken for DNA from the caudal fin of the study fish released.

Study Design and Methods

Study Fish

Steelhead were obtained and tagged at the MKRH. Tagging equipment was set up in early February and tag training took place between March 16 and March 21, 2014. The weight criteria for fish used in the study was a minimum of 13 g and a maximum of 200 g. However, one fish was tagged that weighed 235.2 g which was outside of the established criteria. All others were within the weight criteria. Their average weight was 136.6 g (SD = 28.9), range 31.4 g to 235.2 g and the average length

was 242.2 mm fork length (FL; SD = 18.6 mm), range 119 mm to 283 mm. The average fish weights during Week 1 (mean = 121.5 g, SD = 27.3), Week 2 (mean = 139.0 g; SD = 26.8 g), and Week 3 (mean = 149.3 g; SD = 25.7 g) were significantly different from one another (ANOVA and Holm-Sidak test, $P < 0.001$). The fork lengths of fish released in Week 1 (mean = 232.4 mm, SD = 19.3 mm), Week 2 (mean = 244.1 mm, SD = 16.7 mm), and Week 3 (mean = 250.0 mm, SD = 15.1 mm) were also significantly different from one another (Kruskal-Wallis and Tukey tests, $P < 0.001$).

Prior to tagging fish for each transport truck, 17 fish (68 total) were netted into each of four 166 L (44 gal) perforated cans that were located in the raceway near the tagging trailer (Figure 1). Each can contained a minimum of 151 L (40 gal) 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 density recommendations were met for the duration of each tagging session.

Tags (Transmitters) and Activation

Steelhead were tagged with VEMCO V5 180 kHz transmitters that weighed on average 0.674 g in air (SD = 0.006 g), range (0.654 g to 0.730 g). Tags were 12.7 mm long, 4.3 mm in height, and 5.6 mm in width (http://vemco.com/products/v4-v5-180khz/; accessed June 15, 2015). The percentage of tag weight to body weight averaged 0.5% (SD = 0.1%) and ranged between 0.003 to 0.021 for the 1,440 study fish; well below the recommended 5%.

Tags were custom programmed with three separate codes: a traditional Pulse Position Modulation (PPM) style coding that pulsed every 60 s (average), along with one hybrid PPM/High Residence (HR) coding every 60 s. Eight high residency IDs were superimposed and transmitted on each hybrid transmission every 60 s. Each tag transmitted IDs, on average, every 30 s. All data was transmitted at 180 kHz. The HR component of the coding allowed for detection in areas where collisions were anticipated caused by many tags emitting signals at the same time to the same receiver. Battery life of the V5 tags was estimated by the manufacturer to be 58 d (95% probability) and 69 d (50% probability). However, during the first week of releases, tags had a software bug that resulted in premature failure. The software bug was fixed for fish released in weeks two and three but was not discovered or addressed until after the first week of tags had been used and fish were released.

Tags were soaked in saline water for at least 24 h prior to tag activation. Tags were activated approximately 24 h 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 estimated to the nearest minute.

Surgeon Training

Steelhead tag training was conducted from March 16–21, 2014, with the first day at the Lodi FWS office and the remaining days 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). Four surgeons

were trained (three from USFWS and one from NOAA-Fisheries), but only three were used for the actual tagging (two from USFWS and one from NOAA-Fisheries). One surgeon from USFWS received refresher training and was required to tag fewer fish than the new surgeons during the training. The three new surgeons received more extensive training on surgical techniques and were required to tag more fish during training. Training included sessions on knot tying, mock surgery on bananas, tagging dead fish, and finally tagging live fish. A sample of live fish were held overnight and necropsied the next day to evaluate techniques and recovery. A mock tagging session was held on March 21 to practice logistical procedures, establish a flow to the operations, and to identify and discuss solutions to potential problems that could occur during tagging.

Tagging

A total of 1,584 juvenile steelhead were tagged at MKRH over the course of the 2014 study, including 1,440 study fish and 144 dummy-tagged fish (Table 1). Fish were tagged over three tagging weeks: March 25–27, April 23–25, and May 20–22, 2014. Days were further divided into three sessions; each with one transport truck per session.

During each week of tagging, 480 study fish were tagged and 48 fish were dummy-tagged. 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; those to be processed for fish condition were also tagged with Passive Integrated Transponder (PIT) tags to allow for individual identification. Dummy-tagged fish were held at the release site for 48 h, after which they were euthanized and examined for condition or given to the California/Nevada Fish Health Center (CA/NV FHC) for pathogen screening. Steelhead given to the CA/NV FHC were not tagged with PIT tags.

The present standard operating procedure (SOP; Appendix A) 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 was made during each tagging day to ensure compliance with the SOP (Table 2). The difference in water temperature between the raceway and the gravity feed was out of compliance once (5/22/14); however, it exceeded the established range by only 0.2^0 C (Table 3). Dissolved oxygen concentrations were above the desired range defined in the SOP multiple times. Corrective actions were taken immediately after discovering non-compliant temperatures or DO concentrations.

Fish were taken off feed 24 h prior to the beginning of each tagging day. Fish were held in the MKRH raceway (Figure 1) and were corralled 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 removed from the raceway garbage can and placed into an 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, it was 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 during surgery. Fish were then measured (FL) to the nearest mm and weighed to the nearest 0.1 g before tag implantation (Figure 2). Average surgery times were 2:22 (m:ss; SD= 0:21; range: 1:24 to 4:12). A clip of the caudal fin was taken for DNA (Clemento et al. 2017). Tissue

for DNA was taken from study fish in 2014 to answer two primary questions: 1) Do resident and anadromous *O. mykiss* adult phenotypes consistently differ at certain parts/regions of the genome? and 2) Is there an equal probability of juvenile entrainment at the salvage facility for each of the four hatcheries?

Once the tag was inserted into the fish's body cavity, the fish was transferred to and held for 10 minutes in a 19 L (5 gal) non-perforated bucket filled with 10 L of water supersaturated with oxygen (130–150%) to recover from anesthesia (Figure 3). 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.

Transmitter Validation

Tagged fish were monitored by two VR100 acoustic receivers with 180 kHz hydrophones placed in the recovery buckets to confirm the operational status of each transmitter prior to transportation to the release sites (Figure 3). 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-min recovery period, the (generally) three fish held across two buckets were combined into a 68 L (18 gal) perforated tote and immediately loaded into the tank on the transport truck. Totes were perforated starting 15 cm from the bottom to allow water exchange in the transport truck's holding tank. Each tote was covered with a labeled snap-on lid that facilitated the placement of each tote into the transport truck, as well as into the 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. The transport tanks for steelhead were designed to securely hold twenty-four 68-L perforated totes. Tanks had an internal frame that held totes in individual compartments to minimize contact between containers and to prevent tipping (Figure 4). 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 (26-ft) flatbed truck that was equipped with an oxygen tank and hosing to deliver oxygen to each of the tanks during transport (Figure 5).

The oxygen system consisted of an oxygen tank (282 ft^3 capacity) mounted to a metal frame. A Weldmark (Model #RC250-80-540) medium-duty regulator was used to regulate pressure from the tank to a Victor (Model #1000-0189) 7 LPM flow meter. The oxygen flow rate was maintained at 2 LPM during transport. Dissolved oxygen (DO) levels were checked twice per transport: once after fish were completely loaded into the transport tank and again when the transport truck reached the holding site at Durham Ferry. The oxygen flow rate was reduced if DO levels were above 10 mg/L (100% saturation).

Each truck made one trip to the holding/release site per tagging day, for a total of three trips per day (Table 1). The trip from the MKRH to Durham Ferry took approximately 75 min.

Water temperature and DO in the transport tanks was measured using a YSI 85 or ProODO meter prior to loading totes, after loading totes into transport tanks but before leaving the MKRH, and at the release site after transport but prior to unloading totes (Appendix B:). The water temperature and DO were also measured in the river at the holding site prior to moving the fish into containers in the river. If the difference between the temperature of the transport tank water and the river was >5˚C, the fish would have required tempering. .

During transport, water temperature in each tank was recorded using Onset TidbiT v2 Temp Loggers, one per tank. These temperature loggers were set take a reading every 60 s. The loggers remained in their respective transport tanks for up to seven days; however, only the temperature data from the actual transport periods were utilized. Temperature data were downloaded and exported at the end of each transport period.

The transport tank plug was not completely closed during transport of the second group of steelhead from the MKRH to Durham Ferry on April 25, 2014. Upon the truck's arrival at the holding site, it was observed that the water in the tank had drained to a point where the fish were inside of the totes with an unacceptable amount of water. These fish were not used for the study and were returned to the hatchery where the tags were surgically removed, disinfected, and used to tag a new batch of fish. The new fish were transported to the holding site and placed into their holding cans after the third transport for that day was complete. As a result of this issue, the second set of tagged fish in transport truck number two arrived at the holding location at 1936 hours.

One fish from the second truck on March 27 was culled after transport and prior to release in 2014 (Table 4). Water temperatures prior to transport averaged 14.0 °C and ranged from 12.6 to 16.1 °C (Table 4). Temperatures taken after transport averaged 15.0 °C and ranged between 12.5 to 17.7 °C (Table 4). Temperatures during some transports appear to have decreased; however, a decrease of a tenth of a degree is not a significant reduction in temperature and may be attributable to variation within the temperature monitoring device. There was no need to add ice to the transport tanks during transport as the water temperatures did not increase significantly (Appendix C ; Table 4). None of the fish required tempering in 2014 (Table 4)

Transfer to Holding Containers

Once the transport truck arrived at the holding site, fish were removed from the transport tank and placed into holding cans in the river. The use of "clean" waders or hip boots was required of staff on the flatbed of the transport truck to minimize bio-contamination when the truck returned to MKRH. The river's water temperature and DO levels were measured soon after the transport truck arrived at the holding site. To begin transferring fish to holding cans, approximately ten 68-L non-perforated totes ("sleeves") were placed into the back of a pickup truck and filled halfway with river water. The pickup was then driven from the river's edge to where the transport truck was parked on the levee road and positioned alongside the transport truck. The perforated totes were then unloaded from the transport truck into the partially filled sleeves in the pickup truck. Transporting the perforated totes in sleeves allowed the water level of the totes to rise above the tote perforations, providing fish with more access to water during transport outside of the transport tank. 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 between two and three trips between the transport truck and the river before transfer of all totes to the river's edge was complete.

Once at the river's edge, the tagged steelhead were transferred from the totes into 166 L (44 gal) plastic garbage cans ("holding cans") held in the river (Figure 6). Each holding can was perforated with 1.27 cm diameter holes. Generally, four totes containing three fish each were emptied into each perforated holding can. Each holding can was labeled to ensure that fish in each labeled tote were loaded into the correct holding can for later release at the correct time.

After transfer to the holding cans, the 68 L totes were collected and placed on a clean tarp (4.2 m x 4.2 m) and allowed to dry. At the end of the day, all 68 L totes were transported back to the Lodi Fish and Wildlife Office. These totes were then transported to the Mokelumne River Hatchery on the next day, where they were placed into a -20˚C freezer for 24 h prior to reuse.

Water temperature in the river at the release site ranged from 14.8 to 21.6°C, with an average during the first week of 16.6°C, an average during second week of 15.5°C, and an average during the third week 20.0 °C. Differences in water temperature between the transport tank when arriving at the release site and in the river had a difference of between 0.1 and 4.6°C, with the water temperature at the release site being consistently warmer than in the transport tank (Table 4). However, tempering was not required during the 2014 steelhead study as the difference between the water temperature in the transport truck and the river was not greater than 5^0 C.

Dissolved oxygen levels varied during transports, with some of the DO readings increasing (Table 4). This is most likely due to our infusion of oxygen into the transport tanks during transport. Transport drivers were directed to decrease the inflow of oxygen if levels appeared to be at saturation (greater than 10 mg/l). In some cases, flow rates were decreased from 2.0 LPM to as low as 0.5 LPM to reduce the level of DO during transport.

Upon arrival at the release location, DO concentration varied from a low of 9.61 mg/L to a high of 13.33 mg/L (Table 4). Dissolved oxygen levels ranged between 8.4 and 15.9 mg/L for all measurements in the transport tanks or in the river.

Fish Releases

Tagged study fish were held in the perforated holding cans for approximately 24 h prior to release, at which time they were transported downstream by boat to the release location (Figure 7). The release location was in the middle of the channel approximately 200 m downstream of the holding cans. A Lodi USFWS research vessel (G3, 16 ft aluminum with 25 HP Honda outboard, tiller steer) was used to transport the holding containers to the specified release site. During each release, two to three holding containers were unclipped from the tether line and placed into non-perforated sleeves (Figure 7). Non-perforated sleeves and downstream releases were used to potentially reduce initial predation of tagged fish immediately after release. The potential existed for predators to have congregated near the holding location and followed the smell of the water originating from within the perforated holding cans as the holding cans were moved downstream, which would result in high initial mortality from predation. Once the holding containers were placed into the sleeves they were clipped to the gunnel of the research vessel. These holding containers were then transported to the specified release site; a handheld GPS unit (Garmin 76c) was used to mark the exact release location. Releases were made every

four hours after the beginning of the 24 h 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). Fish were released per this schedule to allow them to distribute over the 24 h tidal cycle.

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

Once the release was completed, the date and release time for any dead fish was recorded and the tags were removed. The tags were returned to the tagging location or office to have the individual tag identified. There were four steelhead mortalities that occurred after holding and prior to release (Table 4). One group of fish was inadvertently released prior to being held for 24 h. These were fish transported in the third truck on 5/22/14 and released approximately two hours later on the same day (Table 1).

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 the first two days of each week of tagging and transport, 12 fish were implanted with dummy transmitters and included in the tagging process (Table 1). An additional 24 dummy-tagged fish per week (tagged on Day 3) were retained for assessment of pathogens and disease by the CA/NV FHC (discussed in the next section).

Groups of dummy transmitters (consisting of three 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 and they did not have a fin clip for taking a tissue sample. The conditions of their transport to and holding at the release site were the same as for fish with active transmitters. However, unlike the tagged fish for the study, 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 h, after which they were evaluated for mortality and condition (Figure 8). 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 (Appendix B:). Water temperature and DO were taken in the perforated holding can after returning from the mimicked boat transport in the river just prior to assessment (Table 6).

The dummy-tagged fish from each week (tagged on Day 1 and Day 2; Table 1) were examined to determine if any fish had died or were compromised 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, including percent scale loss, body color, fin hemorrhaging, eye quality, and gill coloration (Table 5).

One of the 72 dummy-tagged fish was found dead when evaluated after 48 hours (Table 6). Sixty of the remaining 71 had normal gill coloration, 52 of the 71 had normal eye quality, 56 of 71 had normal body coloration and no fish had fin hemorrhaging (Table 6). Mean scale loss for all fish assessed ranged from 9.6 to 25.0% (). Mean fork length (FL) of fish ranged from 228.3 to 253.3 mm (Table 6)

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 (Table 7a). A score of 0-2 was applied to each suture. A composite score (0–12) was calculated from seven parameters to consider possible tagging effects that could affect survival of study fish (T. Liedtke, personal communication).

A mean composite score for surgical condition of all the fish was 2.7, also indicating these fish were somewhat compromised (Table 8). These data indicate that the fish used for the 6 year study in 2014 were in less than optimal condition.

Fish Health

Fish used for fish health examinations were held for 48 h at the Durham Ferry release site on the San Joaquin River before sampling. Groups of 24 MKRH yearling steelhead were sampled on March 29, April 27, and May 24, 2014. Fish were euthanized, fork length (FL) was recorded, any abnormalities were noted, and tissue was sampled for lab assays (Appendix D 1.).

Lab assays were conducted for bacteriology, virology, and histopathology. For bacteriology, a sample of kidney tissue was collected aseptically and inoculated onto brain-heart infusion agar. Bacterial isolates were screened by standard microscopic and biochemical tests (Appendix D 1.: USFWS and AFS-FHS 2010). These screening methods would not detect *Flavobacterium columnare.* *Renibacterium salmoninarum* (the bacteria that causes bacterial kidney disease) was screened by fluorescent antibody tests of kidney imprints.

For virology, three fish were pooled for samples of kidney and spleen and were inoculated onto EPC and CHSE-214 at 15°C as described in the AFS Bluebook (Appendix D.:USFWS and AFS-FHS 2010) with the exception that no blind pass was performed.

For histopathology, the tissues were removed from the fish and immediately fixed in Davidson's fixative. In the lab, the tissues were processed and cut into 5 µm paraffin sections and stained with hematoxylin and eosin (Appendix D: Humason 1979). All tissues for a given fish were placed on one slide and identified by a unique code number. Each slide was examined under a light microscope and observations of abnormalities were noted. Gill tissues from all 24 fish were examined for signs of external parasite infection.

Gill Na^+/K^+-adenosine triphosphatase (gill ATPase) activity was assessed by assays following the methods of McCormick (1993; Appendix D). Gill ATPase activity is correlated with osmoregulatory ability in saltwater, and high concentrations are found in the chloride cells of the lamellae.

There was one dummy-tagged steelhead found dead on March 29 that was to be used for the fish health assessments (Appendix D). One other dummy-tagged steelhead died on 5/24 during preparation for the health assessments. Steelhead survival over the 24 h holding period was high, and no significant pathogen infections were detected. Gill ATPase levels were stable or increasing over the study period, suggesting levels of smolt development were not a factor in fish performance.

Tag Retention Study

Fifty steelhead (17 fish each for Surgeons A and B, 16 fish for Surgeon C) were implanted with an inactive VEMCO acoustic V5 transmitter and a PIT tag and had a fin clipped. Tissue was collected from the tag retention fish to be comparable to the study fish.

Incisions were closed with two simple interrupted stitches tied with square knots with non-absorbable suture; the same as for the study fish. A control sample of 10 fish did not undergo surgery, insertion of a PIT tag, or have a tissue sample taken. After tagging on March 24, 2014, the fish were taken to the Tracy Fish Collection Facility (TFCF) and housed in two indoor tanks. Each tank held 25 dummy tagged fish and 5 untagged control fish, with an approximately equal number of fish from each surgeon. A non-lethal assessment was done on May 9, 2014 (46 days after tagging), which consisted of scoring for presence and condition of sutures, suture pattern, irritation around suture site, incision apposition, incision healing, and fungus presence. The fish were held for a total of 68 days and necropsied on May 30, 2014.

On May 30, fish were euthanized with a lethal dose of MS-222 and necropsied to assess if tags were retained and encapsulated. Control and tagged groups were compared for differences in mortality, the proportion of tags retained, and for dummy tagged fish, overall condition and condition of incisions and sutures, similar to assessments at 46 days after tagging (Table 7b).

At the time of the non-lethal assessment (46 d post-surgery), 3 of 50 fish (6%) were missing one suture, and 1 fish (2%) did not retain any sutures. In addition, 10 fish (20%) displayed some degree of irritation around at least one of the suture sites. Incision apposition was poor in three fish (6%), and the incision was not yet healed in 8 fish (16%). An additional eight fish (16%) displayed some degree of fungus growth.

Tag retention fish were necropsied on May 30, 2014, 68 days after they were tagged at the MKRH. Both control and dummy-tagged fish had 100% survival at the end of the holding period. Tag retention was also 100%, and fish tagged by Surgeons A, B, and C displayed signs of tag expulsion at a rate of 0.00 ± 0.00 (mean ± standard deviation), 0.06 ± 0.24, and 0.06 ± 0.25, respectively (Table 9). Dummy-tagged retention fish increased in fork length by 39 ± 13 mm over the course of the holding period; final sizes of control and dummy-tagged fish were similar (Table 9). Control and dummy-tagged fish also scored similarly for body color, gill color, fin hemorrhaging, and eyes. Composite scores (potential range 0–27) for these tag retention dummy-tagged fish were 3.12 ± 2.47. This score is not directly comparable to those obtained with the dummy tagged fish held at the release site, since some of the parameters are different (Table 7). However, it does show the loosening of sutures and an increase in tag expulsion and fungus over time. The results from the tag retention fish indicate that any mortality in study fish was not likely, directly caused by tag implantation.

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Table 1. The tagging, transport, holding date and times, and numbers of steelhead released during the 2014 Steelhead Survival Study. Fish were released over a 24 hour period after being held for a minimum of 24 hours.

TAGGING DATE	TRANSPORT DATE TIME	START HOLDING DATE TIME	TOTAL RELEASED (A+B+C +D+E+F)	EARLY RELEASES - NOT HELD 24 HOURS DATE TIME	EARLY RELEASES - NOT HELD 24 HOURS NUMBER RELEASED	RELEASE A DATE TIME	RELEASE A NUMBER RELEASED	RELEASE B DATE TIME	RELEASE B NUMBER RELEASED	RELEASE C DATE TIME	RELEASE C NUMBER RELEASED	RELEASE D DATE TIME	RELEASE D NUMBER RELEASED	RELEASE E DATE TIME	RELEASE E NUMBER RELEASED	RELEASE F DATE TIME	RELEASE F NUMBER RELEASED	FISH HEALTH DUMMY TAGGED
3/25/2014	3/25/14 1030-1145	3/25 1254	162			3/26 1517, 1519	24	3/26 1925, 1927, 1929	30									3
3/25/2014	3/25/14 1245 - 1355	3/25 1445		3/25 1440	2 (b)			3/26 1929	6	3/26 2311, 2315	24	3/27 0300	22					3
3/25/2014	3/25/14 1530 - 1625	3/25 1711												3/27 0700	36	3/27 1059	18	6
3/26/2014	3/26/14 1030 - 1155	3/26 1250	162			3/27 1511, 1517	24	3/27 1907, 1909	24	3/27 2302	6							3
3/26/2014	3/26/14 1230 - 1345	3/26 1430								3/27 2302, 2304,2305	30	3/28 0257	24					3
3/26/2014	3/26/14 1500 - 1559	3/26 1640												3/28 0703	36	3/28 1059	18	6
3/27/2014	3/27/14 1025 - 1130	3/27 1235	155			3/28 1507, 1509	24	3/28 1903, 1904	24	3/28 2302	4							6***
3/27/2014	3/27/14 1210 - 1328	3/27 1425								3/28 2302, 2304, 2305	28 (2)	3/29 0255	23**					6***
3/27/2014	3/27/14 1430 - 1526	3/27 1625								3/28 2302	4			3/29 0656	24	3/29 1053	24	12***
4/23/2014	4/23/14 1200 - 1330	4/23 1415	162			4/24 1500	24	4/24 1904, 1905	30									3
4/23/2014	4/23/14 1410 - 1520	4/23 1600						4/24 1904	6	4/24 2301	24	4/25 0258	24					3
4/23/2014	4/23/14 1622 - 1723	4/23 1812												4/25 0657	36	4/25 1059	18	6
4/24/2014	4/24/14 1000-1130	4/24 1230	162			4/25 1500	24	4/25 1900	24	4/25 2302	6							3
4/24/2014	4/24/14 1225 - 1338	4/24 1416								4/25 2301, 2302	30	4/26 0257	24					3
4/24/2014	4/24/14 1443 - 1543	4/24 1632												4/26 0702	36	4/26 1056	18	6
4/25/2014	4/25/14 1015-1120	4/25 1209	156			4/26 1501	24	4/26 1900	24	4/26 2300	4							6***

4/25/2014	4/25/14 1835-1936	4/25 2030 (a)									4/26 2300	28	4/27 0257	24						6***
4/25/2014	4/25/14 1442-1552	4/25 1640									4/26 2300	4			4/27 0658	24	4/27 1057	24		12***
5/20/2014	5/20/14 1015 - 1125	5/20 1250	161			5/21 1503	23(1)	5/21 1900, 1901	30											3
5/20/2014	5/20/14 1220-1330	5/20 1428						5/21 1901	6	5/21 2300	24	5/22 0255	24							3
5/20/2014	5/20/14 1449- 1548	5/20 1655													5/22 0658	36	5/22 1055	18		6
5/21/2014	5/21/14 1030 - 1140	5/21 1224	162			5/22 1500	24	5/22 2320	6	5/23 0009	24									3
5/21/2014	5/21/14 1210-1340	5/21 1425						5/22 2320, 2321	30			5/23 0302	24							3
5/21/2014	5/21/14 1444 - 1542	5/21 1630													5/23 0656	36	5/23 1055	18		6
5/22/2014	5/22/14 1015 -1140	5/22 1235	155			5/23 1505	24	5/23 1900	23(1)	5/23 2259	4									6***
5/22/2014	5/22/14 1235-1400	5/22 1435								5/23 2259	28	5/24 0257	24							6***
5/22/2014	5/22/14 1521-1619	5/22 1720		5/22 1900, 1901	36 (b)					5/23 2259	4				5/24 1055	12				12***

*pre-release mortalities were observed; ( ) number of mortalities entered in parenthesis. ** One fish culled after transport. *** Fish given to the CA/NV Fish Health Center for disease assessments

(a) Fish in transport truck #2 were dewatered, resulting in their rejection. Transport truck returned to MKRH where additional fish were tagged and transported to the holding site. (b) Fish were inadvertently released prior to being held for 24 hours.

Table 2. Parameters and criteria assessed during the quality assurance and quality control inspections of the 2014 Steelhead Survival Study. Parameters were provided by T. Liedtke, USGS.

Parameter Assessed	Assessment Criteria
Anesthesia bucket temperature	Was temp in anesthesia bucket <2°C different than fish source?
Gravity feed temperature	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 temperature	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 2014 Steelhead Survival Study. Highlighted values are those falling outside accepted criteria.**

Date	Time	Raceway Temp (°C)	Anesthesia Bucket Temp (°C)	Diff. from Raceway (°C)	Gravity Feed Temp (°C)	Diff. from Raceway (°C)	Pre-Recovery Bucket DO (%)	Post-Recovery Bucket DO (%)	Pre-Recovery Bucket Temp (°C)	Post-Recovery Bucket Temp (°C)	Pre-Diff. from Raceway (°C)	Post-Diff. from Raceway (°C)
3/25/2014	-----	12.4	13.8	1.4	13.2	0.8	176	155	12.5	12.6	0.1	0.2
3/26/2014	-----	12.1	-----	-----	-----	-----	139.3	123.8	12.8	13.1	0.7	1
3/27/2014	1349	12.5	12.8	0.3	13	0.5	133.8	119.2	12.5	13	0	0.5
4/23/2014	1511	13.6	13.8	0.2	14.5	0.9	147.6	116.4	13.6	15	0	1.4
4/24/2014	1104	13.1	13.8	0.7	13.7	0.6	139.2	120.8	13.6	13.9	0.5	0.8
4/25/2014	-----	13	13.2	0.2	13.2	0.2	114.5	137.6	13.1	14.7	0.1	1.7
5/20/2014	1110	14.2	14.8	0.6	14.8	0.6	142.6	123.8	14.7	15.1	0.5	0.9
5/21/2014	1341	14.6	15.8	1.2	16.1	1.5	138.3	133.2	15.6	15.6	1	1
5/22/2014	1410	14.9	16.8	1.9	17.1	2.2	132	111.8	16.1	16.5	1.2	1.6
Average		**12.8**	**13.5**	**0.6**	**13.5**	**0.6**	**141.7**	**128.8**	**13**	**13.7**	**0.2**	**0.9**
SD		**0.55**	**0.46**	**0.51**	**0.61**	**0.27**	**20.13**	**14.82**	**0.5**	**0.98**	**0.29**	**0.56**

Table 4. Water temperature and dissolved oxygen (DO) concentration 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 2014 Steelhead Survival Study. Averages (AVG) are provided at the end of each week.

Transport Date	Loading time	Tank after loading Temp (˚C)	Tank after loading DO (mg/L)	Tank after transport Temp (˚C)	Tank after transport DO (mg/L)	# Morts after transport	River Temp (˚C)	River DO (mg/L)	Mortalities just prior to release
3/25/2014	845	12.9	9.65	13.4	9.61	0	16.5	14.23	0
3/25/2014	1050	13.3	11.27	16.1	10.04	0	17.3	14.89	0
3/25/2014	1331	13.6	11.61	14.1	12.38	0	18.1	15.9	0
3/26/2014	900	12.8	10.25	12.5	11.27	0	15.5	9.9	0
3/26/2014	1041	12.7	11.35	13.1	11.49	0	16.2	10.2	0
3/26/2014	1314	13.2	11.67	13	12.35	0	16.8	10.7	0
3/27/2014	900	12.6	10.39	13.7	9.89	0	15.7	10.2	0
3/27/2014	1042	12.7	11.37	14.5	10.87	1	16.4	10.6	2
3/27/2014	1257	13.2	12.02	13.7	13.33	0	17	10.8	0
AVG		13	11.06	13.8	11.25		16.6	11.93	
4/23/2014	955	13.4	10.46	14.7	10.18	0	15.5	10.5	0
4/23/2014	1250	13.6	11.69	15.9	11.15	0	16.1	10	0
4/23/2014	1450	13.9	12.05	14.7	13.15	0	16.1	9.6	0
4/24/2014	830	13.9	10.36	15	9.9	0	15.1	9.5	0
4/24/2014	1030	13.5	11.72	15.4	11.22	0	15.7	11.8	0
4/24/2014	1313	14.1	12.14	14.9	13.08	0	16.2	12.6	0
4/25/2014	845	13.3	10.34	14	10.32	0	15	10.6	0
4/25/2014	1715	13.5	12.79	13.2	13.33	0	14.8	10.6	0
4/25/2014	1307	13.3	11.97	13.3	12.72	0	15.2	10.9	0
AVG		13.6	11.5	14.6	11.67		15.5	10.68	
5/20/2014	845	15.9	9.85	15.7	10.18	0	18.6	8.4	1
5/20/2014	1055	14.4	9.9	15.7	10.17	0	19.4	8.7	0
5/20/2014	1318	14.8	11.9	15.6	12.17	0	20.2	8.7	0
5/21/2014	845	16.2	9.7	16.9	9.65	0	18.6	8.4	0
5/21/2014	1050	14.7	9.87	17.3	10.18	0	20	9	0
5/21/2014	1313	16.1	11.71	17.5	11.86	0	21.1	9.3	0
5/22/2014	830	15.8	9.89	16.7	10.02	0	19.3	9.5	1
5/22/2014	1110	15.4	9.85	17.6	9.69	0	20.9	10.1	0
5/22/2014	1342	15.8	11.71	17.7	11.86	0	21.6	10.1	0
AVG		15.5	10.49	16.7	10.64		20	9.13	

Table 5: External characteristics assessed for steelhead smolt condition and short-term survival during the 2014 Steelhead Survival Study. Percent scale loss was also assessed and was the scale loss as a percentage of the total on both sides of the fish. Numerical scores are given in parentheses.

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. Results from dummy-tagged fish held at Durham Ferry for 48 h prior to release during the 2014 Steelhead Survival Study. Criteria are defined in Table 5. Fish that died prior to assessment did not have condition characteristics assessed. Values for fork length and scale loss are presented as mean (standard deviation).

Examination Date, Time	Temperature (0C) and Dissolved oxygen (DO) mg/l	Fork Length (mm)	Mortality	Scale Loss (%)	Normal Body Color	No Fin Hemorrhaging	Normal Eye Quality	Normal Gill Color
3/27/14, 1116	15.5; 10.3	236.7 (23.5)	0/12	24.2 (19.3)	8/12	12/12	12/12	10/12
3/28/14, 1105	15.3; 10.2	228.3 (21.8)	0/12	25.4 (22.0)	10/12	12/12	12/12	11/12
3/29/14, 1130			0/24*					
4/25/14, 1115	15.0; 10.3	247.3 (19.5)	1/12	15.4 (11.0)	10/11	11/11	10/11	10/11
4/26/14, 1115	14.6; 10.2	240.8 (13.9)	0/12	13.3 (8.1)	12/12	12/12	8/12	10/12
4/27/14, 1130			0/24*					
5/22/14, 1115	20.2 9.0	253.3 (10.9)	0/12	9.6 (5.8)	10/12	12/12	5/12	12/12
5/23/14, 1115	20.5 8.9	248.3 (11.5)	0/12	25.0 (14.5)	6/12	12/12	7/12	7/12
5/24/14, 1130			0/24*					

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

Table 7: Necropsy variables for assessing composite scores for dummy tag fish held for 48 hours (a) and for tag retention at (b) 46 and 68 days.

a.

Variable Name	**Score**	**Description**
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%)
Fungus Present?	0	No fungus present
	1	Fungus present
Fungus Location		
	Suture	Fungus on the suture material itself
	Incision	Fungus on skin in/around incision
	Tail	Fungus on skin on the tail
	Body	Fungus on skin on the body
Organ Damage	0	No organ damage present
	1	Some organ damage present
Peritoneal Apposition	0	Peritoneum completely closed, perfect apposition
	1	Peritoneum partially closed
	2	Peritoneum completely open (>75%)
Signs of Expulsion	0	No signs of tag expulsion
	1	Some bulging or lateral pressure
	2	Expulsion process obvious or complete

b.

Variable Name	**Score**	**Description**
Suture Present?		
	0	Yes
	1	Yes, but untied or becoming untied
	2	No
Suture Pattern	0	Pattern intact
	1	Pattern not intact
Suture Irritation	0	No irritation
	1	Mild irritation (redness or swelling)
	2	Moderate irritation (redness or swelling)
	3	Severe irritation (purulent discharge)
	4	Ulceration
Incision Apposition	0	Completely closed, perfect apposition
	1	Incision partially open due to gape or overlap
	2	Incision completely open (>75%)
Incision Healing	0	Completely healed
	1	Partially healed
	2	Not healed
Fungus Present?	0	No fungus present
	1	Fungus present
Disease Present?	0	No signs of disease
	1	Signs of disease present
Organ Damage	0	No organ damage present
	1	Some organ damage present
Signs of Tag Expulsion	0	No signs of tag expulsion
	1	Some bulging or lateral pressure
	2	Expulsion process obvious or complete

Table 8. Results of characteristics assessed on dummy-tagged steelhead before necropsy during the 2014 Steelhead Survival Study. The parameters, 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 confounding effects of compromised parameters on survival. Values are presented as mean (standard deviation).

Date, Time	Ant. Suture present? (0-2)	Suture present? (0-2)	Incision Apposition (0-2)	Incision Apposition	Organ Damage (0-1)	Peritoneal Apposition (0-2)	Signs of Tag Expulsion (0-2)	Composite Score (0-12)
3/27/14, 1116	0.1 (0.3)	0 (0)	0.5 (0.7)	0 (0)	0.7 (0.5)	1.2 (0.8)	0 (0)	2.4 (1.0)
3/28/14, 1105	0 (0)	0 (0)	0.4 (0.7)	0.3 (0.5)	0.5 (0.5)	1.75 (0.5)	0 (0)	3.0 (1.3)
3/29/14, 1100	*	*	*	*	*	*	*	*
4/25/14, 1115	0 (0)	0 (0)	0.2 (0.6)	0 (0)	0.2 (0.4)	1.8 (0.4)	0 (0)	2.2 (0.6)
4/26/14, 1115	0 (0)	0 (0)	0.8 (1.0)	0 (0)	0.3 (0.5)	2 (0)	0 (0)	3.1 (0.9)
4/27/14, 1100	*	*	*	*	*	*	*	*
5/22/14, 1115	0 (0)	0 (0)	0.3 (0.6)	0 (0)	0.5 (0.5)	2 (0)	0 (0)	2.8 (0.8)
5/23/14, 1115	0 (0)	0 (0)	0.4 (0.8)	0 (0)	0.5 (0.5)	2 (0)	0 (0)	2.9 (0.8)
5/24/14, 1130	*	*	*	*	*	*	*	*

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

Table 9. Results of fish condition (a), surgical characteristics (b), mortality, tag retention, air time, initial weight, fork length (initial, final, and increase) and scale loss (c) after fish were retained for 68 days (between March 24 and May 30, 2014).

A

Group	*Body Color*	*Gill Color*	*Fin Hemorrhaging*	*Eyes*
Control	0.2	0.1	0	0
Pooled Dummy	0.14	0.02	0	0.02
Surgeon A	0.06	0	0	0
Surgeon B	0.12	0.06	0	0
Surgeon C	0.25	0	0	0.06

b

Treatment Group	**Pooled Dummy**	**Surgeon A**	**Surgeon B**	**Surgeon C**
Signs of Tag Expulsion	0.04 (0.20)	0.00 (0.00)	0.06 (0.24)	0.06 (0.25)
Anterior Sutures	0.20 (0.40)	0.29 (0.47)	0.29 (0.47)	0.00 (0.00)
Posterior Sutures	0.24 (0.43)	0.41 (0.51)	0.18 (0.39)	0.13 (0.34)
Suture Pattern	0.42 (0.50)	0.35 (0.49)	0.47 (0.51)	0.44 (0.51)
Suture Irritation	0.40 (0.63)	0.43 (0.68)	0.44 (0.68)	0.33 (0.54)
Incision Apposition	0.04 (0.20)	0.00 (0.00)	0.12 (0.33)	0.00 (0.00)
Incision Healing	0.18 (0.44)	0.24 (0.56)	0.24 (0.44)	0.06 (0.25)
Organ Damage	0.30 (0.46)	0.24 (0.44)	0.18 (0.39)	0.50 (0.52)
Fungus Presence	0.04 (0.20)	0.06 (0.24)	0.06 (0.24)	0.00 (0.00)
Disease	0.06 (0.24)	0.00 (0.00)	0.06 (0.24)	0.13 (0.34)
Composite Score	4.72 (4.45)	5.00 (4.78)	5.18 (4.67)	3.94 (4.01)

C

Treatment Group	**Survival (%)**	**Tag Retention (%)**	**Air Time (mm:ss)**	**Initial Weight (g)**	**Initial Fork Length mm (SD)**	**Final Fork Length mm (SD)**	**Increase Fork Length mm (SD)**	**Scale Loss % (SD)**
Control	100	-----	-----	-----	-----	263 (16)	-----	9 (15)
Pooled Dummy	100	100	03:16 (00:38)	127.5 (31.2)	233 (21)	272 (22)	39 (13)	11 (11)
Surgeon A	100	100	03:11 (00:44)	125.1 (40.1)	229 (28)	270 (25)	40 (11)	10 (9)
Surgeon B	100	100	03:36 (00:36)	133.3 (26.5)	239 (17)	275 (23)	36 (12)	11 (12)
Surgeon C	100	100	02:59 (00:19)	123.8 (25.6)	231 (16)	272 (20)	41 (14)	12 (13)

Figures

Figure 1: Perforated garbage cans placed in the raceway for steelhead in prior to tagging.

Figure 2: Tagging set up for steelhead at Mokelumne River Hatchery in 2014. Fish in picture is a Chinook salmon, but the set up for steelhead was similar. Fish were weighed to the nearest 0.1 g and measured (FL) to the nearest mm immediately prior to tag implantation.

Figure 3. The recovery bucket and tag verification staging area at the Mokelumne River Hatchery during the 2014 Steelhead Survival Study. Recovery buckets, which each contained 1–2 tagged fish and 130–150% oxygen saturation, were placed to the left of the staging area immediately after surgery. Their tags were validated and the recovery buckets were moved to the right side of the staging area to await completion of 10 min in the oxygenated recovery buckets. After recovery, 2–3 buckets were combined into a perforated tote in a sleeve of water. Photo credit: USFWS.

Figure 4. Perforated totes containing tagged steelhead in the transport tank on a transport truck.

a)

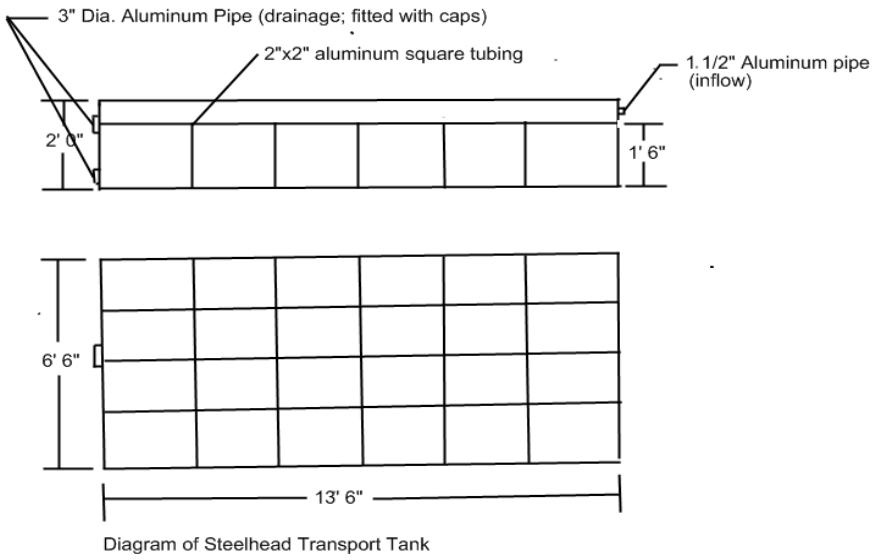


Diagram of Steelhead Transport Tank

b)

Figure 5. Dimensions and diagram of steelhead transport tanks (a) and the prepared transport truck used to transport tagged steelhead during tagging operations of the 2014 Steelhead Survival Study (b). 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. Photo credits: USFWS.

Figure 6. Release site at Durham Ferry in Manteca, CA with holding cans in the river.

Figure 7a. **A fish release at Durham Ferry. Tagged fish were held in perforated holding cans in the river for approximately 24 h after tagging. The cans were then transported to the middle of the channel approximately 200 m downstream of the holding cans where the fish were released (a).**

7b.Immediately before release, the perforated cans were placed into non-perforated sleeves to reduce the chance of predators smelling fish in the cans and following them to the release site. Photo credits: Pat Brandes/USFWS.

Figure 8: Dummy tagged fish in holding can being processed in 2014.

Appendices

Appendix A

Date : 3/19/14
Doc Version: V3_FINAL

Standard Operating Procedure

Acoustic Tagging for Steelhead 2014 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 dessicator 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 NET** to capture source fish and place them into an anesthesia bucket.

Appendix B:

Date : Final 04/7/14

Standard Operating Procedure

Holding and Releasing Acoustically Tagged Fish 2014 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.

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 release crew and let them know he is leaving the hatchery and let the release crew know the temperature in the transport truck after loading so the unload crew 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 for the fish being transported 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 crew/set-up crew will drive a pick-up down to the river once the fish arrive and fill up eight tote sleeves 1/2 full of clean river water and place them into the bed of the pick-up truck.

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

They 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

Once the transport tank temperature is taken and recorded, the perforated totes will be transferred, one at a time, by the truck driver and one unloader from the transport tank to two other unloaders in the pick-up truck who place the perforated totes into the partially-filled sleeves in the pick-up truck. Start unloading with the highest group tote number first. After transfer there should be approximately 12 gallons of water in each perforated tote, within a tote sleeve. Repeat for 8-9 totes prior to delivering to the river holding site.

Transport water filled tote sleeves back to the transport truck for additional loads of perforated totes with study fish and dummy tagged fish and bring down to the river's edge.

Holding site tempering

If the difference, between the water temperature in the transport tank and the river is greater than 5˚C, check the water temperature in the totes, after unloading them to the river's edge.

If water temperature in the totes is within the 5˚C difference of the river temperature, start loading totes into holding perforated garbage cans in the river.

If the water temperature in the totes at the river's edge is still different from that in the river by more than 5˚C add an additional bucket of river water and bubbler to each tote and hold fish for approximately 15 minutes prior to retaking water temperature in totes. If water temperature in the totes is now within the 5˚C difference of the river temperature, start loading totes into holding perforated garbage cans in the river. Otherwise repeat procedure by adding an additional bucket ½ full of river water to tote and wait an additional 15 minutes prior to loading into the perforated garbage cans in the river and repeat as necessary. Once water temperatures in the totes is less than 5˚C different than in the river, move fish to perforated holding cans as quickly and

carefully as possible. Twenty-four tote sleeves are available, if needed at the holding site, for use for tempering all totes in the each transport tank.

Transfer of tagged fish to the River

Before fish are transferred into their in-river holding container, fish must be observed to check if the number of fish in each tote to agrees with what is written on the datasheet. If there are any mortalities or any fish 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 to perforated garbage cans in the river have been completed, lay rinsed, perforated totes on a tarp for drying, prior to loading on a pick-up truck for return to Lodi where they will be driven to Mokelumne River Hatchery by tagging personnel and placed into the freezers at the hatchery 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 rinsed 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

Field crew 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, 1100.

Release crews should wear all appropriate field gear. This includes; waders with boots, safety belt, appropriate outerwear, and PFD with safety strobes when on the boat and head lamp 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 2014 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.

Prior to leaving shore, confirm security guard or other crew member is on site and observing the release. He/she is on site to call for help, or assist if boat capsizes or other emergency-type event occurs.

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. If cans appear to be tipping or submerging, slow down the rate of speed. 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 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 from the container and place it into a zip-lock bag. Record the number of mortalities for each container 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 of 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. Also, 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 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 encountered 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. Do a necropsy on the morts and fill out a necropsy data sheet on the fish that were mortalities. Please make sure it is clear on the datasheet what fish you are necropsying (can obtained from and bag number if there is more than one mort 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 health):

1. **NEW FOR 2014**: Take cans containing dummy tagged fish prior to processing, halfway to release site by boat and return to shore, duplicating the release process for the dummy tagged fish.
2. 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.
3. Euthanize the 12 dummy tagged steelhead
4. Note PIT tag for each fish, the date, and time on Fish Condition Assessment data sheet.
5. 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.
6. Take picture of each fish showing sutures (turn camera date and time stamp on). Record picture number on Fish Condition Assessment data sheet
7. Do necropsy per training protocol and score tagging for each fish.
8. Put PIT tags and dummy tags in a ziplock 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, check the 24 dummy tagged steelhead for mortalities and **keep them 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.

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 fish, place the carcass into a Ziploc 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.

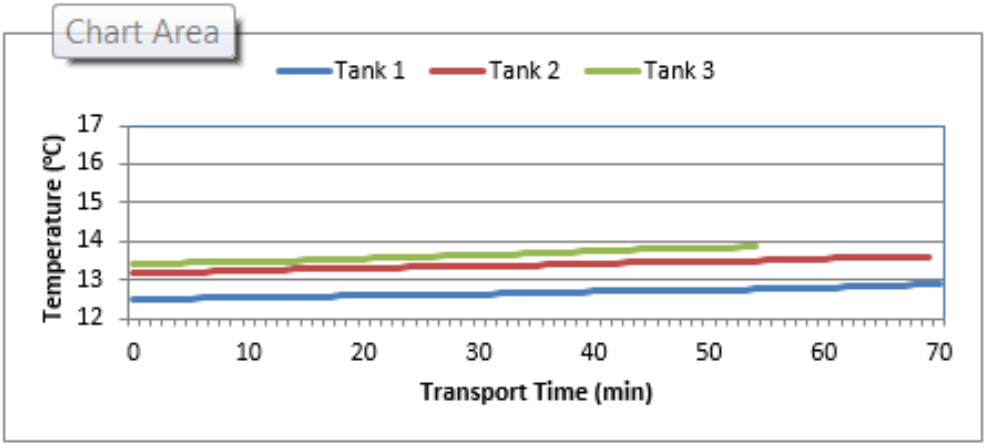
Data Entry:

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. Night crews should enter data and day crews should QA/QC database 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.

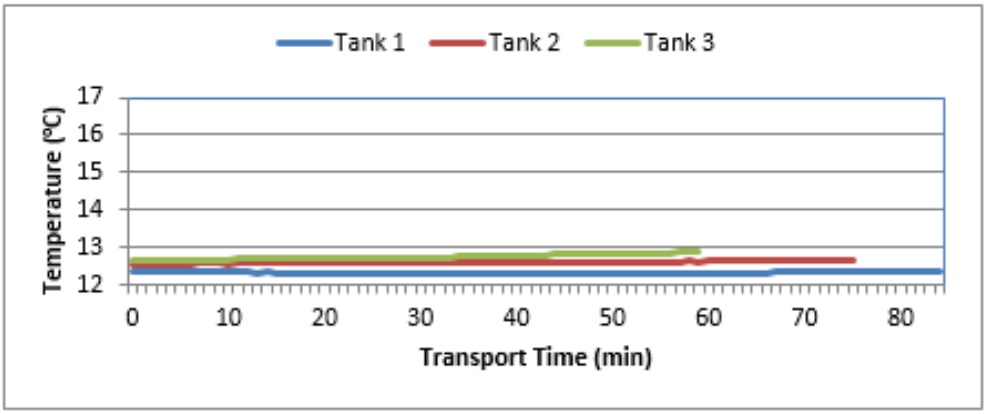
Appendix C.

Water temperatures in transport tanks during transport from Mokelumne River Hatchery to the release site or holding location at Durham Ferry during the spring of 2014. Note axis is on a different scale on **5/20/14.**

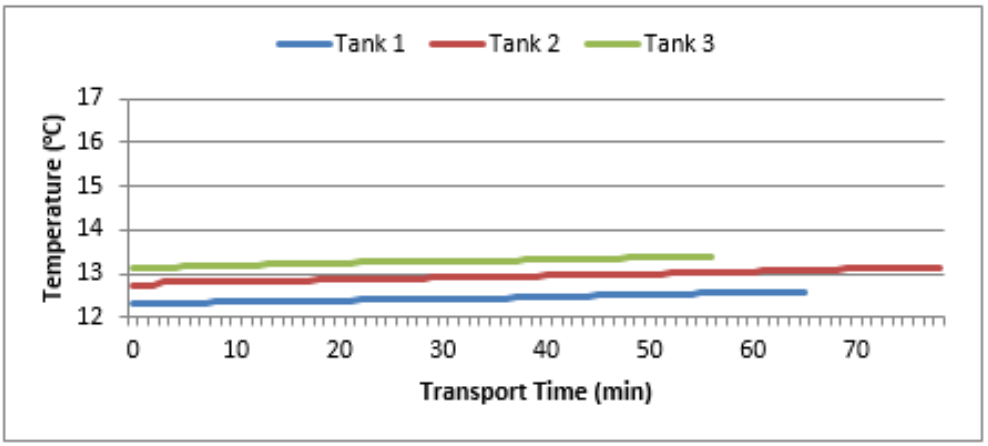
3/25/14



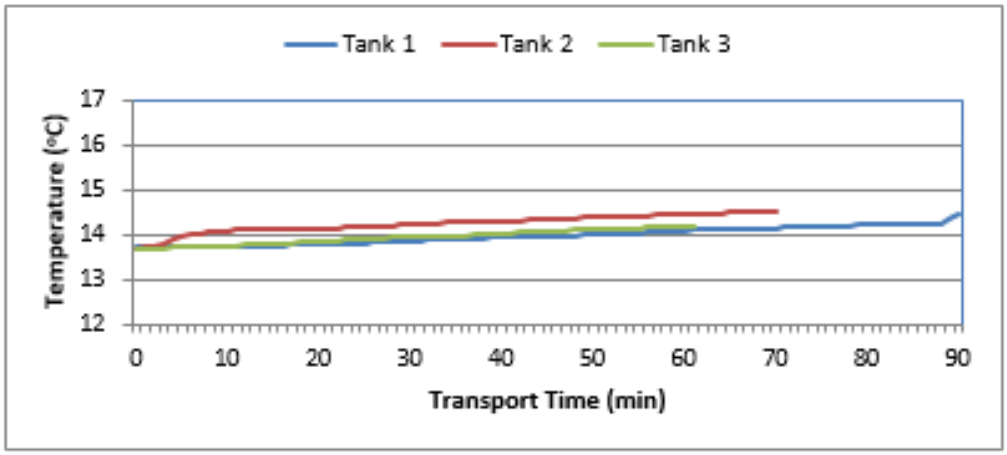
3/26/14



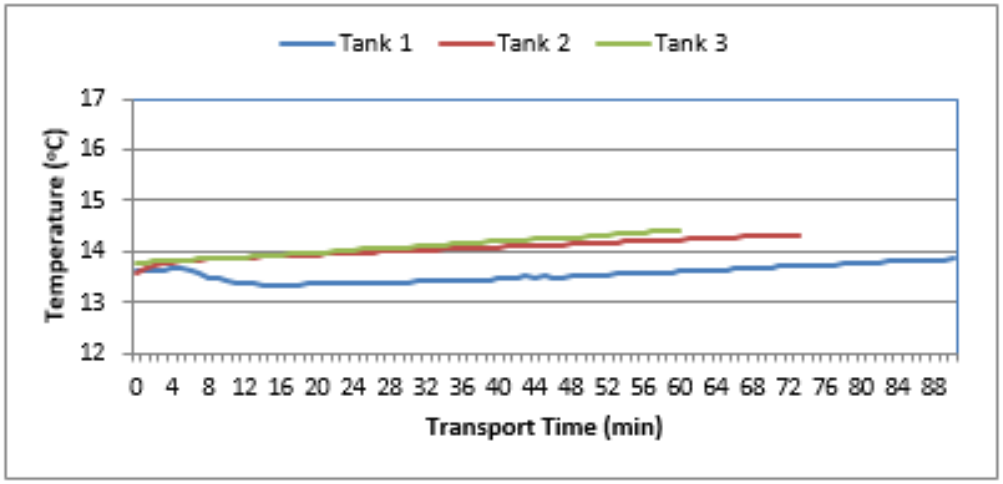
3/27/14



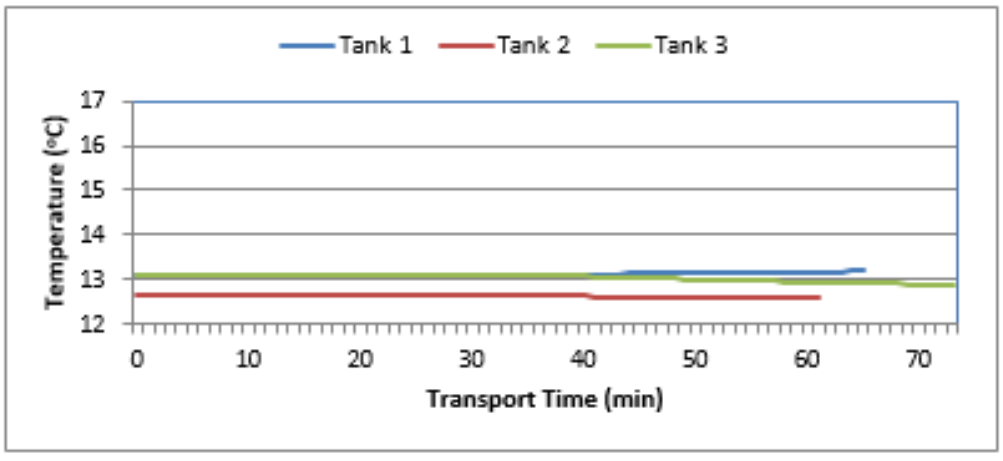
4/23/14



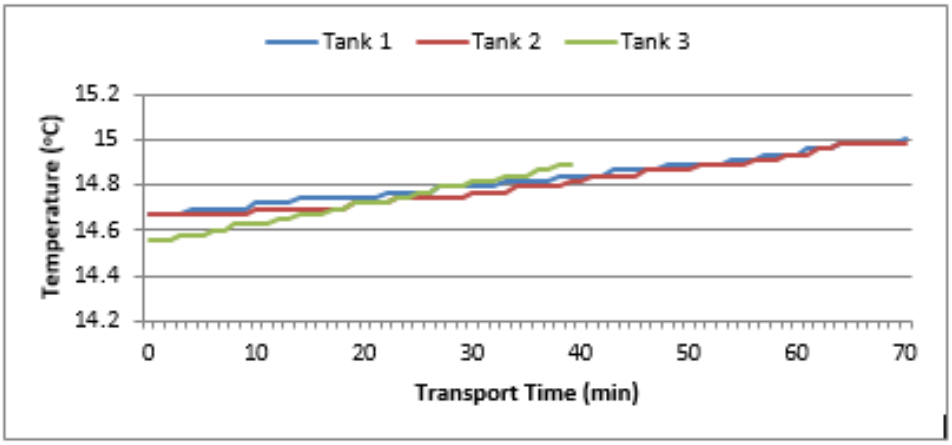
4/24/14



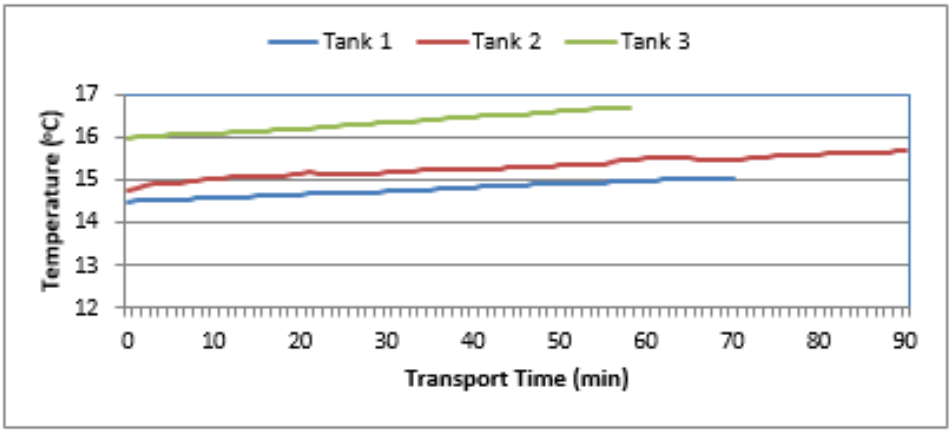
4/25/15



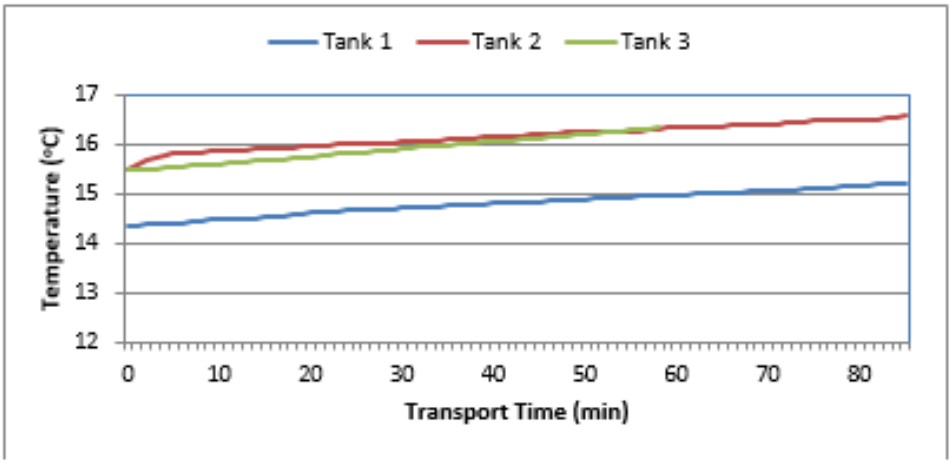
5/20/14



5/21/14



5/22/14

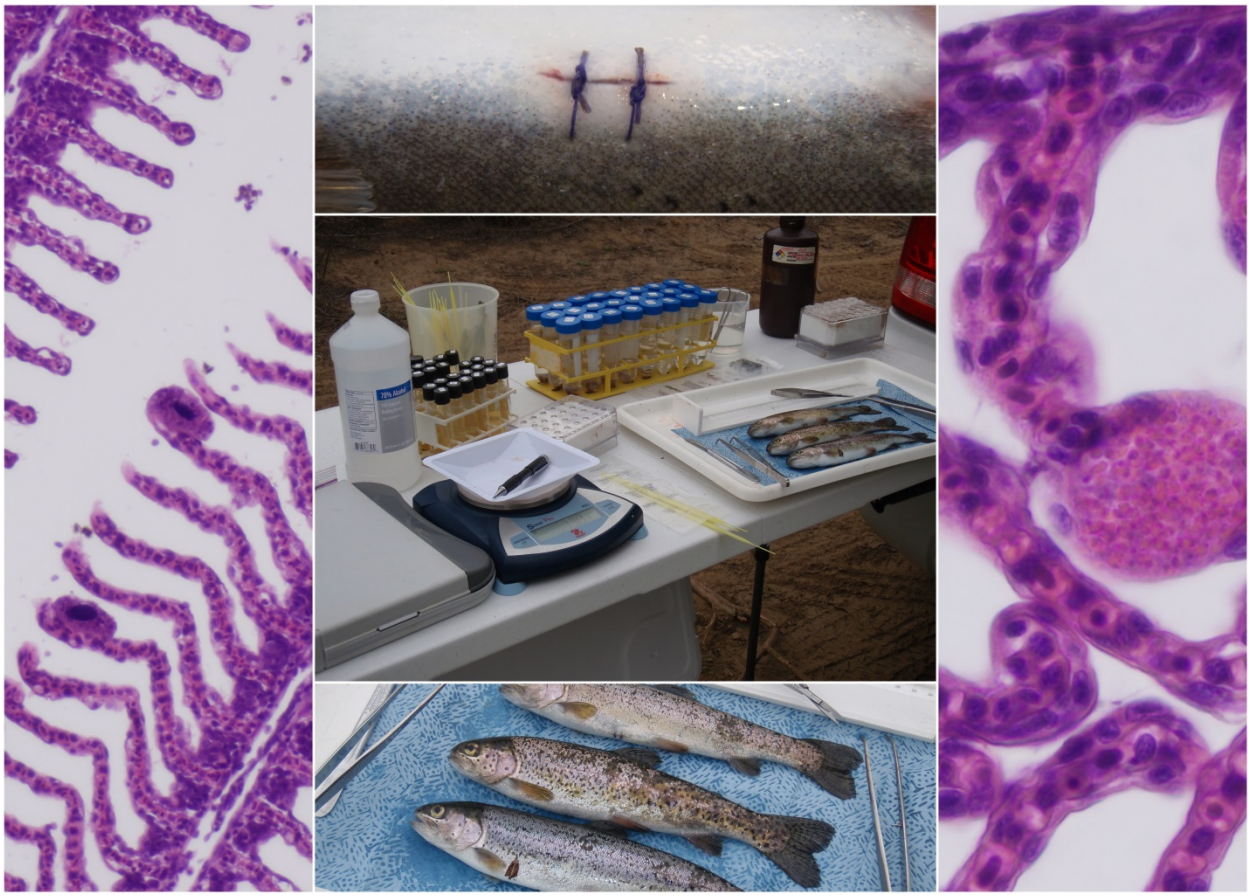


Appendix D 1.

U.S. Fish & Wildlife Service

Pathogen Screening and Gill Na^+/K^+- ATPase Assessment of South Delta Chinook and Steelhead 2014 Release Groups

Ken Nichols



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SUMMARY

The health and physiological condition of the study fish can help explain their performance and survival during the studies. Juvenile Chinook salmon and steelhead trout were surveyed for specific fish pathogens and smolt development using gill Na^+/K^+-ATPase (gill ATPase) activity levels. In both steelhead and Chinook release groups, survival over the 24 holding period was high. No significant pathogen infections were detected in Chinook or steelhead release groups. Gill ATPase levels were stable or increasing over the study period suggesting levels of smolt development would not be a factor in fish performance.

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BACKGROUND

As a component of studies examining the reach-specific survival and distribution of migrating juvenile Chinook salmon and steelhead in the San Joaquin River and Delta, the CA-NV Fish Health Center conducted a general pathogen screening and smolt physiological assessment. Steelhead trout were examined in support of the 6-year Study required by the 2009 Biological Opinion on Central Valley Project and State Water Project operations (RPA IV.2.2). The health and physiological condition of the study fish can help explain their performance and survival during the studies. Similar pathogen screening and physiological assessments have been conducted on Chinook used in various south delta studies since 1996. Juvenile Chinook from Merced River Hatchery used in the majority of these past examinations had varying levels of infections with the myxozoan parasite *Tetracapsuloides bryosalmonae*, the causative agent of Proliferative Kidney Disease (PKD). In 2014, severe PKD in Merced River Chinook required a shift to juvenile Chinook from Mokelumne River Fish Hatchery.

METHODS

FISH SAMPLING

All study fish were cohorts of acoustic tagged release groups and shadowed each release group through handling, tagging (dummy tagged), transport, and in-river holding. Study fish were held for 48 hours at the Durham Ferry release site on the San Joaquin River before sampling. Groups of 30 juvenile Mokelumne River Hatchery Chinook salmon were sampled on 19 April, 4 May and 19 May, 2014. Groups of 24 Mokelumne River Hatchery yearling steelhead trout were sampled on 29 March, 27 April and 24 May, 2014. Fish were euthanized, fork length (FL) was recorded, any abnormalities were noted and tissue sampled for lab assays.

LAB ASSAYS

Bacteriology – A sample of kidney tissue was collected aseptically and inoculated onto brain-heart infusion agar. Bacterial isolates were screened by standard microscopic and biochemical tests (USFWS and AFS-FHS 2010). These screening methods would not detect *Flavobacterium columnare*. *Renibacterium salmoninarum* (the bacteria that causes bacterial kidney disease) was screened by fluorescent antibody test of kidney imprints.

Virology – Three fish pooled samples of kidney and spleen were inoculated onto EPC and CHSE-214 at 15°C as described in the AFS Bluebook (USFWS and AFS-FHS 2010) with the exception that no blind pass was performed.

Histopathology – The tissues were removed from the fish and immediately fixed in Davidson's fixative. In the lab, the tissues were processed for 5 µm paraffin sections and stained with hematoxylin and eosin (Humason 1979). All tissues for a given fish were placed on one slide and identified by a unique code number. Each slide was examined under a light microscope and observations of abnormalities were noted. In steelhead release groups, gill tissues from all 24 fish were examined for signs of external parasite infection. In Chinook release groups, gill, kidney, liver and intestine tissues from 10 fish per group were examined for parasite infection or other abnormalities.

Gill ATPase – Gill Na^+/K^+-Adenosine Triphosphatase (gill ATPase) activity was assayed by the method of McCormick (1993). Gill ATPase activity is correlated with osmoregulatory ability in saltwater, and high concentrations are found in the chloride cells of the lamellae.

RESULTS

FISH CONDITION

Chinook – Prior to the health assessment, one fish died in 19 April group and no mortality occurred in the 4 May or 19 May release groups (Table 1). A penetrating abdominal wound (external abnormality) and degenerated intestine (internal abnormality) were noted on this single mortality. No significant scale loss or pale gills were noted in any of the Chinook health sample groups. Overall, sutures from tagging surgery were in good conditions with minor inflammation noted in 3% (1/30) of fish sampled 19 April; a loose suture noted in 3% of (1/30) fish sampled 4 May; and minor hemorrhaging noted in 13% (4/30) of fish sampled 19 May.

Table1. Chinook release group mean (± sd) fork length (FL), mortality over the 48 hr. holding period, fish with external abnormalities (Ext Abn), fish with internal abnormalities (Int Abn) and number of fish sampled for lab assays (N).

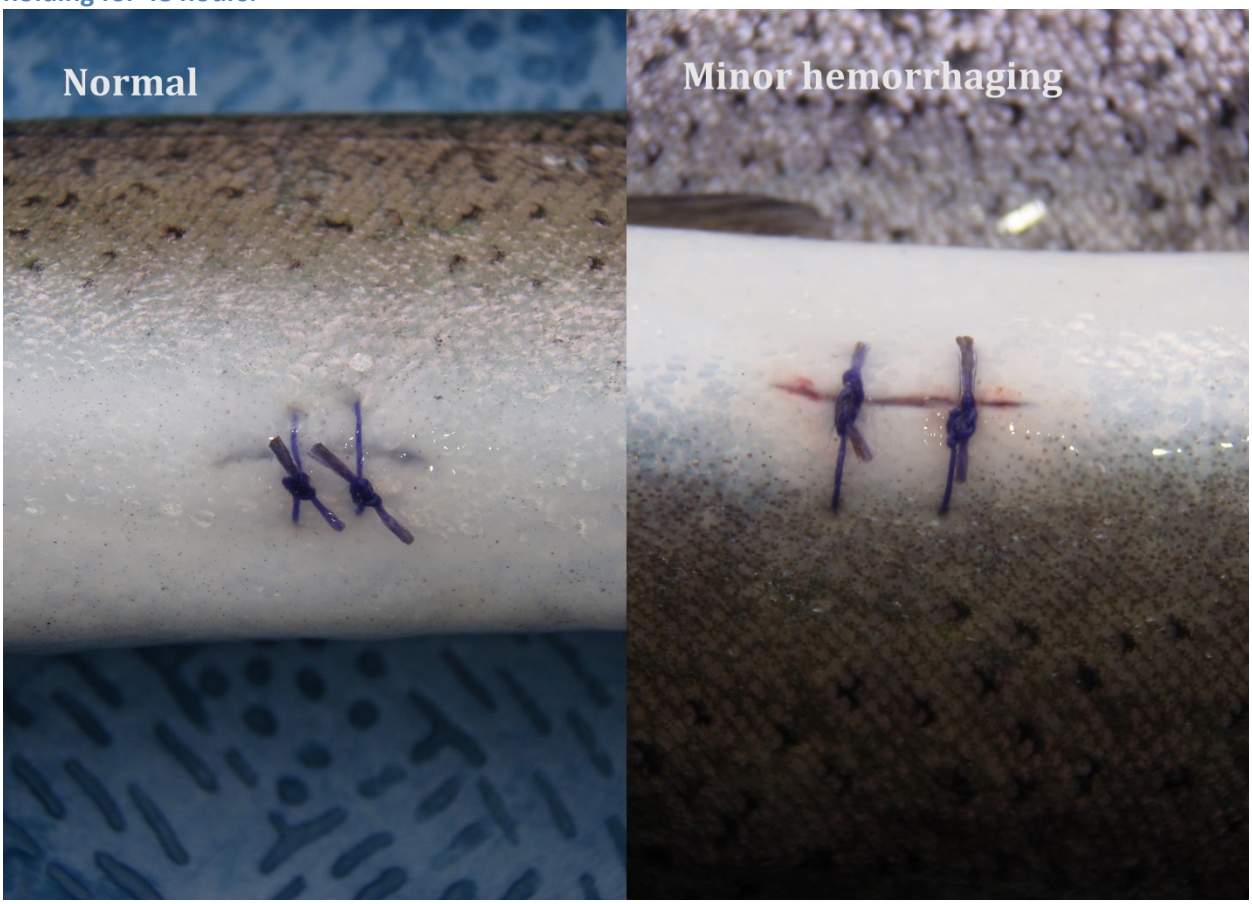
Group	FL (mm)	Mortality	Ext Abn	Int Abn	N
19 April	96.2 ±5.1	1/30 (3%)	0/30 (0%)	0/30 (0%)	29
4 May	101.2 ±4.4	0/30 (0%)	0/30 (0%)	0/30 (3%)	30
19 May	99.5 ±5.2	0/30 (0%)	0/30 (0%)	0/30 (3%)	30

Steelhead – One fish died prior to the health assessment in the 29 March release group, and no mortality occurred in the 27 April or 24 May groups (Table 2). No wounds or clinical signs of infection were observed on the single mortality. In the 29 March health assessment group, no significant external or internal abnormalities were noted, and minor hemorrhaging or inflammation (Figure 1) at the suture site was observed in 21% (5/24) of fish. In the 27 April health assessment group, cloudy eyes were noted in 4% (1/24) of fish, and partly open or bleeding sutures were observed in 8% (2/24) of fish. In the 24 May group, significant scale loss (>50% of body) was noted in 13% (3/24) of fish; 4% (1/24) of fish had eye abnormalities; and minor hemorrhaging or partly open sutures were noted in 21% (5/24) of fish.

Table 2. Steelhead release group mean (± sd) fork length (FL), mortality over the 48 hr. holding period, fish with external abnormalities (Ext Abn), fish with internal abnormalities (Int Abn) and number of fish sampled for lab assays (N).

Group	FL (mm)	Mortality	Ext Abn	Int Abn	N
29 March	240 (±14)	1/24 (4%)	0/24 (0%)	0/24 (0%)	23
27 April	250 (±13)	0/24 (0%)	1/24 (4%)	0/24 (0%)	24
24 May	249 (±17)	0/24 (0%)	4/24 (17%)	0/24 (0%)	24

Figure 1. Examples of normal sutures and minor hemorrhaging at suture site in fish assessed after holding for 48 hours.



BACTERIOLOGY AND VIROLOGY

In both Chinook and steelhead sample groups, no virus or other cytopathic effects were observed by cell culture over the 21 day incubation period. No obligate bacterial pathogens were detected, and other isolates were isolated in 3-24% of sample groups (Table 3). These other isolates were common fauna in the environment and fishes GI tract (Aoki 1999) and were likely contaminates due to field sampling conditions.

Table 3. Summary of bacteria isolated from the kidneys of dummy tagged fish.

Species	*Aeromonas* /*Pseudomonas*	various Gram positive bacteria
Chinook	3% (3/87)	17% (15/87)
Steelhead	11% (8/71)	24% (17/71)

HISTOPATHOLOGY

Chinook – No significant abnormalities or signs of infection were detected in tissues from the 30 fish examined.

Steelhead – No significant abnormalities were observed on the gills of 69 fish examined; however, subclinical parasite infections were observed. Light infections with *Capriniana piscium* (Figure 2A, formerly known as *Trichophrya*, presumptive identification) were observed in 75% (52/69) of gills. Cyst-like xenoma (Figure 2B) caused by an unidentified microsporidian were observed in 3% (2/69) of gill samples. There was no associated lesion or other sign of impairment associated with these infections.

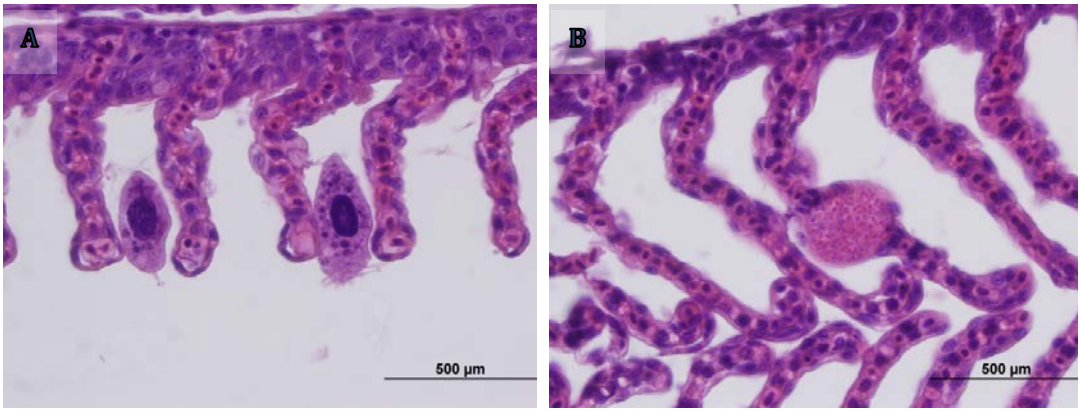


Figure 2. Histology sections (H&E stained) of steelhead gills with (A) *Capriniana piscium* (formerly *Trichophrya*) infections, and (B) Cyst-like xenoma. Note the absence of significant inflammation or lesion in both infections.

GILL ATPASE ACTIVITY

Chinook – Gill ATPase activity levels (µmol ADP*mg protein^{-1}*hr^{-1}) ranged from 0.6 to 14.3. Two fish from the 19 April sample group were excluded from the analysis due extremely high activity levels which were likely errors in the protein measurement. The activity levels in the 4 May release group were lower than the 19 April and 19 May groups (Figure 3, $P<0.001$, ANOVA).

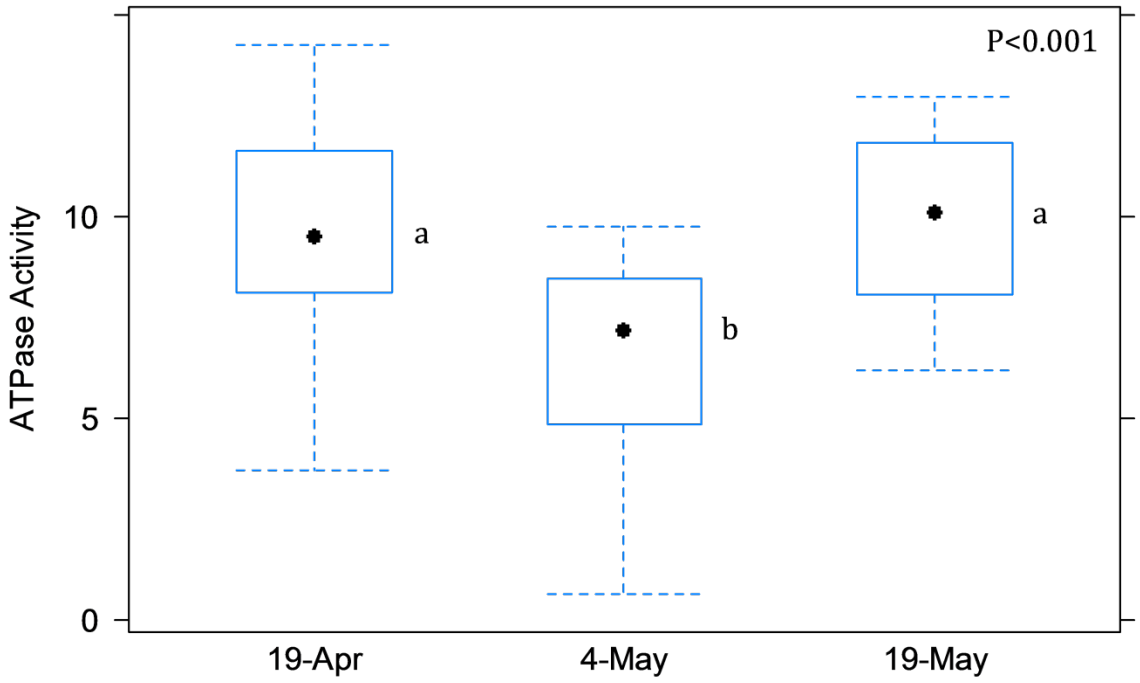


Figure 3. Boxplot of median gill ATPase activity (µmol ADP·mg protein^{-1}·hr^{-1}) in juvenile Chinook salmon sampled from the 19 April, 4 May and 19 May release groups. Groups with letter subscripts in common were not significantly different (P<0.001, ANOVA).

Steelhead – Gill ATPase activity levels (µmol ADP*mg protein^{-1}*hr^{-1}) ranged from 0.2 to 5.7. Activity levels tended to increase with the highest levels observed in the May release group (Figure 4, P=0.008, ANOVA).

DISCUSSION

No significant health issues were observed in either the Chinook or steelhead release groups in 2014. The Chinook salmon from Mokelumne River Hatchery used in the study this year did not have any signs of *T. bryosalmonae* infections common in the Merced River Hatchery Chinook during past years. The minor suture issues observed in both Chinook and steelhead release groups were observed in only a few individuals and did not impact overall health of the fish. Several steelhead from the 24 May release group were observed to have significant scale loss which may have been an indication of higher smolt development.

Gill ATPase activity levels were stable or increasing over the study period suggesting smolt development would not be a significant factor in fish performance. Gill ATPase activity in salmonids typically increases and peaks near the time of most active

migratory behavior (Duston, Saunders and Knox 1991; Ewing, Ewing and Satterthwaite 2001; Wedemeyer 1996). In Chinook sample groups, gill ATPase levels were similar in the first (19 April) and last (19 May) release groups suggesting these fish were not yet past time peak smolt development. The cause of the lower median gill ATPase levels observed in the second (4 May) Chinook release group was not apparent. While in steelhead sample groups, gill ATPase levels increased over time the relationship with migration behavior may not be consistent. In unpublished CA-NV Fish Health Center data, steelhead have demonstrated the ability to significantly increase activity levels in only a few days following hatchery release.

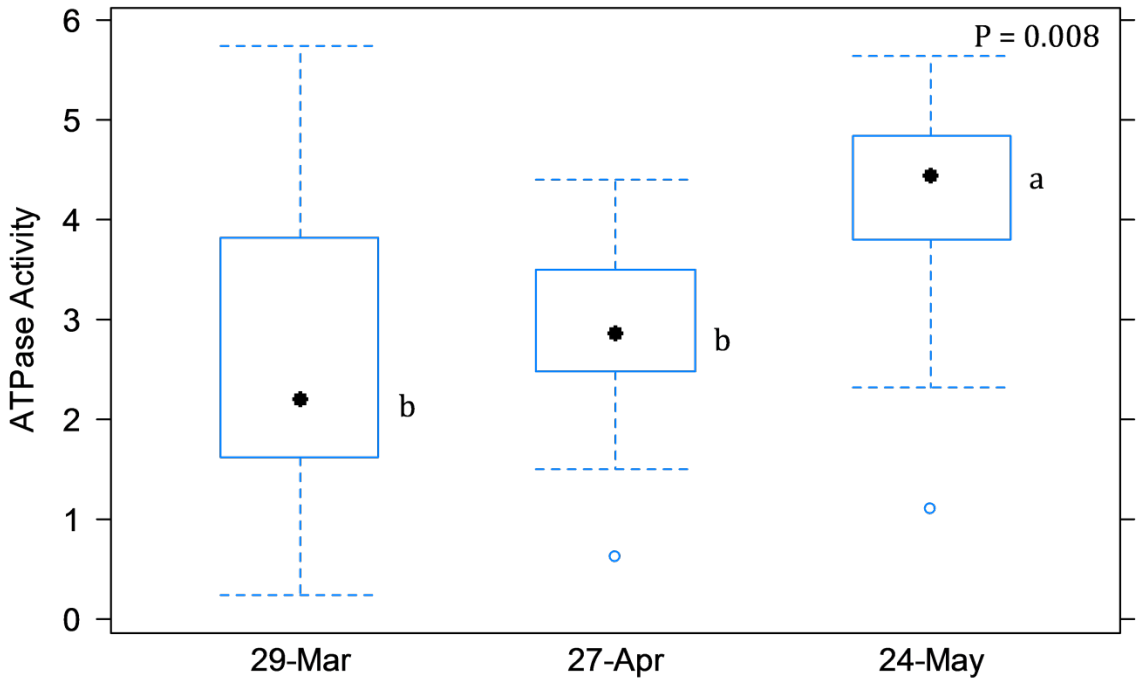


Figure 4. Boxplot of median gill ATPase activity (µmol ADP·mg protein^{-1}·hr^{-1}) in juvenile steelhead from the March, April and May release groups. Groups with letter subscripts in common were not significantly different (P=0.008, ANOVA).

ACKNOWLEDGMENTS

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